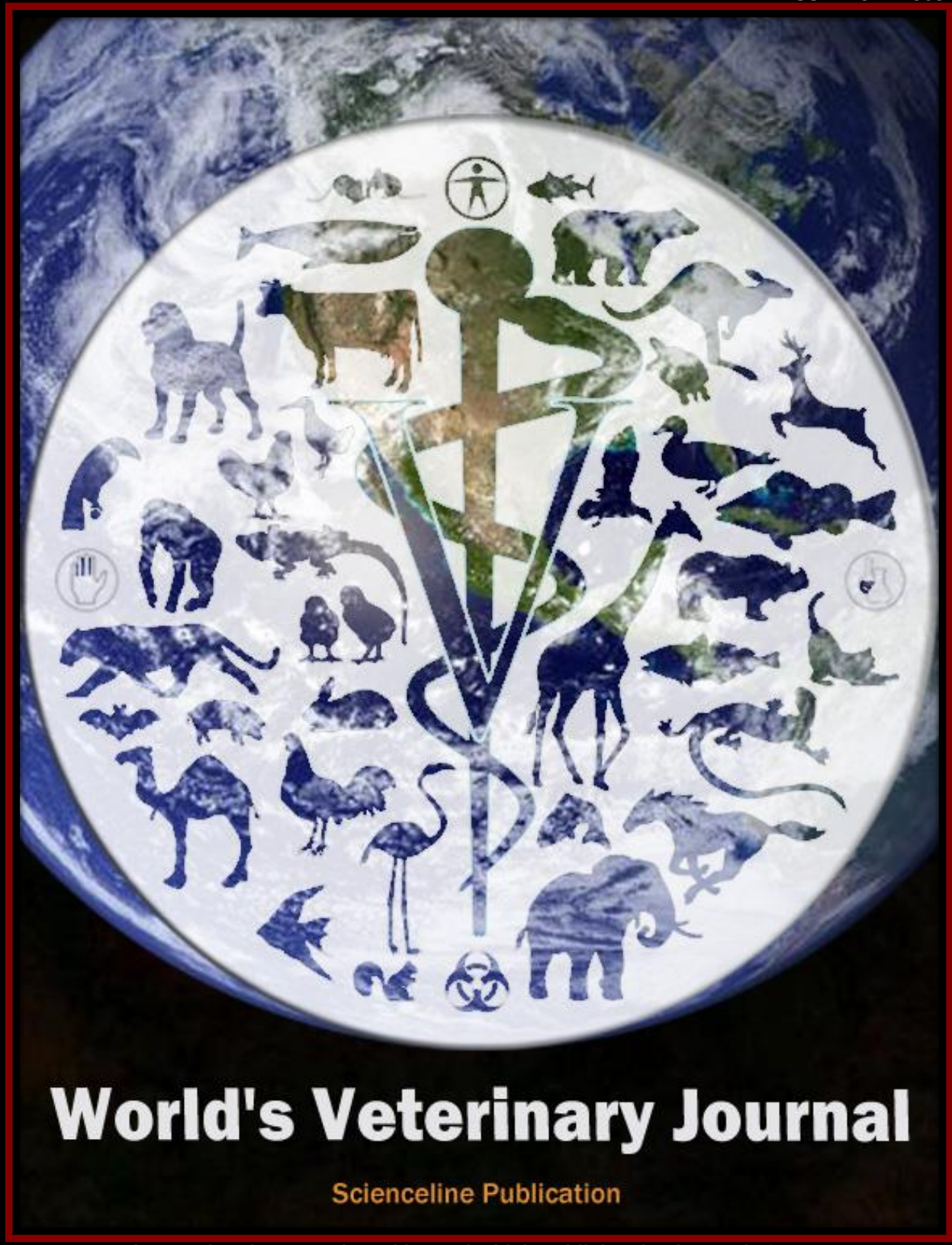
In the Name of God

ISSN: 2322-4568



World's Veterinary Journal

Scienceline Publication

An international peer-reviewed journal which publishes in electronic format

Volume 10, Issue 4, December 2020

WORLD'S VETERINARY JOURNAL

ISSN: 2322-4568

World Vet. J., 10 (4): 465-480; December 25, 2020

Advisory Board

Archive

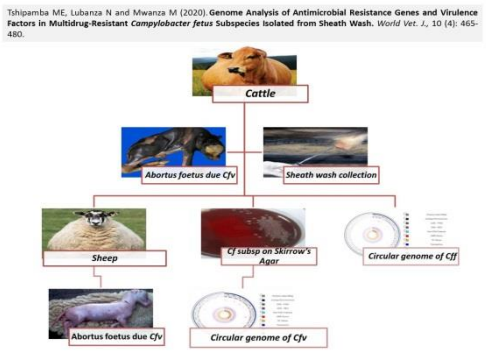
Volume 10 (4); December 25, 2020

Research Paper

Genome Analysis of Antimicrobial Resistance Genes and Virulence Factors in Multidrug-Resistant Campylobacter fetus Subspecies Isolated from Sheath Wash

Tshipamba ME, Lubanza N and Mwanza M.

World Vet. J. 10(4): 465-480, 2020; pii:S232245682000057-1-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj57



ABSTRACT: *Campylobacter fetus* subspecies are mostly characterized by reproductions problems in cattle and sheep. This study aimed to study the genetic profile and assess the genes mechanism of resistance and their virulence factors using genome sequence analysis. A total of 59 confirmed *Campylobacter fetus* subspecies based on molecular assays and DNA sequencing were subjected to antimicrobial susceptibility test against 14 antibiotic agents representing the five classes of antibiotics using the disc diffusion method. In addition, sequencing the genome of all strains induced complete resistance against all tested antibiotics. The results of the antimicrobial test indicated that 54.4% had a resistance profile, 26.3% were intermediate, while 19.3% were observed to be susceptible. The Whole Genome Sequencing (WGS) result revealed the presence of different genes, such as Broad-specificity multidrug efflux pump and 16S rRNA (guanine^{527}-N^{7})-methyltransferase (gidB), efflux pump conferring antibiotic resistance (MacA and MacB), protein-altering cell wall charge conferring antibiotic resistance (PgsA), which have never been reported in *Campylobacter fetus* subspecies. The WGS also revealed the presence of genes that involved in colonization, adhesion, motility, and invasion, such as type IV secretion system protein (VirD4), S-Layer, cytolethal distending toxin (A, B, and C), *Campylobacter* invasion antigen (CiaB), and fic domain protein (fic) were among important CDS. The presence of these uncommon genes explains the resistance of *Campylobacter fetus* subspecies against different tested antibiotics. The results of this study can be used to implement molecular surveillance of *Campylobacter fetus* subspecies and conduct further studies on the resistance mechanism in these subspecies.

Keywords: Broad-specificity multidrug efflux pump, *Campylobacter fetus* subspecies, Genome analysis, Methyltransferase gidB, Multidrug resistance.

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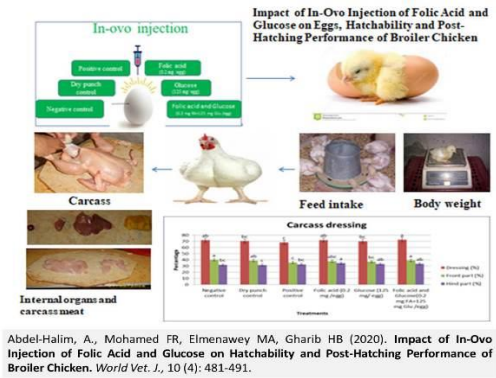
Research Paper

Impact of In-Ovo Injection of Folic Acid and Glucose on Hatchability and Post-Hatching Performance of Broiler Chicken

Abdel-Halim A, Mohamed FR, Elmenawey MA, Gharib HB.

World Vet. J. 10(4): 481-491, 2020; pii:S232245682000058-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj58



ABSTRACT: The present study was designed to investigate the impact of in-ovo injection of folic acid and glucose on hatching eggs from 55 weeks old broiler breeders. A total number of 900 hatching eggs were collected from Arbor Acres broiler breeders, then, eggs were divided into 6 groups including 1) Negative Control (non-injected, NC), 2) Dry Punch Control (pricked without injecting any solution, DPC), 3) Positive Control (eggs were injected with 0.5 mL normal saline, PC), 4) Folic Acid group (eggs were injected with 0.2 mg/ egg folic acid, FA), 5) Glucose group (eggs were injected with 125 mg/ egg glucose, Glu), and 6) Folic Acid with Glucose group (eggs were injected with 0.2 mg folic acid with 125 mg/ egg glucose, FA+Glu). Each treatment was divided into five replicates of 30 eggs each. Eggs were injected into the albumen under the air sac. After in-ovo injection, the eggs were stored for four days before hatching. After hatching, the chickens were reared in groups according to the treatments. All treatments were divided into 10 replications of 9 chickens in each. In-ovo injection with folic acid decreased the albumen pH significantly to 9.19 after 4 days of injection, while the negative control was 9.43. Hatching quality was severely affected by all in-ovo injection treatments, but no significant differences were found between the treatment groups concerning the hatchability of fertile eggs. Injection treatments had no significant effect on the growth rate or the production number in any of the weeks. Injection of folic acid and (FA+Glu) significantly increased chickens' body weight at two and four weeks of age. Also, the dressing percentage when using folic acid and (FA+Glu) was significantly increased to 72.1% and 72.5%, respectively, compared to the positive control group (68.3%). In conclusion, our data suggested that in-ovo injection with a mixture of folic acid and glucose (0.2 mg folic acid+ 125 mg/ egg glucose) could be

used to enhance carcass characteristics. Further studies should be conducted to find the effects of in-ovo injection folic acid and glucose on different incubation days and at different sites of injection.
Keywords: Broilers, Folic Acid, Glucose, Hatchability, In- Ovo injection, Old breeders, Post-hatch

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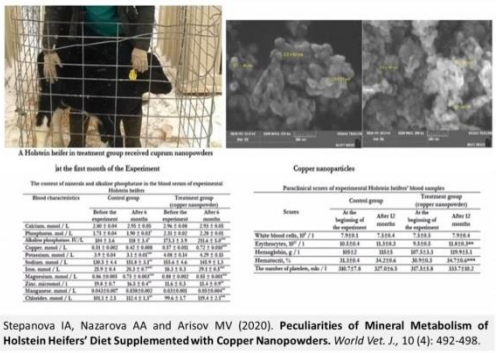
Research Paper

Peculiarities of Mineral Metabolism of Holstein Heifers' Diet Supplemented with Copper Nanopowders

Stepanova IA, Nazarova AA and Arisov MV.
World Vet. J. 10(4): 492-498, 2020; pii:S232245682000059-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj59



Stepanova IA, Nazarova AA and Arisov MV (2020). **Peculiarities of Mineral Metabolism of Holstein Heifers' Diet Supplemented with Copper Nanopowders.** *World Vet. J.*, 10 (4): 492-498.

ABSTRACT: The current study aimed to investigate the effect of copper nanopowder on physiological and mineral metabolism indicators of Holstein cattle during the growth process of the animal. There were two experimental groups (control and treatment) and each one included seven Holstein heifers. From the first month of each heifer in the experimental group, a suspension of copper nanoparticles in a dose of 0.04 mg/kg was orally administered daily to the usual diet. The biologically active additive was administered to the animal's diet at intervals. The weight of the animals was measured monthly, a clinical blood test was performed, the mineral composition of the blood was studied, the mineral composition of animal hair was also examined. The findings indicated a positive dynamics in the increase of body weight in the treatment group, compared to the control group. As a result of clinical blood tests, it was noted that the number of erythrocytes, the level of hemoglobin, and hematocrit increased in the experimental animals due to copper nanoparticles compared to these parameters in these animals at the beginning of the tests. Mineral analysis of blood samples in the treatment group presented an increase in the levels of copper, potassium, iron, zinc, and manganese while in animals of the control groups there was an increase in sodium, calcium, and phosphorus. According to the blood serum and hair analysis, copper in the nanodispersed state indicated antagonistic effects on boron, silicon, antimony, molybdenum of the treatment group and there was a synergist in aluminum, titanium, manganese, cobalt, iron, and potassium levels compared to the beginning of the experiment. The obtained results indicated that the addition of copper nanopowder to the diet of experimental animals increased the growth, stimulated the function of hematopoiesis, and improved the characteristics of mineral metabolism of the Holstein heifers.
Keywords: Copper nanopowder, Cattle, Mineral metabolism, Physiological characteristics

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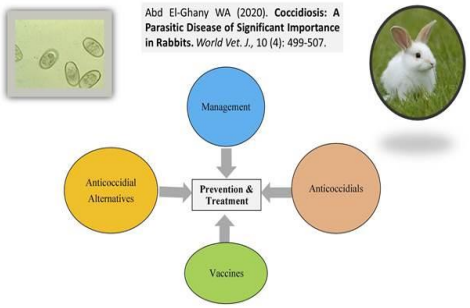
Review

Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits

Abd El-Ghany WA.
World Vet. J. 10(4): 499-507, 2020; pii:S232245682000060-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj60



ABSTRACT: Rabbits are considered an important and healthy source of animal protein all over the world. They are susceptible to important diseases that can reduce their productivity, causing severe economic losses. Coccidiosis is one of the important protozoon diseases caused by Eimeria species. Rabbits are highly susceptible to coccidiosis, especially after weaning time. Coccidiosis in rabbits has two forms, namely hepatic and intestinal. Affected animals indicated the symptoms of diarrhea, reduced appetite, dehydration, and weight loss as well as liver and intestinal lesions. Diagnosis is based on the detection of the infective stages of the protozoon in feces or affected tissues. Prevention and control are achieved by adopting hygienic measures and using different anticoccidial drugs. The use of natural alternatives for the prophylaxis of coccidiosis in rabbits indicated promising results. Vaccine production trials are still under investigation. Accordingly, this review article aims to shed light on coccidiosis in rabbits considering pathology, diagnosis, and control.
Keywords: Eimeria, Intestine, Liver, Rabbits, Treatment
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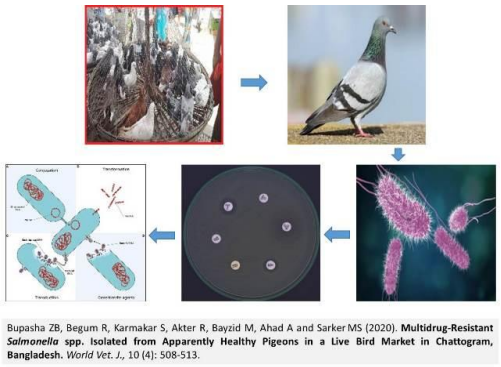
Research Paper

Multidrug-Resistant Salmonella spp. Isolated from Apparently Healthy Pigeons in a Live Bird Market in Chattogram, Bangladesh

Bupasha ZB, Begum R, Karmakar S, Akter R, Bayzid M, Ahad A and Sarker MS.
World Vet. J. 10(4): 508-513, 2020; pii:S232245682000061-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj61

ABSTRACT: Multidrug-resistant *Salmonella* could pose a severe public health threat. The current study aimed to investigate the prevalence of antibiotic resistance and some antibiotic-resistant genes in *Salmonella* spp. isolated from pigeons in a live bird market, Chattogram, Bangladesh. A total of 100 cloacal swab samples were collected aseptically from apparently healthy pigeons in the live bird market, namely Riazuddin Bazar in Chattogram city, Bangladesh. Different bacteriological and biochemical tests were used for the isolation and identification of *Salmonella* spp. The susceptibility test of *Salmonella* isolates to different antibiotics was performed by the disk diffusion method. PCR assay using specific primers was used for antibiotic resistance genes detection. The results indicated that the prevalence of *Salmonella* spp. was 29% in sampled birds. The highest antibiotic resistance rate was found to be ampicillin (93.1%), followed by both sulfamethoxazole-trimethoprim and tetracycline (86.2%). In contrast, 65.5% of isolates were found sensitive to ciprofloxacin, followed by colistin (62.1%), kanamycin (55.2%), and gentamicin (48.3%). 96.6% of *Salmonella* isolates were classified as multidrug-resistant and harbored *bla*TEM, *tet*A, *sul*1, and *sul*2 genes. In conclusion, pigeons as carriers of antibiotic-resistant *Salmonella* spp. may pose a health risk to other birds and humans.
Keywords: Antibiogram, Antibiotic resistance genes, Pigeons, Prevalence, *Salmonella*



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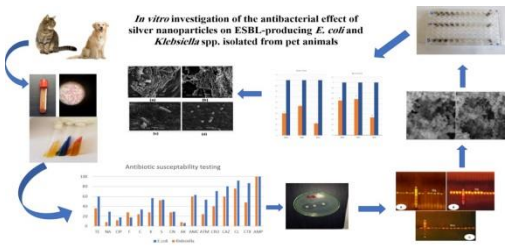
Research Paper

In Vitro Investigation of the Antibacterial Effect of Silver Nanoparticles on ESBL-producing *E. coli* and *Klebsiella* spp. Isolated from Pet Animals

Khalil OA, Enbaawy MI, Salah T, Mahmoud H and Ragab E.

World Vet. J. 10(4): 514-524, 2020; pii:S232245682000062-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj62

ABSTRACT: Despite the presence of modern antibacterial drugs, bacterial infections are still a major threatening problem due to the enormous increase in multi-drug-resistant bacteria. Nanoparticles have been extensively used as an applicable and safe alternative to antibiotics. The present study aimed to explore the inhibitory effect of silver nanoparticles on Extended Spectrum Beta lactamase (ESBL) producing *E. coli* and *Klebsiella* spp. in vitro as well as their effect on the expression of antibiotic resistance genes. Different samples (i.e., wound swabs, Fecal swabs, and urine samples) were collected from dogs and cats. Phenotypic and molecular identification, antibiotic susceptibility testing, and double-disk synergy test were carried out for the identification of ESBL producing *E. coli* and *Klebsiella* spp. Silver nanoparticles were tested for their in vitro antibacterial potential and there were reports of their minimum inhibitory concentration and minimum bactericidal concentration. Moreover, the effect of silver nanoparticles on the expression of antibiotic resistance genes (i.e., *blaTEM, blaSHV,* and *blaCTX*) was assessed as well as their effect on the structural integrity of the bacterial cells using Scanning Electron Microscope (SEM). Results revealed that 23 isolates (19.16%) (*E. coli*=17, *Klebsiella* spp.=6) were confirmed as ESBL producing. Silver nanoparticles indicated a promising antibacterial effect where the minimum inhibitory concentration of AgNPs for ESBL producing *E. coli* was measured as 0.31 mg/ml, and 0.62 mg/ml for ESBL-producing *Klebsiella* spp., while the minimum bactericidal concentration of ESBL-producing *E. coli* and *Klebsiella* spp. was reported as 0.15 mg/ml and 0.3 mg/ml, respectively. Consequently, the expression of antibiotic resistance genes was downregulated in both bacteria species and there was a noticeable toxic effect of AgNPs on *E. coli* and *Klebsiella* spp. cells which was investigated using SEM. It can be concluded that silver nanoparticles have a promising antibacterial activity and could be considered an applicable alternative for the control of ESBL producing bacteria.
Keywords: *E. coli*, ESBLs*, Klebsiella* spp., Pets, Silver nanoparticles



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Research Paper

Polymorphism Association of Pituitary Positive Transcription Factor-1 Gene with Body Weight Traits in BC1 Hybrid Chicken (*Gallus gallus gallus Linnaeus*, 1758) from Cross Breeding between Female F1 Broiler and Male Pelung

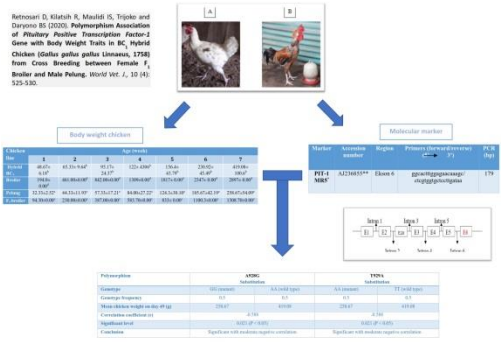
Retnosari D, Kilatsih R, Maulidi IS, Trijoko and Daryono BS.

World Vet. J. 10(4): 525-530, 2020; pii:S232245682000063-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj63

ABSTRACT: Pituitary Positive Transcription Factor-1 gene is closely related to chicken growth and productivity. This research was conducted to detect Single Nucleotide Polymorphism in the exon 6 Pituitary Positive Transcription Factor-1 gene and its association with the bodyweight growth in the first backcross hybrid chicken. Procedures of the research included crossbreeding female first filial broiler chicken with male Pelung chicken to obtain first backcross hybrid chicken, Day Old chick hatched were maintained during 49 days, the bodyweight on the Day-Old chick measured every seven

days, DNA was isolated by Chelex 5% method, Pituitary Positive Transcription Factor-1 gene was amplified by PCR, DNA band was visualized utilizing electrophoresis, and the PCR product was sequenced using Sanger method. The DNA sequence was aligned using Clustal omega software to gain Single Nucleotide Polymorphism. The Single Nucleotide Polymorphism was analyzed using the Pearson correlation test between chicken body weights of 49-days-old chickens with the polymorphism points. The conclusion indicated that the bodyweight of the first backcross hybrid chicken was higher than the Pelung chicken but lower than the first filial broiler chicken. Single Nucleotide Polymorphism was not found on the exon 6 Pituitary Positive Transcription Factor-1 gene in the first backcross hybrid chicken.

Keywords: Growth, Hybrid chickens, PIT-1 gene, SNP



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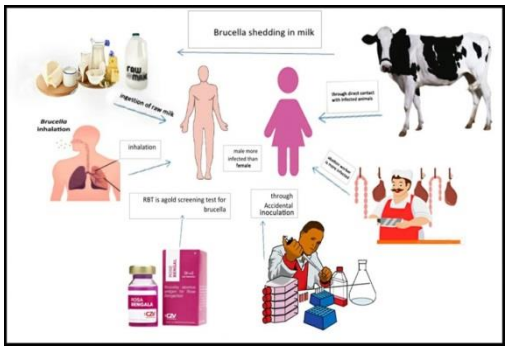
Research Paper

Seroprevalence and Associated Risk Factors of Brucellosis in Livestock and Residents of New Valley Governorate, Egypt

Diab MS, Zidan ShAA, Hassan NAA, Elaadli H and Bayoumi AM.

World Vet. J. 10(4): 531-539, 2020; pii:S232245682000064-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj64



ABSTRACT: Brucellosis is a worldwide zoonotic disease which is now considered endemic in most parts of Egypt. A cross-sectional study was carried out from December 2018 to February 2020 to investigate the seroprevalence of brucellosis in humans and livestock residing in two regions located in New Valley Governorate, Egypt. A total of 1254 animals (673 cattle, 348 sheep, and 233 goats) and 523 human serum samples were examined for brucellosis using Rose Bengal test (RBT) and then randomly selected sera (15 from cattle, 7 from sheep, 3 from goats, and 45 from humans) were further analyzed by complement fixation test, enzyme-linked immunosorbent assay to compare and detect the sensitivity and specificity of RBT. The prevalence of brucellosis was 0% in cattle, sheep, and goats while it was 23.9% in humans using RBT. Concerning humans, there was a higher percentage of infection in EL Kharga (33.6%). The prevalence of this infection was also at a higher level among individuals aged above 40 years (28.57%). Furthermore, men (26.11%) were more inclined to be inflicted, compared to women (22.5%) with no significant difference. Considering the human occupation, abattoir workers were the most predominant group of people at risk (33.3%), followed by farmers (31.25%) and animal keepers (20.6%) while the lowest prevalence was demonstrated in the housewives where the prevalence was 18.8 %. As a result, risk factors of the age range, locality, time of infection, contact with animals, and occupational groups could significantly affect the prevalence of human brucellosis in the New Valley Governorate. In conclusion, brucellosis is an alarming problem among residents of the New Valley Governorate. Thus, reducing the prevalence in humans and animals in the region of study may include restriction of the marketing the raw milk and enhancing public health awareness.

Keywords: Brucellosis, Cattle, Complement fixation test, ELISA, Human, Rose Bengal test, Sheep and goats.

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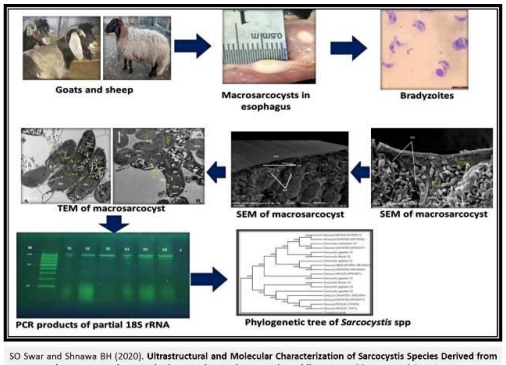
Research Paper

Ultrastructural and Molecular Characterization of Sarcocystis Species Derived from Macroscopic Sarcocysts of Domestic Sheep and Goats in Soran City, Erbil, Iraq

Swar SO and Shnawa BH.

World Vet. J. 10(4): 540-550, 2020; pii:S232245682000065-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj65



SO Swar and Shnawa BH (2020). **Ultrastructural and Molecular Characterization of Sarcocystis Species Derived from Macroscopic Sarcocysts of Domestic Sheep and Goats in Soran City, Erbil, Iraq.** *World Vet. J.*, 10 (4): 540-550.

ABSTRACT: This study aimed to identify *Sarcocystis* species isolated from macroscopic sarcocysts from naturally infected domestic sheep and goats using the molecular method, as well as investigating the morphological and the ultrastructural characteristics of the isolated species. A total of 1000 esophagi were collected from sheep and goats and examined for the presence of sarcocysts. Macroscopic sarcocysts were isolated from the infected esophagi, and *Sarcosystis* species were identified molecularly by 18S rRNA gene sequence analysis. Moreover, the ultrastructure of the sarcocysts was investigated by both scanning and transmission electron microscopy. The macroscopic sarcocysts were detected in 9.1% (91/1000) of the esophagi. The results of electron microscopy indicated the characteristic features of the macroscopic sarcocysts. The cysts contained numerous merozoites and banana-shaped bradyzoites. The bradyzoites were characterized by possessing a double-membrane pellicle and

consisted of a conoid in one of the apices, numerous micronemes, two rhoptries, as well as a long, convoluted mitochondrion, subterminal nucleus, and several amylopectin granules. The partial analysis of the 18S rRNA gene presented that all isolates produced bands of expected sizes on gel electrophoresis. The findings from the phylogenetic analysis revealed that the identified *Sarcocystis* species were most closely related to *S. gigantea, S. moulei,* and *S. medusiformis*. To the authors' knowledge, this is the first time *S. medusiformis* has been recorded in goats. Goats and sheep can be proposed as alternative intermediate hosts for *S. gigantea* and *S. moulei,* respectively, cross-infection may also occur between them and the host specificity of these species of *Sarcocystis* is questionable.
Keywords: Goats, Phylogeny, *Sarcocystis*, Sheep, Ultrastructure, 18S rRNA

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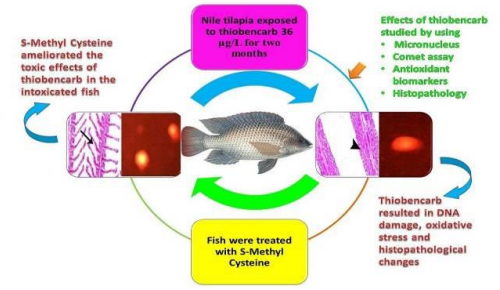
Research Paper

S-Methyl Cysteine Protective Effects in Oreochromis Niloticus Fish Contaminated by Thiobencarb Herbicide

Elmadawy MA, Abdo W, Omar AA and Mahfouz NB.

World Vet. J. 10(4): 551-561, 2020; pii:S232245682000066-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj66

ABSTRACT: Thiobencarb which is a carbamate herbicide is used for managing undesirable weeds during rice cultivation in Egypt. This study was designed to investigate the adverse effects of a field dose of thiobencarb on Nile tilapia and ameliorating the role of the low dose of S-methyl cysteine (SMC). Experimental fishes were divided into four groups; first group was reared without any treatments and served as a control group; the second group was exposed to thiobencarb (36µg/L); the third group was fed on a commercial feed containing 200 mg of SMC/Kg in conjunction with thiobencarb added to aquarium (36µg/L) while, the fourth group was fed on a feed containing 200 mg of SMC/Kg only. Fishes were sacrificed at the end of the experimental course (two months) and sampling was carried out. Catalase, Glutathione S Transferase activities, Glutathione reduced, and Malondialdhyde levels were assayed. Genotoxic effect of thiobencarb and SMC on treated fish was investigated in erythrocytes, gills, and liver tissues using micronucleus and comet assay. Histopathological examination of livers, gills, and brain was also carried out. The results indicated that fish exposed to thiobencarb indicated herbicide dependent oxidative stress and genotoxic effect justified by a significant difference in antioxidant biomarkers as well as nuclear abnormalities and comet parameters compared to control values. Moreover, histopathological findings were in line with other results. SMC ameliorated the adverse effects which were effective in the improvement of DNA and oxidative damage in thiobencarb intoxicated fish.
Keywords: Carbamate, Fish, Genotoxic damage, Histopathology.



Elmadawy MA, Abdo W, Omar AA and Mahfouz NB (2020). **S-Methyl Cysteine Protective Effects in *Oreochromis Niloticus* Fish Contaminated by Thiobencarb Herbicide.** *World Vet. J.*, 10 (4): 551-561.

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Research Paper

Microbiological Studies on Naturally Present Bacteria in Camel and Buffalo Milk

Abdou AM, Hedia RH, Omara ST, Kandil MM, Bakry MA and Effat MM.

World Vet. J. 10(4): 562-570, 2020; pii:S232245682000067-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj67

ABSTRACT: The aim of current study was to isolate and identify naturally occurring probiotic *Lactobacillus* species in buffalo milk, camel milk, and camel urine to investigate their susceptibility to antibiotics and their antibacterial activity against pathogenic bacteria. A total number of seven samples which included three milk samples from buffalo, three milk samples from camel, and one urine sample from camel were collected and used in this study. The samples were cultured, and 18 isolated strains were identified by using 16S rRNA multiplex Polymerase Chain Reaction analysis, which was performed following DNA extraction from the isolated bacteria. Buffalo and camel milk were different in their *Lactobacilli* content. All *Lactobacilli* strains that were found in both camel milk and camel urine, were also found in buffalo milk, *Lactobacilli* strains in camel milk and urine were generally more resistant to the antibiotic. *Lactobacilli* isolated from buffalo milk, camel milk, and also camel urine presented variable degrees of antibacterial activity against pathogenic bacteria. Further studies should be conducted with more samples to gain more information in the field of antibacterial activity of probiotic *lactobacilli* and to understand the mechanisms of their activity. Hopefully, they can be used as natural alternatives instead of synthetic antibiotics.
Keywords: Antibacterial, Antibiotics, *Lactobacillus*, Probiotics.

Abdou AM, Hedia RH, Omara ST, Kandil MM, Bakry MA and Effat MM (2020). **Microbiological Studies on Naturally Present Bacteria in Camel and Buffalo Milk.** *World Vet. J.*, 10 (4): 562-570.

[Full text-PDF] [XML] [Google Scholar] [Crossref Metadata]

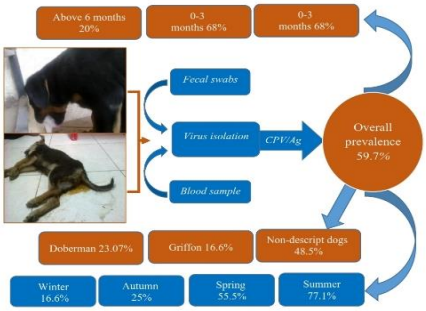
Research Paper

Canine Parvovirus Infection in Dogs: Prevalence and Associated Risk Factors in Egypt

Sayed-Ahmed MZ, Elbaz E, Younis E and Khodier M.

World Vet. J. 10(4): 571-577, 2020; pii:S232245682000068-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj68

ABSTRACT: Canine parvovirus (CPV) infection is a global infectious and contagious viral disease of canine, especially in dogs infected by three variants of CPV type. This study aimed to investigate the prevalence and potential risk factors of parvovirus infection in dogs residing in Egypt. A total of 122 dogs suffering from vomiting and diarrhea were screened by antigen rapid CPV/Canine Coronavirus Ag test kit for the diagnosis of CPV infection from March 2012 to February 2013. Age, breed, season, and vaccination of each dog were recorded to study the prevalence of CPV. The overall prevalence of CPV infection in dogs was reported as 59.7%. Dogs between 0 and 3 months of age indicated the highest prevalence of 68% followed by 4-6 months of age which was 53.3%. The lowest prevalence of CPV was reported in dogs above 6 months of age (20%). The maximum prevalence was noticed in non-descript dogs (48.5%) followed by German shepherds (26.7%), Doberman (23.07%), and Griffon (16.6%). Among different risk factors, young, unvaccinated puppies and exotic breeds were more prone to CPV infection. Regarding the season, the higher prevalence was noticed in summer (77.1%) followed by spring (55.5%), autumn (25%), and winter (16.6%). Thus, CPV is an infectious and highly contagious viral disease of dogs. Age and seasonal variations are risk factors in the prevalence of CPV infection. Identification of the potential risk factors associated with the disease may be helpful to construct the ideal preventive measures.



Sayed-Ahmed MZ, Elbaz E, Younis E and Khodier M (2020). **Canine Parvovirus Infection in Dogs: Prevalence and Associated Risk Factors in Egypt.** *World Vet. J.*, 10 (4): 571-577.

Keywords: Canine parvovirus, Egypt, Epidemiology, Prevalence, Risk factors

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Research Paper

Survival and Productivity of Culinary Herb Species in a Nutrient Film Technique-type Aquaponic System with Nile Tilapia

Valdez-Sandoval C, Guerra-Centeno D, Lepe-López M, Díaz-Rodríguez M and Pineda-Alvizuris L.

World Vet. J. 10(4): 578-586, 2020; pii:S232245682000069-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj69

ABSTRACT: Aquaponics is an evolving technology for producing plants and fish (or other aquatic organisms) in an integrated water recirculating system. However, the survival and productivity of terrestrial plants in aquaponic systems have not been evaluated for most plant species. The present study aimed to analyze the survival rate, growth, and biomass production of eight culinary herbs, commonly used in Guatemala, in a Nutrient Film Technique-type (NFT) aquaponic system with Nile tilapia (*Oreochromis niloticus*). The investigated herbs included coriander (*Coriandrum sativum*), parsley (*Petroselinum crispum*), peppermint (*Mentha spicata*), thyme (*Thymus vulgaris*), samat (*Eryngium foetidum*), oregano (*Plectranthus amboinicus*), dill (*Anethum graveolens*), and basil (*Ocimum basilicum*). A total of 50 individuals of each herb species and 150 juvenile Nile tilapias were distributed in 5 aquaponic modules. The survival rate, growth, and biomass production were measured for herbs and tilapias. All the herb species survived against the NFT aquaponic conditions. The findings indicated that the herb survival was species-dependent and ranged 42-98%. There was a significant effect of the herb species both on height and biomass gains. Post hoc comparison showed interspecific differential abilities to grow biomass in NFT aquaponics conditions. Among the investigated herbs, *M. spicata* and *O. basilicum* were the most productive species. Refinement in the selection of initial plants and aquaponic management could improve plant performance.

Valdez-Sandoval C, Guerra-Centeno D, Lepe-López M, Díaz-Rodríguez M and Pineda-Alvizuris L (2020). **Survival and Productivity of Culinary Herb Species in a Nutrient Film Technique-type Aquaponic System with Nile Tilapia.** *World Vet. J.*, 10 (4): 578-586.

Keywords: Ecological production, Hydroponics, *Oreochromis*, Recirculating water, Sustainable aquaculture

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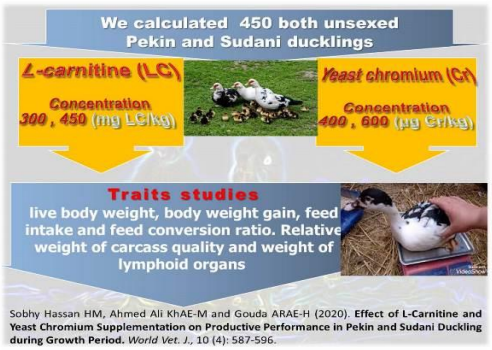
Research Paper

Effect of L-Carnitine and Yeast Chromium Supplementation on Productive Performance in Pekin and Sudani Duckling during Growth Period

Sobhy Hassan HM, Ahmed Ali KhAE-M and Gouda ARAE-H.

World Vet. J. 10(4): 587-596, 2020; pii:S232245682000070-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj70

ABSTRACT: The present study aimed to evaluate the effect of L-carnitine and Yeast chromium supplementation on the productive performance of Pekin and Sudani duckling breeds. A total number of 450 both unsexed Pekin and Sudani ducklings (225 per each breed) one-day-old were investigated in the current study. The experimental period lasted 12 weeks of age. Experimental ducklings were randomly divided into the 5 equal treatments with 90 ducklings (45 number from both Pekin and Sudani ducklings per each). Each experimental treatment was randomly divided into 3 equal replicates of 30 ducklings (15 ducklings in each breed). The five experimental treatments were as follows: the first treatment was the control with basal diets, treatments 2 and 3 received basal diets supplemented with 300 and 450 mg/kg diet L-carnitine (LC), respectively, while treatments 4 and 5 received basal diets supplemented with 400 and 600 µg/kg diets Yeast chromium (Cr), respectively. The results indicated that growing duckling fed diets supplemented with LC and Cr were significantly improved in live body weight, body weight gain, feed intake, and feed conversion ratio. The relative weight of carcass quality and weight of lymphoid organs significantly increased with supplemented diets. Therefore, both duckling breeds fed on diets supplemented with 450 mg LC/kg resulted in better performance without any adverse effect on carcass quality as well as economic efficiency.
Keywords: L-carnitine, Pekin ducks, Productive Performance, Sudani ducks, Yeast chromium.



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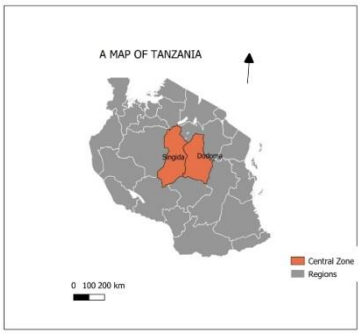
Research Paper

Epidemiological Assessment of Contagious Bovine Pleuropneumonia in Central Tanzania

Mngumi S, Makungu S and Mdetele D.

World Vet. J. 10(4): 597-601, 2020; pii:S232245682000071-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj71

ABSTRACT: A retrospective study was conducted to determine the epidemiology of Contagious Bovine Pleuropneumonia (CBPP) in the Central Zone of Tanzania. The present study used data from archived information of Central Zone Veterinary Centre (CZVC) for the past five years in the forms of weekly, monthly, and slaughterhouse reports, as well as Event Mobile Application (EMA-i) reports submitted to the zone. The present study found that out of 14 Local Government Authorities (LGAs) in the Central Zone,10 reported the disease in the past five years. Moreover, 56, 426, and 11147cases were reported as deaths, and the cattle at risk respectively. Therefore, 3.8%, 13%, and 0.5%were reported as CBPP prevalence, case fatality rate, and mortality rate, respectively. It was also revealed that there was a clear temporal pattern of CBPP occurrence, with more cases being reported between August to December. In conclusion, CBPP was a seasonal problem in Central Tanzania. Therefore, the present research recommended the strengthening of control measures against this disease in the central zone of Tanzania. In order to be able to assess the actual burden of the disease on-site, this extensive study must be carried out, since the parameters obtained during the study were lower compared to the situation on-site.
Keywords: Central zone, Contagious bovine pleuropneumonia, Prevalence and distribution



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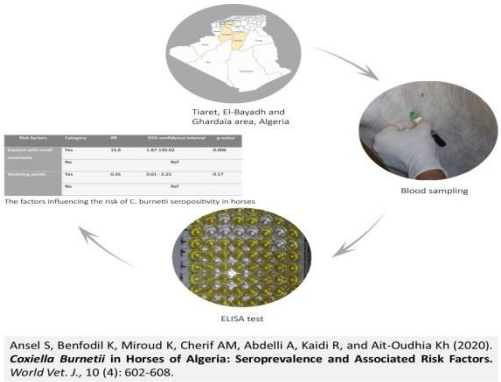
Research Paper

Coxiella Burnetii in Horses of Algeria: Seroprevalence and Associated Risk Factors

Ansel S, Benfodil K, Miroud K, Cherif AM, Abdelli A, Kaidi R, and Ait-Oudhia Kh.

World Vet. J. 10(4): 602-608, 2020; pii:S232245682000072-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj72

ABSTRACT: The Q fever is a worldwide zoonotic disease caused by *Coxiella burnetii* (an obligate intracellular bacterium). This pathogen affects humans, ruminants, equines, carnivores, rodents, and birds. A cross-sectional study was carried out from March 2017 to May 2018 to assess the seroprevalence and identify the risk factors of *C. burnetii* infection in horses (*Equus Caballus*) residing in three districts of Algerian, namely Tiaret, El-Bayadh, and Ghardaia. Serum samples collected from 182 horses were analyzed via enzyme-linked immunosorbent assay



(ELISA). Association of seropositivity with potential risk factors related to animals (e.g., age, gender, breed, housing, and presence of ticks), breeding characteristics (e.g., geographical localization, contact with animals), and environmental characteristics (i.e., presence of water source) was analyzed by univariate and multivariate logistic regression. An overall seroprevalence of 9.9% (18/182) was obtained. The univariate analysis of risk factors for *C. burnetii* seroprevalence demonstrated higher seropositivity in horses that had contact with small ruminants (p=0.004) and dromedaries (p=0.002) as well as in those living near a water source (p=0.036) and in El-Bayadh district (p=0.005). The multivariate logistic regression analysis indicated that the risk of *C. burnetii* infection was significantly higher in horses that were in contact with small ruminants (RR: 15.6). Algeria is endemic for Q fever in horses and prophylactic measures must be taken to reduce /prevent its transmission to animals and humans.
Keywords: Algeria, *Coxiella burnetii,* ELISA, Horses, Q fever, Seroprevalence

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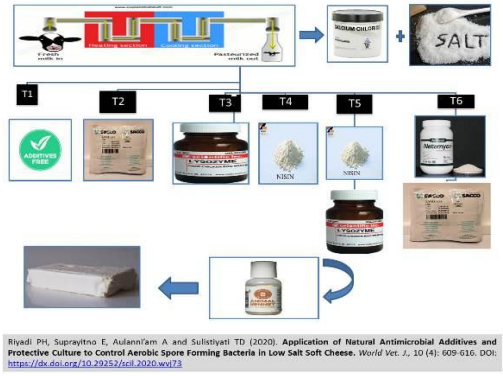
Research Paper

Application of Natural Antimicrobial Additives and Protective Culture to Control Aerobic Spore Forming Bacteria in Low Salt Soft Cheese

Abo Shaala EK, Awad SA and Nazem AM.

World Vet. J. 10(4): 609-616, 2020; pii:S232245682000073-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj73

ABSTRACT: There is an increasing interest in the application of natural antimicrobials instead of chemical ones to enhance the microbiological quality of dairy products. The objective of this study was to assess the effect of some natural antimicrobial additives and protective culture for reducing the usage of chemical preservatives, shelf-life extension, retarding microbial spoilage in low-salt soft cheese. The antimicrobial agents (protective culture, nisin, lysozyme, and natamycin) were studied on the activity of 28 isolates of spore-forming bacteria. Inhibitory effect of different natural antimicrobial additives as protective culture (*Lactobacillus rhamnosus*, 40 mg kg $^{-1}$), nisin (25 mg kg $^{-1}$), lysozyme (100 mg kg $^{-1}$), combination of nisin and lysozyme (25 mg kg $^{-1}$/100 mg kg $^{-1}$), and combination of protective culture and natamycin (40 mg kg $^{-1}$/25 mg kg $^{-1}$) were studied on the growth of aerobic spore-forming bacteria in low-salt soft cheese during the storage period (30 days) at 4±1°C. The results revealed that the addition of different natural antibacterial additives with various concentrations had a significant effect on aerobic spore-forming bacteria, compared to other treatments and control. The growth pattern of aerobic spore-forming bacteria gradually decreased in all treatments along the storage period with variable reduction percentages in comparison with control cheese which was in continuous increment. The application of a combination of nisin and lysozyme had the most significant reduction of aerobic spore-forming bacteria, compared to control and other treatments.
Keywords: Aerobic spore-forming bacteria, Lysozyme, Nisin, Natamycin, Protective culture



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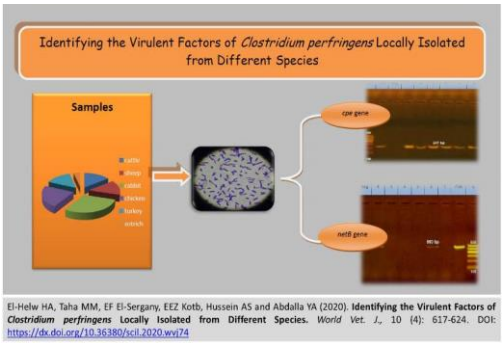
Research Paper

Identifying the Virulent Factors of Clostridium perfringens Locally Isolated from Different Species

El-Helw HA, Taha MM, EF El-Sergany, EEZ Kotb, Hussein AS and Abdalla YA.

World Vet. J. 10(4): 617-624, 2020; pii:S232245682000074-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj74

ABSTRACT: *Clostridium perfringens* incriminated in many diseases among different species of animals due to its ability to produce many virulence factors. In the current study, 135 intestinal samples were collected from different animal species of different localities in Egypt. Samples were subjected to isolation and identification (morphologically and biochemically) for obtaining *Clostridium perfringens* isolates (n=26, 19.25%). The PCR was carried out to elucidate the virulence factors. It was indicated that all the 26 *Clostridium perfringens* isolates had CPA gene and *Clostridium perfringens* enterotoxin (CPE gene), whereas 23% of isolates of chicken and cattle intestinal samples contained CPA, Net B, and CPE genes as virulence factors. Consequently, those isolates are highly recommended to be used in the preparation of enterotoxemia and necrotic enteritis vaccines as they are more virulent strains.
Keywords: *Clostridium perfringens*, CPA gene, CPE gene, Net B gene



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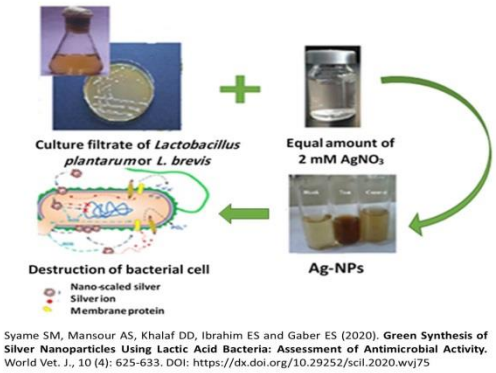
Research Paper

Green Synthesis of Silver Nanoparticles Using Lactic Acid Bacteria: Assessment of Antimicrobial Activity

Syame SM, Mansour AS, Khalaf DD, Ibrahim ES and Gaber ES.

World Vet. J. 10(4): 625-633, 2020; pii:S232245682000075-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj75

ABSTRACT: The biosynthesis of silver nanoparticles (Ag-NPs) is a new methodology in nanotechnology with a hopeful implementation in medicine, food control, and pharmacy. The objective of the present research was to conduct a green synthesis of Ag-NPs using the cell-free supernatant of *Lactobacillus plantarum* and *Lactobacillus brevis* and evaluate their antibacterial and antifungal activities. The production of Ag-NPs was confirmed by the color alteration from yellow to brown. Using the UV-visible spectrophotometer, the biosynthesized Ag-NPs indicated an absorption peak at 410 nm. The transmission electron microscope was used for the determination of the size and morphology of the nanoparticles. Nanoparticles appeared in spherical or polyhedral form, poly-dispersed and their diameter ranged from 5 to 40 nm. The X-ray diffraction analysis exhibited the crystalline nature of the particles with a face-centered cubic (FCC) structure. The biosynthesized Ag-NPs were evaluated for their antimicrobial efficiency using the agar well diffusion method. The antibacterial activity of Ag-NPs was more potent against Gram-negative bacteria than Gram-positive bacteria. Ag-NPs synthesized from *Lactobacillus plantarum* recorded the maximum activity against *Escherichia coli* (ATCC® 10536™) and *Pseudomonas* (ATCC® 27853™) bacteria, while those synthesized from *Lactobacillus brevis* recorded the maximum activity against *Escherichia coli* (ATCC® 35218™). Ag-NPs synthesized from *Lactobacillus plantarum* and *Lactobacillus brevis* showed antifungal activity against *Candida albicans* (ATCC® 10231™). The effect of these nanoparticles on *Escherichia coli* (ATCC® 10536™) was examined and imaged by a transmission electron microscope that indicated damage to the plasma membrane and cell wall. In conclusion, the biosynthesized Ag-NPs have applications as antimicrobial agents in the medicine and food industry.
Keywords: Antimicrobial activity, *Lactobacillus brevis, Lactobacillus plantarum*, Silver nanoparticles, Transmission electron microscope



Syame SM, Mansour AS, Khalaf DD, Ibrahim ES and Gaber ES (2020). **Green Synthesis of Silver Nanoparticles Using Lactic Acid Bacteria: Assessment of Antimicrobial Activity.** World Vet. J., 10 (4): 625-633. DOI: https://dx.doi.org/10.29252/scil.2020.wvj75

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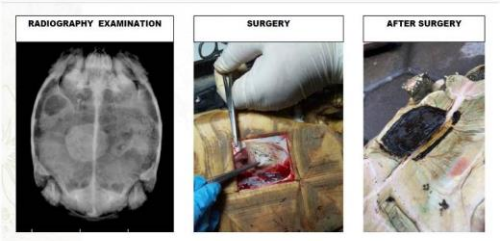
Research Paper

Urinary Bladder Stone Removal Surgery in Sulcata tortoise (Geochelon sulcata) with Lateral Plastron Osteotomy Technique

Kartika Sari DA and Apritya D.

World Vet. J. 10(4): 634-637, 2020; pii:S232245682000076-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj76

ABSTRACT: This paper aimed to examine the management of urinary calculi of a sulcata tortoise. A 5-year-old Sulcata tortoise (*Geochelon sulcata*) was presented with a history of dehydration, loss of appetite, and lameness. Clinical signs and radiographic examination indicated urinary calculi in the urinary bladder. Radiographic results revealed that there was a radiopaque urinary calculi mass. Plastron osteotomy and cystotomy techniques were used to remove urinary calculi. The appetite of the tortoise returned to normal in a week after the surgery. The lateral plastron is an appropriate osteotomy technique, especially for the immediate opening of the plastron with a stone-filled bladder. This was a safe area to open plastron since it was far from the heart. This method was not beneficial for the tortoise in their infancy since it would interrupt the development of plastron formation resulting in the postoperative asymmetrical plastron structure.
Keywords: Urinary calculi, Cystotomy, Plastron osteotomy, Sulcata tortoise



Kartika Sari DA and Apritya D (2020). **Urinary Bladder Stone Removal Surgery in Sulcata tortoise (*Geochelon sulcata*) with Lateral Plastron Osteotomy Technique.** *World Vet. J.*, 10 (4): 634-637. DOI: https://dx.doi.org/10.29252/scil.2020.wvj76

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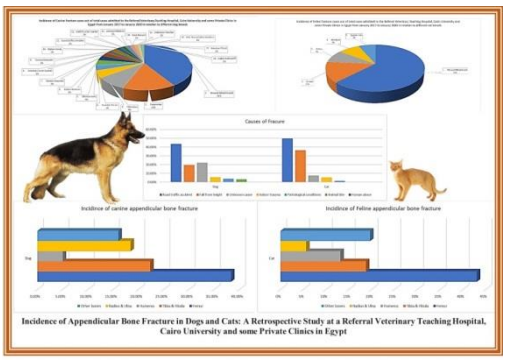
Research Paper

Incidence of Appendicular Bone Fracture in Dogs and Cats: Retrospective Study at Veterinary Hospital of Cairo University and some Private Clinics in Egypt

Abo-Soliman AAM, Ahmed AE and Farghali HAMA.

World Vet. J. 10(4): 638-652, 2020; pii:S232245682000077-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj77



ABSTRACT: Appendicular bone fractures in small animal practice constitute a major challenge facing veterinary orthopedic surgeons concerning affected limb and bone as well as the extent of tissue damage, site, and shape of the fracture line. Therefore, this retrospective study was designed to provide descriptive data at referral veterinary teaching hospital, faculty of veterinary medicine, Cairo University, and some private pet clinics in Cairo district, Egypt to identify and determine the prevalence of appendicular fractures arising from trauma in dogs and cats treated from January 2017 to January 2020, and emphasizing the information that characterized the population (breed, age, gender, and animal size). The investigated fractures were classified according to the specific limb (forelimbs / hind limbs), specific bone fractures (Humerus, radius and ulna, femur, tibia and fibula, and the other bones), extent of tissue damage (open or closed and incomplete or complete), site (proximal, diaphyseal or distal zones), number (single or comminuted), and the direction of the fracture line (transverse, oblique or spiral). From the obtained data, it could be concluded that there was a high incidence of the appendicular long bones concerning the different bone fractures with significantly higher records in dogs, compared to cats. The highest records of fracture were in mongrel dogs, and cats as rescued animals. Excluding mongrel dogs and cats, the highest incidence of fracture-cases in dogs was recorded in Miniature breeds and svelte breeds for cats. Male dogs and cats showed a higher incidence than females. The bone fracture mostly occurred in dogs younger than one-year-old, and cats aged one to three years. A fracture in the hindlimbs was more significant than forelimbs with the highest incidence in femoral bone among both dogs and cats. The percentage of open fractures were more common in cats than dogs. Incomplete fractures were recorded more frequently in dogs than cats. In dogs, the most common fractures in the femur, tibia/fibula, humerus, and radius/ulna were complete comminuted diaphyseal femoral, complete oblique diaphyseal tibial/fibular, complete transverse distal humoral, and complete transverse diaphyseal radial/ulnar fractures respectively. Moreover, cats were complete transverse distal femoral, complete oblique diaphyseal tibial/fibular, complete spiral diaphyseal humoral, and complete transverse distal radial/ulnar fractures. In conclusion, appendicular bone fracture among dogs and cats referred to the veterinary teaching hospital, Cairo University and some private clinics in Egypt showed high incidence (87% in dogs and 71.8% in cats) out of total fracture cases and this incidence correlated with some predisposing factors (including breeds, weight, age, and gender) and causative agents that resulted in different types of appendicular fractures.
Keywords: Cat, Dog, Femur, Fracture, Orthopedic

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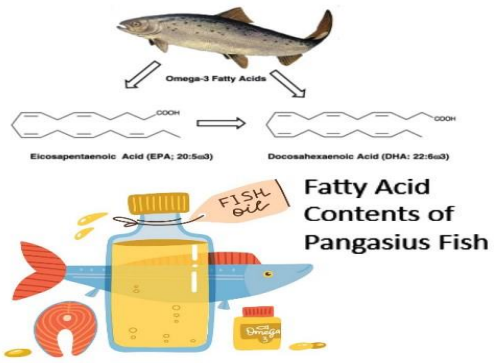
Research Paper

The Effects of Adding Lysin Essential Amino Acid to Commercial Feed on Fatty Acid Contents of Pangasius Fish

Nopita W, Lamid M and Agustono.

World Vet. J. 10(4): 653-657, 2020; pii:S232245682000078-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj78



ABSTRACT: Pangasius is a medium to very large freshwater shark catfish primarily used for consumption with high economic value. The content of pangasius fatty acids is higher than in marine fish, since marine fish have a lower saturated fatty acid composition than freshwater fish. The present research aimed to determine the effects of adding lysine essential amino acid to commercial feeds on the saturated and unsaturated fatty acids contents of pangasius fish. In the present research, an experimental method with completely randomized design was used. The treatment was done by adding lysine with different doses including P0 (0%), P1 (1.2%), P2 (2.2%), and P3 (3.2%). Each treatment was repeated five times. The main parameters studied were the content of saturated and unsaturated fatty acids in pangasius fish meat. The observed parameter was water quality. The present results indicated the use of lysine in commercial feed caused significant differences in the content of saturated fatty acids, Monounsaturated Fatty Acids (MUFA) and Polyunsaturated Fatty Acids (PUFA) in pangasius meat; a decrease in the saturated fatty acids content was found in P3 with 3.2% (3.5882 mg/dl). In P2, an increase in the MUFA content of 2.2% (5.9630 mg/dl) was found. An increase in the PUFA content was found in P3 treatment with 3.2% Lysin (23.1082 mg/dl). P1, P2 and P3 indicated lower results than control treatments (P0). The use of lysine in commercial feed indicated significant differences in the content of saturated fatty acids, MUFA and PUFA in pangasius.
Key words: Lysine essential amino acid, Saturated fatty acids, Unsaturated fatty acids.

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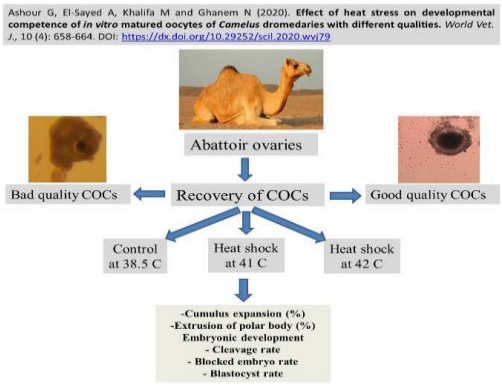
Research Paper

Effect of heat stress on developmental competence of in vitro matured oocytes of Camelus dromedaries with different qualities

Ashour G, El-Sayed A, Khalifa M and Ghanem N.

World Vet. J. 10(4): 658-664, 2020; pii:S232245682000079-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj79



ABSTRACT: The deleterious effect of heat stress on cumulus-oocytes complexes (COCs) competence is well recognized in different livestock species. Therefore, the present study aimed to investigate the effect of physiologically relevant heat stress on the developmental competence of camel COCs during *in vitro* maturation (IVM). A total of 1548 COCs were divided into six groups in this study. The groups were named K1 and K2 representing good and low-quality COCs incubated at 38.5°C for 30 hours. While K3 and k4 represent good and low-quality COCs exposed to 41°C for the first 6 hours of IVM. Finally, K5 and k6 represent the groups of good and low-quality COCs exposed to 42°C for the first 6 hours of IVM. After exposure of COCs to heat stress at 41°C and 42°C during the first 6 hours of *in vitro* maturation, the COCs were incubated at 38.5°C for 24 hours of IVM. The *in vitro* matured COCs were activated to cleave using ethanol followed by 4 mM 6-DMAP and developed embryos were cultured *in vitro* for 7 days post parthenogenetic activation. The results of this study indicated that heat stress at 42°C significantly decreased the Pb (polar body) extrusion rate in K4 and K6, compared to other groups. Additionally, the embryo cleavage rate was significantly lower for good and low-quality oocytes exposed to heat stress (K2, K3, K4, K5, and K6), compared to good quality COCs of the control group (K1). The cleavage rate was lower for low quality (K2; 63 ± 1.28) than good quality COCs (K1; 53 ± 1.85). The percentages of oocytes that developed to the blastocyst stage were lower for K2, K3, K4, K5, and K6 than K1. Moreover, the blastocyst rate was lower for K2 (9 ± 0.22) than K1 (15 ± 0.22). The results of this study indicated that exposure of camel oocytes to heat stress for 6 hours during *in vitro* maturation severely reduced extrusion of polar body, cleavage, and blastocyst rates. The low-quality camel COCs were reduced developmental capacity than good quality oocytes.
Keywords: Camel, Embryo development, Heat stress, Oocyte

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Research Paper

The Effect of Dietary Supplementation of Cod Liver Oil on Ratio of Saturated and Unsaturated Fatty Acids in Giant Prawn (*Macrobrachium rosenbergii*) Meat

Marzuki L, Agustono and Rahardja BS.

World Vet. J. 10(4): 665-669, 2020; pii:S232245682000080-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj80

ABSTRACT: The existence of feed plays an important role in aquaculture activities. This is due to the dominant influence on fish feed growth. Feeding with the right nutritional components can produce healthy and high-quality fish products. One of the nutrients needed by fish is fatty acids. In fact, the provision of fatty acids, one of which is not in the meat, has an important influence. So, this study aims to determine the effect of adding cod liver oil to commercial feed on the ratio of saturated and unsaturated fatty acids to the meat of giant prawn. This research was conducted experimentally with a completely randomized design. The treatment is given a dose of cod liver oil 0% (control), and treatments 1-4 use 3% dose addition to each treatment. On the other hand, in the data analysis stage, the researchers used ANOVA and continued with the Duncan's test. Based on the results, the study notes that the administration of cod liver oil in commercial feed does not affect the decreasing content of saturated fatty acids in giant prawn meat. On the other hand, the results also showed that the best ratio was found in treatment 4 at a dose of 12%. Therefore, it is concluded that the provision of nutrients for feed related to fatty acids in the pole has not reduced the content of saturated fatty acids. As well as, the best ratio of saturated fatty acids and unsaturated fatty acids was 1.21:1 with cholesterol content of 88.34 mg/dl and this ration resulted by the greatest growth rate in present study.
Keywords: Cod liver oil, Feed, Giant prawn, Saturated fatty acids

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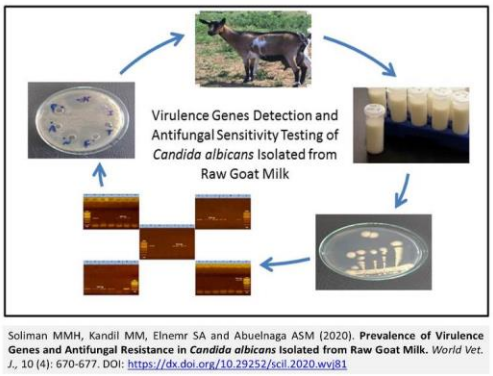
Research Paper

Prevalence of Virulence Genes and Antifungal Resistance in Candida albicans Isolated from Raw Goat Milk

Soliman MMH, Kandil MM, Elnemr SA and Abuelnaga ASM.

World Vet. J. 10(4): 670-677, 2020; pii:S232245682000081-10; DOI: https://dx.doi.org/10.54203/scil.2020.wvj81

ABSTRACT: The contamination of goat milk with pathogenic fungi can cause health hazards for the consumers either they consume it raw or even in the processed form. Since there are few studies concerning yeasts in raw goat milk, the present study aimed to determine the prevalence of yeasts and isolate *Candida albicans* from raw goat milk samples. Also, this study determined the distribution of virulence genes and the antifungal susceptibility profile of *Candida albicans* isolates. A total of 30 goat milk samples (collected from free-grazing goats) were mycologically examined. The confirmed *Candida albicans* isolates were subjected to PCR assay to detect the virulence genes (SAP4, RAS1, ALS1, HWP1, and PLB1). Also, antifungal sensitivity testing was performed against the commercially available antifungal agents and probiotics (*Lactobacillus acidophilus* and *Lactobacillus plantarum*). The mycological examination revealed that 14 out of 30 (46.7%) goat milk samples were positive for yeasts and only 4 (13.3%) isolates were confirmed as *Candida albicans*. The results from the PCR assay showed that RAS1 and ALS1 were found in 4 (100%) isolates, HWP1 and SAP4 were found in 2 (50%) isolates, while PLB1 was not detected in tested *Candida albicans* isolates (0%). Antifungal sensitivity testing results showed that ketoconazole gave the best activity against *Candida albicans* isolates, followed by fluconazole, nystatin, and itraconazole. All isolates were resistant to terbinafine. Moreover, both *Lactobacillus acidophilus* and *Lactobacillus plantarum* showed antifungal effects against *Candida albicans*, but *Lactobacillus plantarum* was more effective than *Lactobacillus acidophilus*. Antifungal resistance is a major problem that can lead to failure of candidiasis treatment. Regular antifungal sensitivity testing and searching for an alternative bio-eco-friendly approach for proper control and treatment of candidiasis are strongly needed to prevent treatment failure and emergence of resistant isolates.



Soliman MMH, Kandil MM, Elnemr SA and Abuelnaga ASM (2020). **Prevalence of Virulence Genes and Antifungal Resistance in *Candida albicans* Isolated from Raw Goat Milk.** *World Vet. J.*, 10 (4): 670-677. DOI: https://dx.doi.org/10.29252/scil.2020.wvj81

Keywords: Antifungal sensitivity testing, *Candida albicans*, Goat milk, Virulence genes, Probiotics.

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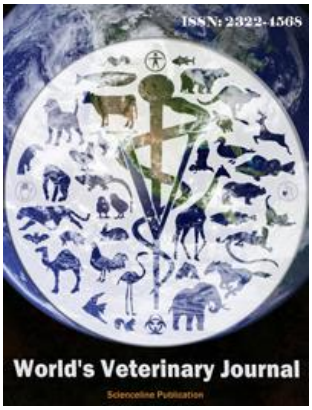

World's Veterinary Journal

ISSN: 2322-4568

Frequency: Quarterly

DOI Prefix: https://dx.doi.org/10.54203/scil.2020.wvj

Current Issue: 2020, Vol: 10, Issue: 4 (December 25)

Publisher: SCIENCELINE

www.wvj.science-line.com

World's Veterinary Journal (ISSN 2322-4568) is an international, English language, peer reviewed open access journal aims to publish the high quality material from veterinary scientists' studies ... View full aims and scope

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- WVJ indexed/covered by SCOPUS, NLM Catalog (NLM ID: 101688928), ScopeMed, RICeST-ISC, Ulrich's™/ProQuest, NAAS (Score: 3.96), UBTIB, SHERPA/RoMEO, Genamic, INFOBASE, Index Copernicus International (ICV 2019= 115.91) (full index information)
- Open access full-text articles is available beginning with Volume 1, Issue 1.
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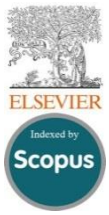

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2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 465-480, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj57

Genome Analysis of Antimicrobial Resistance Genes and Virulence Factors in Multidrug-Resistant *Campylobacter fetus* Subspecies Isolated from Sheath Wash

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ORGINAL ARTICLE
pii: S232245682000057-10
Received: 27 Sept 2020
Accepted: 10 Nov 2020

ABSTRACT

Campylobacter fetus subspecies are mostly characterized by reproductions problems in cattle and sheep. This study aimed to study the genetic profile and assess the genes mechanism of resistance and their virulence factors using genome sequence analysis. A total of 59 confirmed *Campylobacter fetus* subspecies based on molecular assays and DNA sequencing were subjected to antimicrobial susceptibility test against 14 antibiotic agents representing the five classes of antibiotics using the disc diffusion method. In addition, sequencing the genome of all strains induced complete resistance against all tested antibiotics. The results of the antimicrobial test indicated that 54.4% had a resistance profile, 26.3% were intermediate, while 19.3% were observed to be susceptible. The Whole Genome Sequencing (WGS) result revealed the presence of different genes, such as Broad-specificity multidrug efflux pump and 16S rRNA (guanine^{527}-N^{7})-methyltransferase (gidB), efflux pump conferring antibiotic resistance (MacA and MacB), protein-altering cell wall charge conferring antibiotic resistance (PgsA), which have never been reported in *Campylobacter fetus* subspecies. The WGS also revealed the presence of genes that involved in colonization, adhesion, motility, and invasion, such as type IV secretion system protein (VirD4), S-Layer, cytolethal distending toxin (A, B, and C), *Campylobacter* invasion antigen (CiaB), and fic domain protein (fic) were among important CDS. The presence of these uncommon genes explains the resistance of *Campylobacter fetus* subspecies against different tested antibiotics. The results of this study can be used to implement molecular surveillance of *Campylobacter fetus* subspecies and conduct further studies on the resistance mechanism in these subspecies.

Keywords: Broad-specificity multidrug efflux pump, *Campylobacter fetus* subspecies, Genome analysis, Methyltransferase gidB, Multidrug resistance.

INTRODUCTION

Embryonic deaths, temporary infertility with mild endometritis, and abortions can be caused by *Campylobacter fetus* subspecies in the herd, which resulted in substantial production losses and economic hardship for producers worldwide (Bellows et al., 2002; Morrell et al., 2011). Venereal diseases such as bovine genital campylobacterosis are considered as the primary cause of reproductive loss (McCool et al., 1988). Experimental studies revealed that treating carrier bulls has been done by victimization, either vaccination with a monovalent oil-based immunizing agent or recurrent antimicrobial agent. Furthermore, several of the revealed antimicrobial treatment protocols that used antimicrobial agents are not commercially obtainable. What is more, it had been confirmed that none of the revealed studies had provided robust proof supporting a treatment of choice that eliminates shedding in infected bulls (Vasquez et al., 1983; Truyers et al., 2014).

On the other hand, researchers discovered that vaccination itself is not ample to manage a pestilence. However, habitually testing and culling of infected bulls is suggested. Testing and culling procedures have a considerable price related to the premature loss of high price animals; particularly, considering the value of breeding bulls (Guerra et al., 2014; Erickson et al., 2017). A study conducted on the analysis of long action oxytetracycline and a monovalent immunizing agent for the management of *Campylobacter fetus* subsp. *venerealis* disease in bulls ended that there have been no vital variations between treated and untreated bulls. No matter the diagnostic applied, treatment with two label doses of this regime failed to stop the shedding of *Campylobacter fetus* subsp. *venerealis* in treated bulls, therefore, is not suggested as an efficient management strategy (Erickson et al., 2017). The resistance against long action oxytetracycline observed by Erickson et al. (2017) may be correlated with the previous findings obtained by Mak et al. (2014), that researchers indicated that bacteria species contain a variable number of resistance mechanisms against

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different antimicrobial agents for their self-defense to ensure their complete protection. This resistance mechanism is, for the most part, co-regulated through the expression of resistance genes (Mak et al., 2014).

Thus considering the importance of *Campylobacter fetus* subspecies relatedness to food, public, and animal health and their resistance profile to different antibiotics, this study aimed to study the genetic profile and assess genes mechanism of resistance and their virulence factors by using genome sequence analysis.

MATERIALS AND METHODS

Ethical approval

Ethical consideration for this study was submitted and approved by the Animal Production Sciences Research Ethics Committee of the North-West University (NWU-AnimProdRec), South Africa. The study procedures were performed in accordance with the requisite ethical standards.

Study design and area

This was a cross-sectional descriptive study conducted in Dr. Ruth Mompati District Municipality in North-West Province, South-Africa, from January 2018 to December 2019, focusing on positive *Campylobacter fetus* subspecies collected from the state veterinary services and processed at the North-West University Laboratory at Mafikenf Campus for further analysis.

Bacterial identification

Positive *Campylobacter fetus* subspecies isolated from sheath wash and obtained from bulls that were from in the North-West province in South Africa were transported in Steve medium was spread plated on the surface of Skirrow's agar (Tryptose blood Agar Base, Oxoid, UK) mixed with 7% sheep blood and supplemented with *Campylobacter* selective supplement (Skirrow, SR0069E, Oxoid, England). Plates were then incubated anaerobically at 37˚C for 72 hours using an anaerobic Jar 2.5 L (Oxoid, England) containing CampyGenTM sachet CN0025A (Oxoid, England) to produce a microaerophilic atmospheric condition for the growth of *Campylobacter fetus*. All the plates were firstly examined for growth after the 72 hours incubation period, and if growth was not observed, the plates were re-incubated for a further 48 hours. After the incubation period, all the plates were subjected to morphological identification (a small colony, mucoid, greyish, flat colony with irregular edges). The suspected plates of *Campylobacter fetus* were considered for further analysis (Acke et al., 2009).

Molecular assays for the identification of *Campylobacter fetus* subspecies

Genomic DNA extraction

The extraction of genomic DNA was carried out according to the standard molecular methods (Di Giannatale et al., 2014) using Zymo-Research Kit (Bio-Lab, South Africa) following the manufacturer's instructions. The extracted genomic DNA was then quantified spectrophotometrically using a Nanodrop® ND-1000. The genomic DNA was kept in fresh DNA tubes to serve as a DNA template for subsequent PCR.

Polymerase chain reaction

The confirmation of members of the genus *Campylobacters fetus* subsp in this study was performed using Polymerase Chain Reaction (PCR). A total volume of 50 µL containing 20 µL PCR 2xMaster Mix (Bio-Labs, Iso 9001, England), 4 µL template DNA, 22 µL nuclease DNA free water (Bio-concept ltd, ISO 9001, paradiesrain 14, Switzerland) and 4µL of oligonucleotide primer (Forward and Reverse) were prepared and mixed in PCR tubes. The DNA was amplified using a Thermal cycler (Bio-Rad, T100^{TM} Thermal cycler, Model: T100^{TM} Thermal cycler, serial number: 621BR19028, Singapore) as it has been presented in table 1 (Schulze et al., 2006; Wangroongsarb et al., 2011).

The DNA sequencing analysis

The amplified PCR products of isolated bacteria were sent to Inqaba Biotechnology (Pretoria, South Africa) for sequencing. The sequences and chromatograms were observed with Bio-systems. Forward and reverse sequences were compared and corrected for conformity. Blast program tools were applied to search for the sequences (Altschul et al., 1997) to find the closest match for each one in the Gen-Bank. The closest sequences were then downloaded and aligned with the original sequences using clustal and edited using Finch TV version 1.4.0.

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Analysis of the antimicrobial profile of *Campylobacter fetus* subspecies

The evaluation of the antimicrobial profile of *Campylobacter fetus* in this study was carried out using the Kirby-Bauer disc diffusion method (BAKWS and Turck, 1966; Washington and Wood, 1995). The results were interpreted based on the guidelines of the Clinical and Laboratory Standards Institute guidelines (institute, 2013). Multiple drug resistance was defined as resistance to two or more classes of antimicrobials tested. The antibiotics panel selected for screening *Campylobacter fetus* subspecies were the one mostly used in the case of campylobacteriosis in both veterinary and human medicine. The following 14 antibiotics were used: Erythromycin (15 mg), Azithromycin (15 mg), Streptomycin (10mg), Neomycin (30 mg), Gentamicin (10 mg), Ampicillin (10 mg), Amoxicillin (10 mg), Ciprofloxacin (5 mg), Enrofloxacin (5 mg), Norfloxacin (5 mg), Doxycycline (5 mg), Tetracycline (30 mg), Chloramphenicol (30 mg) and Nalidixic Acid (30 mg).

Genome sequencing, assembly, and annotation

The extracted genome DNA samples were fragmented using an enzymatic approach (NEB Ultra II FS Kit, BioLabs, England). The DNA fragmented results were sized selected (200-500 bp), using AMPure XP beads, the fragments were end-repaired, and Illumina specific adapter sequences were ligated to each fragment. Each sample was individually indexed, and a second size selection step was performed. Samples were then quantified, using a fluorometric method, diluted to a standard concentration (4 nM), and then sequenced on Illumina's NextSeq platform, using a NextSeq 300 cycle kit, following a standard protocol per manufacturer's instruction to generate a total number of 43,826,295 reads and 41,141,918 reads with 2X 300bp paired-end read length and the coverage was 40X respectively for *Campylobacter fetus* subsp *venerealis* NW_ME2 and *Campylobacter fetus* subsp. *fetus* NW_ME1.

The sequenced data generated were analyzed using appropriate bioinformatics tools, with default parameters, through the Kbase platform (Arkin et al., 2018). The sequenced data was filtered for low-quality reads and adapter regions using Trimmomatic- v0.36 (Bolger et al., 2014), and Fast QC-version 0.11.5 was employed to assess the quality of data. The genome assembly was performed using SPAdes- version 3.13.0 (Bankevich et al., 2012). The functional annotation of the entire draft assembly was carried out with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Haft et al., 2018) and Rapid Annotations using Subsystems Technology (RAST, version 11) (Aziz et al., 2008).

Statistical analysis

Data were analyzed using SPSS (version 23.0). Descriptive statistics such as frequencies and percentages were applied to determine the occurrence of bacteria and the antimicrobial profile of the isolated bacteria. Pearson's chi-square test of association was used to ascertain the association between the area and occurrence of bacteria and antimicrobial profile. Furthermore, the study used the Kruskal Wallis test and the Mann-Whitney's U to ascertain whether the resistance differed significantly across the antibiotics and whether the resistance differed across the bacterial species. For significant tests, cross-tabulations (Tables 2 and 3) were used to explain the association of the difference in resistance against different antibiotic profile of the isolated bacteria. Based on Kruskal Wallis test results, the p-value <0.05 was considered significant, and they have been presented in tables 4,5, and 6.

Table 1. Primers and PCR conditions

Primers	Conditions	References
MG3F (5'-GGTAGCCGCAGCTGCTAAGAT-3') MG4R (5'-TAG CTACAA TAA CGA CAA CT-3').	-Initial denaturation: 95°C for 15 seconds -Second denaturation: 96°C for 15 seconds - Annealing: 60°C for 60 seconds - Extension: 72°C for 90 seconds The processes of the second denaturation, annealing, and extension were repeated for 35 cycles and a final extension done at 180°C for 10 min	(Schulze et al., 2006)
CFCH57F (GCAAGTCGAACGGAGTATTA) CF1054R (GCAGCACCTGTCTCAACT)	-Initial denaturation: 94°C for 5 minutes - Second denaturation: 94°C for 60 seconds - Annealing at 50°C for 60 sec - Extension: 72°C for 60 seconds - The processes of the second denaturation, annealing, and extension were repeated for 30 cycles and a final extension done at 720C for 10 min	(Wangroongsarb et al., 2011).

DNAse free water, *Staphylococcus aureus* ATCC 25923 and *Campylobacter Jejune* ATCC® BAA-1153™ were used in this study as negative controls for PCR.

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Table 2. The occurrence of antimicrobial profile of *Campylobacter fetus* subsp. *fetus* isolated from sheath wash

Cross-tabulation of the antimicrobial profile of isolated bacteria				
Bacteria	**Antibiotics**	**Resistance**		
		Resistant	**Intermediate**	**Susceptible**
Campylobacter fetus subsp. *fetus*	Tetracycline	59.3%	29.6%	11.1%
	Doxycycline	44.4%	44.4%	11.1%
	Ampicillin	63.0%	29.6%	7.4%
	Amoxicillin	59.3%	29.6%	11.1%
	Erythromycin	25.9%	48.1%	25.9%
	Azithromycin	38.5%	34.6%	26.9%
	Neomycin	57.7%	19.2%	23.1%
	Streptomycin	48.1%	33.3%	18.5%
	Gentamicin	44.4%	25.9%	29.6%
	Ciprofloxacin	63.0%	22.2%	14.8%
	Nalidixic acid	85.2%	11.1%	3.7%
	Norfloxacin	33.3%	29.6%	37.0%
	Enrofloxacin	37.0%	33.3%	29.6%
	Chloramphenicol	51.9%	37.0%	11.1%
	Total	50.8%	30.6%	18.6%

Table 3. Phenotypic antimicrobial profile of *Campylobacter fetus* subsp. *venerealis* isolated from sheat wash

Bacteria	**Antibiotics**	**Cross-tabulation of the antimicrobial profile**		
		Resistant	**Intermediate**	**Susceptible**
Campylobacter fetus subsp. *venerealis*	Tetracycline	71.0%	25.8%	3.2%
	Doxycycline	54.8%	32.3%	12.9%
	Ampicillin	67.7%	16.1%	16.1%
	Amoxicillin	58.1%	22.6%	19.4%
	Erythromycin	48.4%	16.1%	35.5%
	Azithromycin	51.6%	22.6%	25.8%
	Neomycin	64.5%	32.3%	3.2%
	Streptomycin	71.0%	16.1%	12.9%
	Gentamicin	48.4%	29.0%	22.6%
	Ciprofloxacin	51.6%	19.4%	29.0%
	Nalidixic acid	61.3%	22.6%	16.1%
	Norfloxacin	45.2%	29.0%	25.8%
	Enrofloxacin	45.2%	19.4%	35.5%
	Chloramphenicol	67.7%	12.9%	19.4%
	Total	57.6%	22.6%	19.8%

RESULTS

Antimicrobial resistance profile of *Campylobacter fetus* subspecies

The overall results of the antimicrobial resistance profile revealed that 54.4% of the isolated bacteria indicated a resistance profile against the antimicrobial tested, 26.3% presented intermediate profile, while a small portion of isolated bacteria 19. 3% were observed to susceptible as they have been demonstrated in figure 1.

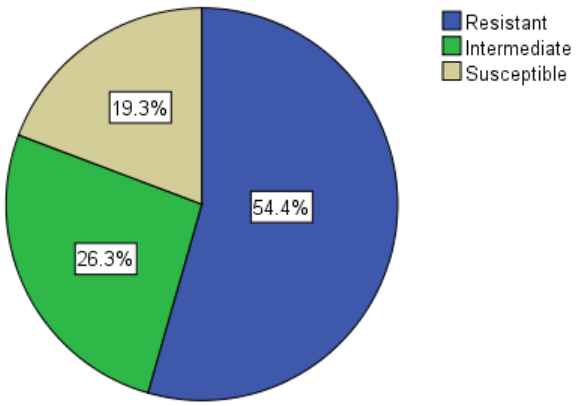

Figure 1. Overall profiles of *Campylobacter fetus* subspecies subjected to the antimicrobial test

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The occurrence of antimicrobial resistance profile of *Campylobacter fetus* subspecies

The analysis revealed that the antibiotic resistance of the *Campylobacter fetus* subsp *fetus* differed across the different antibiotics. For instance, it was observed that more strains of this subspecies were resistant to nalidixic acid (85.2%) than those that were resistant to ampicillin or ciprofloxacin (63%), as presented in table 2. Some strains of these subspecies were found to be more susceptible to norfloxacin (37%) than those that were susceptible to gentamicin (29.6%). It was also observed that 71.0% of *Campylobacter fetus* subsp.*venerealis* were resistant to tetracycline, 71.0% streptomycin, while 61.3% were resistant to nalidixic acid, 67.7% were resistant to chloramphenicol, and 35.5% of the isolates were found to be susceptible to erythromycin (Table 3).

Relationship between resistance profile and antibiotics

Pearson Chi-square test indicated that they were no association between the area from where samples were collected and the occurrences of isolated bacteria ($p>0.05$) as presented in table 5. The Kruskal Wallis test results indicated that the antibiotic resistance of *Campylobacter fetus* subsp. *fetus* differed significantly in accordance with the different tested antibiotics ($p<0.05$). However, there was no significant difference in the antibiotic resistance across the different antibiotics for the *Campylobacter fetus* subsp. *venerealis* ($p>0.05$), as presented in table 4. The Mann-Whitney's U test presented that the antibiotic resistance to nalidixic acid differed significantly across the two bacterial species ($p<0.05$) as presented in table 6.

Encoding of resistance genes in *Campylobacter fetus* subspecies using genome sequencing

The analysis revealed numerous genes involved in the resistance and virulence, as presented in tables 7 and 8. Additionally, it was observed that the present genome *Campylobacter fetus* subsp. *fetus* NW_ME1 contained six mobile element proteins, *Campylobacter fetus* subsp. *venerealis* _NW_ME2 was observed to have nine mobile element proteins. It was also observed that genes MurA (UDP-N-acetylglucosamine 1-carboxyvinyltransferase), Dxr (1-deoxy-D-xylulose 5-phosphate reductoisomerase) were exclusively found in *Campylobacter fetus* subsp. *fetus* NW_ME1. Furthermore, the encoded genes were located at different positions of the DNA strand and had a different DNA length, as is demonstrated in figures 2 and 7.

General genome feature and data availability

Data from our genome shotgun project of *Campylobacter fetus* subsp. *venerealis* NW_ME2 and *Campylobacter fetus* subsp. *fetus* NW_ME1 have been deposited at DDBJ/ENA/Gene Bank under the accession numbers JAATTN000000000 and JAAVIZ000000000. The version published in this paper was JAATTN010000000 and JAAVIZ010000000. The Raw reads were also submitted in NCBI/ SRA and are available in NCBI under Bio project PRJNA614613 and PRJNA614609. The genome of *Campylobacter fetus* subsp. *venerealis* NW_ME2 has a total length of 1,891,894 bp with an average G + C content of 33.2%, while *Campylobacter fetus* subsp. *fetus* NW_ME1 has a total length of 1,845,025 nucleotides with a GC content of 33.4%. Both isolates harbor resistance genes, and virulence factors were demonstrated in figures 8 and 9. The nucleotide sequences with high similarities (≥ 95%) were also submitted in the NCBI Gene bank and they are available under these accession numbers: MT138642; MT138643; MT138644; MT138645; MT138646; MT138647; MT138648; MT138649; MT138650; MT138651; MT138652; MT138653; MT138654; MT138655; MT138656; MT138657; MT138658; MT138659; MT138660; MT138661

Pathogenomics and phylogenetic analysis

The Pathogenomics analysis using Pathogen Finder version 1.1 revealed that our input organism *Campylobacter fetus* subsp. *fetus* NW_ME1 was predicted to be a human pathogen. The probability of being a human pathogen was 0.914, whose input proteome coverage (%) was % 0.16 and matched with pathogenic tree families (Table 9).

Phylogenetic analysis

Phylogenetic analysis constructed by inserting genome into species tree version 2.2.0 through Kbase platform. The phylogenetic analysis revealed that the multidrug-resistant *Campylobacter fetus* subspecies, which were isolated from sheath wash and obtained from bulls that were from the North-West province in South Africa, were highly similar and clustered tightly into one specific phylogenetic subgroups in the phylogenetic tree as it has been presented in figure 10.

Table 4. Kruskal Wallis test for association between the area in which the samples were collected and the occurrence of *Campylobacter fetus* subspecies

Bacteria		Resistance
Campylobacter fetus subsp. *fetus*	Chi-Square	34.652
	df	13
	p-value	0.001
Campylobacter fetus subsp. *venerealis*	Chi-Square	19.705
	df	13
	p-value	0.103

a. Kruskal Wallis Test; b. Grouping Variable: Antibiotic; df: the degree of freedom

Table 5 Association between the area in which samples were collected and the occurrence of *Campylobacter fetus* subspecies.

Chi-Square Tests	Value	df	p-value
Pearson Chi-Square	0.728	3	0.867

df: degree of freedom

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Table 6. Relationship between *Campylobacter fetus* subspecies resistance and different antibiotics tested against

Antibiotic	Statistical test	Resistance
Tetracycline	Mann-Whitney U	361.500
	P-value	0.287
Doxycycline	Mann-Whitney U	384.000
	P-value	0.552
Ampicillin	Mann-Whitney U	413.500
	P-value	0.926
Amoxicillin	Mann-Whitney U	400.000
	P-value	0.744
Erythromycin	Mann-Whitney U	378.500
	P-value	0.508
Azithromycin	Mann-Whitney U	361.500
	P-value	0.475
Neomycin	Mann-Whitney U	348.000
	P-value	0.308
Streptomycin	Mann-Whitney U	328.500
	P-value	0.108
Gentamicin	Mann-Whitney U	390.500
	P-value	0.638
Ciprofloxacin	Mann-Whitney U	356.000
	P-value	0.275
Nalidixic acid	Mann-Whitney U	314.500*
	P-value	0.039
Norfloxacin	Mann-Whitney U	356.000
	P-value	0.300
Enrofloxacin	Mann-Whitney U	410.000
	P-value	.887
Chloramphenicol	Mann-Whitney U	376.000
	P-value	0.448

a. Grouping Variable: Bacteria

Table 7. Genes resistant encoded in *Campylobacter fetus* subsp. *venerealis* NW_ME2 and *Campylobacter fetus* subsp *fetus* NW_ME1

Genes	Name	Function
YkkCD	Broad-specificity multidrug efflux pump *YkkC*	EFP-CAR
YKKCD	Broad-specificity multidrug efflux pump *YkkCD*	EFP-CAR
CmeABC	Multidrug efflux system, membrane fusion component => *CmeA*	EFP-CAR
CmeABC	Transcriptional repressor of CmeABC operon, *CmeR*	RME–ARGe
CmeABC	Multidrug efflux system, outer membrane factor lipoprotein => *CmeC*	EFP-CAR
CmeABC	Multidrug efflux system, inner membrane proton/drug antiporter (RND type) => *CmeB*	EFP-CAR
gidB	16S rRNA (guanine(527)-N(7))-methyltransferase	Ge-CR-Ab
rpoB	DNA-directed RNA polymerase beta subunit	AT-TSsp
rpoC	DNA-directed RNA polymerase beta' subunit	AT-TSsp
rho	Transcription termination factor Rho	AT-TSsp
Ddl	D-alanine ligase	AT-TSsp
MacB	Macrolide export ATP-binding/permease protein MacB	EFP-CAR
MacA	Macrolide-specific efflux protein MacA	EFP-CAR
7a-HSDH-like	Enoyl-[acyl-carrier-protein] reductase, 7-alpha-HSDH-like => refractory to triclosan	AT-REPLp
MurA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	AT-SUSCsp
dxr	1-deoxy-D-xylulose 5-phosphate reductoisomerase	AT-SUSCsp
EF-G	Translation elongation factor G	AT-SUSCsp
EF-Tu	Translation elongation factor Tu	AT-SUSCsp
NimB	Nitroimidazole resistance protein NimB	A-Inc ENZ
Iso-tRNA	Isoleucyl-tRNA synthetase	AT-SUSCsp
S10p	SSU ribosomal protein S10p (S20e)	AT-SUSCsp
inhA, fabI	Enoyl-[acyl-carrier-protein] reductase [NADH]	AT-SUSCsp
Alr	Alanine racemase	AT-SUSCsp
PgsA	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	Prot alt CWCh-CAR
gyrB	DNA gyrase subunit B	AT-SUSCsp
gyrA	DNA gyrase subunit A	AT-SUSCsp
S12p	SSU ribosomal protein S12p	AT-SUSCsp
TolC	Outer membrane protein TolC	Out Mem EF Prot-CAR

EFP-CAR (Efflux pump conferring antibiotic resistance), RME–ARGe (regulator modulating the expression of antibiotic resistance genes), Ge-CR-Ab(Gene conferring resistance via absence), AT-TSsp (antibiotic target in susceptible species), AT-REPLp (antibiotic target replacement protein), AT-SUSCsp (antibiotic target in susceptible species), A-Inc ENZ (antibiotic inactivation enzyme), Prot alt CWCh-CAR (protein-altering cell wall charge conferring antibiotic resistance), Out Mem EF Prot-CAR (Out membrane efflux protein conferring antibiotic resistance).

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Table 8. Virulence factors encoded in *Campylobacter fetus* subspecies Isolated from sheath wash

Property	Genes	Product	Classification
Virulence factor	fliI	Flagellum-specific ATP synthase FliI	Motility, Chemotaxis, Invasion, Phase variation
Virulence factor	CheY	Chemotaxis regulator - transmits chemoreceptor signals to flagellar motor components CheY	Chemotaxis, motility
Virulence factor	GidA	tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA	Invasion
Virulence factor	flgC	Flagellar basal-body rod protein FlgC	Motility, Chemotaxis, Invasion, Phase variation
Virulence factor	pseB	UDP-N-acetylglucosamine 4,6-dehydratase (inverting)	Biosynthesis, synthesis of both functional flagella and lipopolysaccharides
Virulence factor	fliP	Flagellar biosynthesis protein FliP	Motility, Chemotaxis, Invasion, Phase variation
Virulence factor	flhA	Flagellar biosynthesis protein FlhA	Motility, Chemotaxis, Invasion, Phase variation
Virulence factor	fliM	Flagellar motor switch protein FliM	Motility
Virulence factor	fliQ	Flagellar biosynthesis protein FliQ	Motility, Chemotaxis, Invasion, Phase variation
Virulence factor	fliN	Flagellar motor switch protein FliN	Motility, Chemotaxis, Invasion, Phase variation
Virulence factor	CdtA	Cytolethal distending toxin subunit A	Cytotoxin production
Virulence factor	CdtB	Cytolethal distending toxin subunit B	Cytotoxin production
Virulence factor	CdtC	Cytolethal distending toxin subunit C	Cytotoxin production
Virulence factor	MotA	Flagellar motor rotation A	Motility
Virulence factor	MotB	Flagellar motor rotation B	Motility
Virulence factor	VirB3	Inner membrane protein forms channel for type IV secretion of T-DNA complex	Adhesion and colonization
Virulence factor	VirB4	ATPase required for both assembly of type IV secretion complex and secretion of T-DNA complex	Adhesion and colonization
Virulence factor	VirB10	Inner membrane protein of type IV secretion of T-DNA complex, TonB-like	Adhesion and colonization
Virulence factor	VirB8	Inner membrane protein forms channel for type IV secretion of T-DNA complex	Adhesion and colonization
Virulence factor	VirB9	Outer membrane and periplasm component of type IV secretion of T-DNA complex has secretin-like domain	Adhesion and colonization
Virulence factor	VirB9	Forms the bulk of type IV secretion complex that spans outer membrane and periplasm	Adhesion and colonization
Virulence factor	VirB5	Minor pilin of type IV secretion complex	Adhesion and colonization
Virulence factor	VirB1	Bores hole in peptidoglycan layer allowing type IV secretion complex assembly	Adhesion and colonization
Virulence factor	CiaB	*Campylobacter* invasion antigen B	Invasion, colonization
Virulence factor	SLP	S-Layer protein	Adherence, evasion
Virulence factor	VirB11	ATPase required for both assembly of type IV secretion complex and secretion of T-DNA complex	Adhesion and Colonization
Virulence factor	VirD4	Type IV secretion system protein	Adhesion and colonization
Virulence factor	VirD4	Like coupling protein	Adhesion and colonization
Virulence factor	fic	Fic domain protein, BT_4222 type	Adhesion and colonization
Virulence factor	TrbE	Conjugative transfer protein TrbE	Adhesion and colonization
Virulence factor	TrbD	Conjugative transfer protein TrbD	Adhesion and colinization
Virulence factor	TrbC	Conjugative transfer protein TrbC	Adhesion and colonization
Virulence factor	TrbI	Conjugative transfer protein TrbI	Adhesion and colonization
Virulence factor	TrbG	Conjugative transfer protein TrbG	Adhesion and colonization
Virulence factor	TrbF	Conjugative transfer protein TrbF	Adhesion and colonization
Virulence factor	TrbL	Conjugative transfer protein TrbL	Adhesion and colonization
Virulence factor	TrbJ	Conjugative transfer protein TrbJ	Adhesion and colonization
Virulence factor	TraT	IncF plasmid conjugative transfer surface exclusion protein TraT	Adhesion and colonization
Virulence factor	TrbB	Conjugative transfer protein TrbB	Adhesion and colonization

Table 9. Matched pathogenic families with *Campylobacter fetus* subsp. *fetus* NW_ME1

Accession No	Organism	Class	Protein function	Protein number	Identity (%)
CP000792	*Campylobacter. concisus*13826 complete genome	Epsilonproteo bacteria	Thymidylate synthase, flavin dependent	EAT99108	80.1
CP000767	*Campylobacter. curvus* 525.92 Complete genome	Epsilonproteo bacteria	Ribosomal protein S2	EAU00943	81.25
CP000538	*Campylobacter. Jejune* subsp. *jejune* 81 – 176 Complete genome	Epsilonproteo bacteria	Ribosomal protein L22	EAQ72756	85.45

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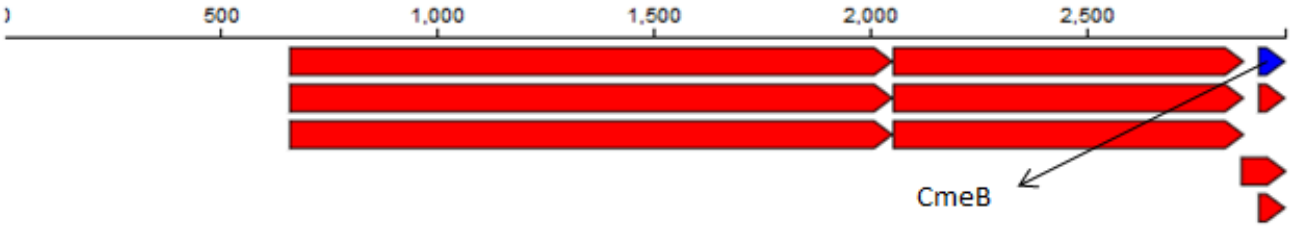

Figure 2. *Campylobacter fetus* subsp. *venerealis*: RND efflux system inner membrane transporter CmeB, Location: 2,897-2,960 (+ Strand). DNA Lengh 63

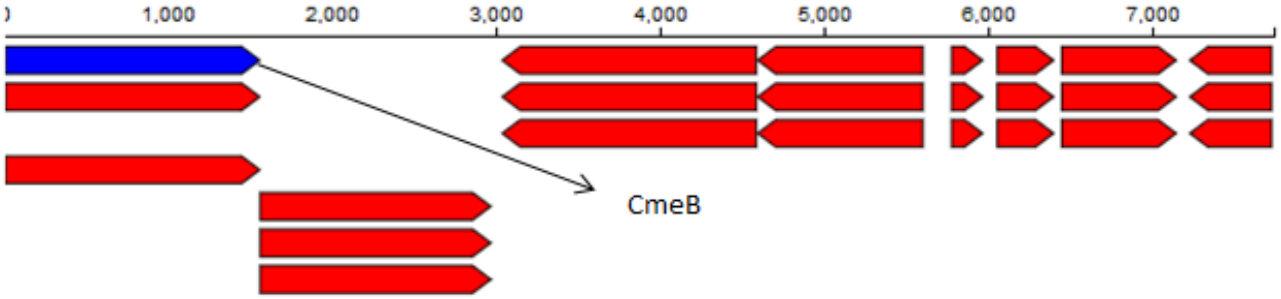

Figure 3. *Campylobacter fetus* subsp. *fetus*: RND efflux inner membrane transporter CmeB, Location: 2-1,562 (+ Strand). DNA Length 1560

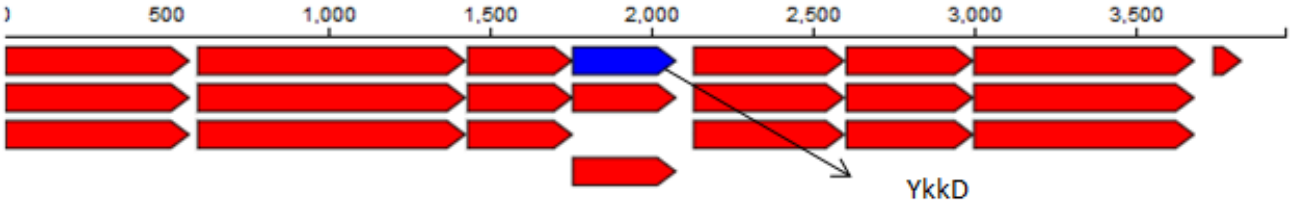

Figure 4. *Campylobacter fetus* subsp. *fetus*: Broad-specificity multidrug efflux pumps YkkD. Location 1, 757 – 2, 078 (+ Strand), DNA Length: 321

Figure 5. *Campylobacter fetus* subsp. *venerealis*: Broad-specificity multidrug efflux pumps YkkD. Location: 2, 471 – 2, 792 (- Strand), DNA Length: 321

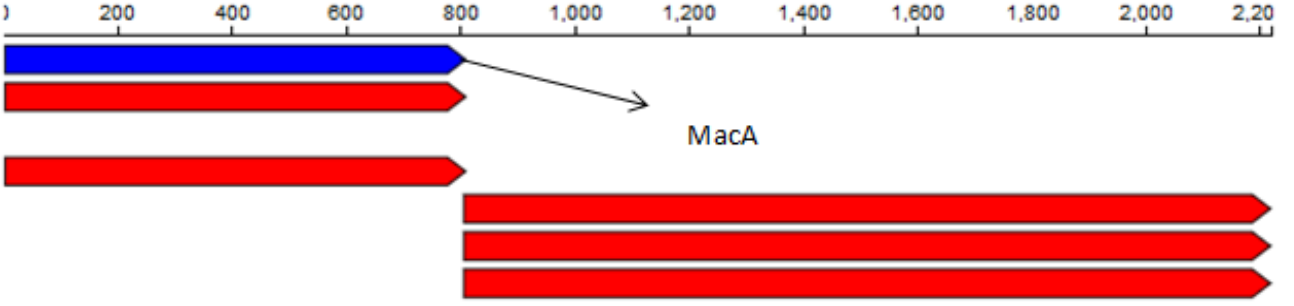

Figure 6. *Campylobacter fetus* subsp. *fetus*: Macrolide efflux protein MacA. Location: 2 – 809 (+ Strand), DNA Length: 807

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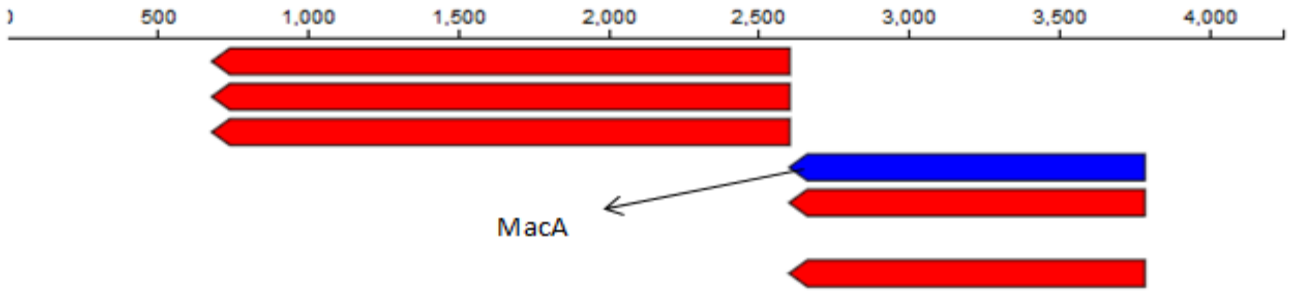

Figure 7. *Campylobacter fetus* subsp. *venerealis*: Macrolide specific efflux protein MacA. Location: 2, 599 – 3, 784 (-Strand), DNA Length: 1, 185

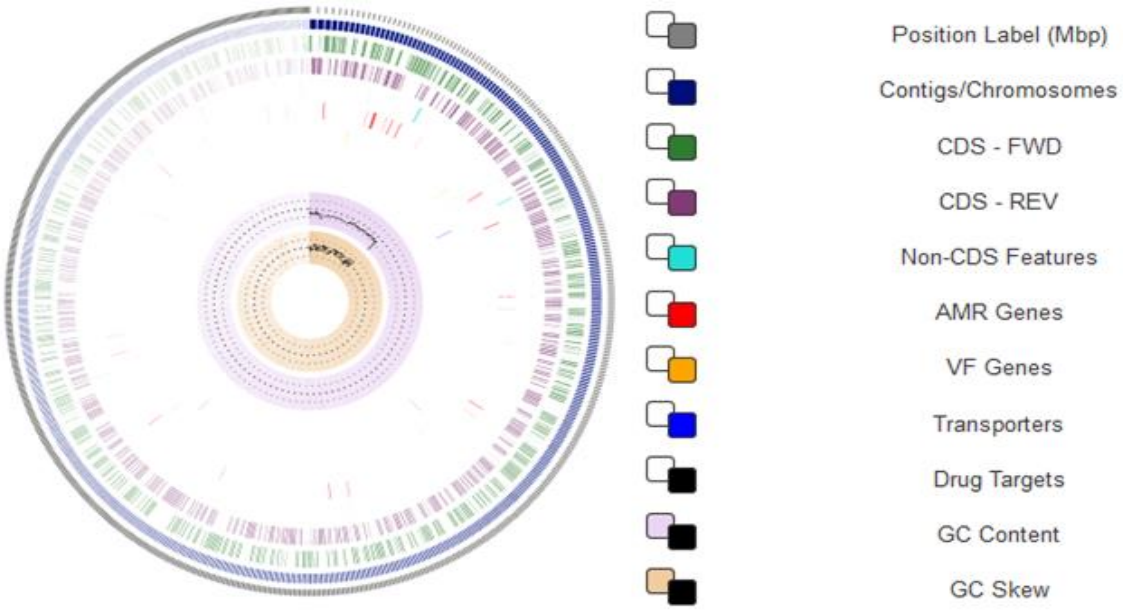

Figure 8. Circular genome map of *Campylobacter*. *fetus* subsp. *fetus* NW_ME1. The overview of the genome map of *Campylobacter fetus* subsp. *fetus* NW_ME1 generated via Patrick annotation version 3.6.3. The tracks on the viewer are displayed as concentric rings, from outermost to innermost: Position, Contigs, CDS-Forwards, CDS- Reverse, Non-CDS features, GC content, and GC skew

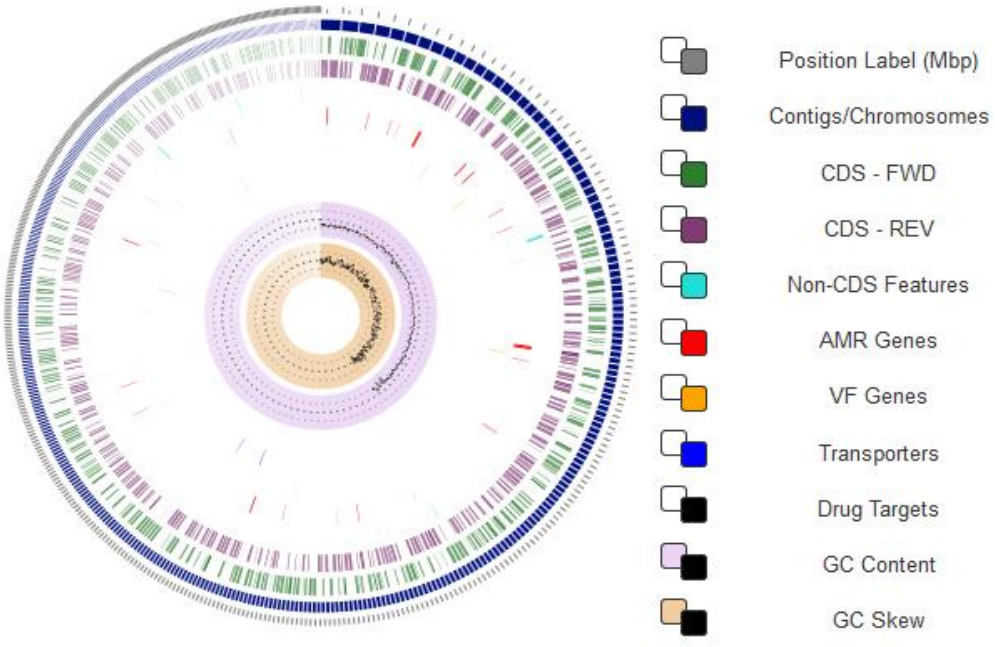

Figure 9. Circular genome map of *Campylobacter fetus* subsp. *venerealis* NW_ME2 generated using Patrick annotation-circular, from outside to inner ring contigs, CDS on the forward, CDS on the reverse, non CDS features, CDS with homology to known antimicrobial resistance genes, virulence genes, transports, drug targets GC content and GC skew.

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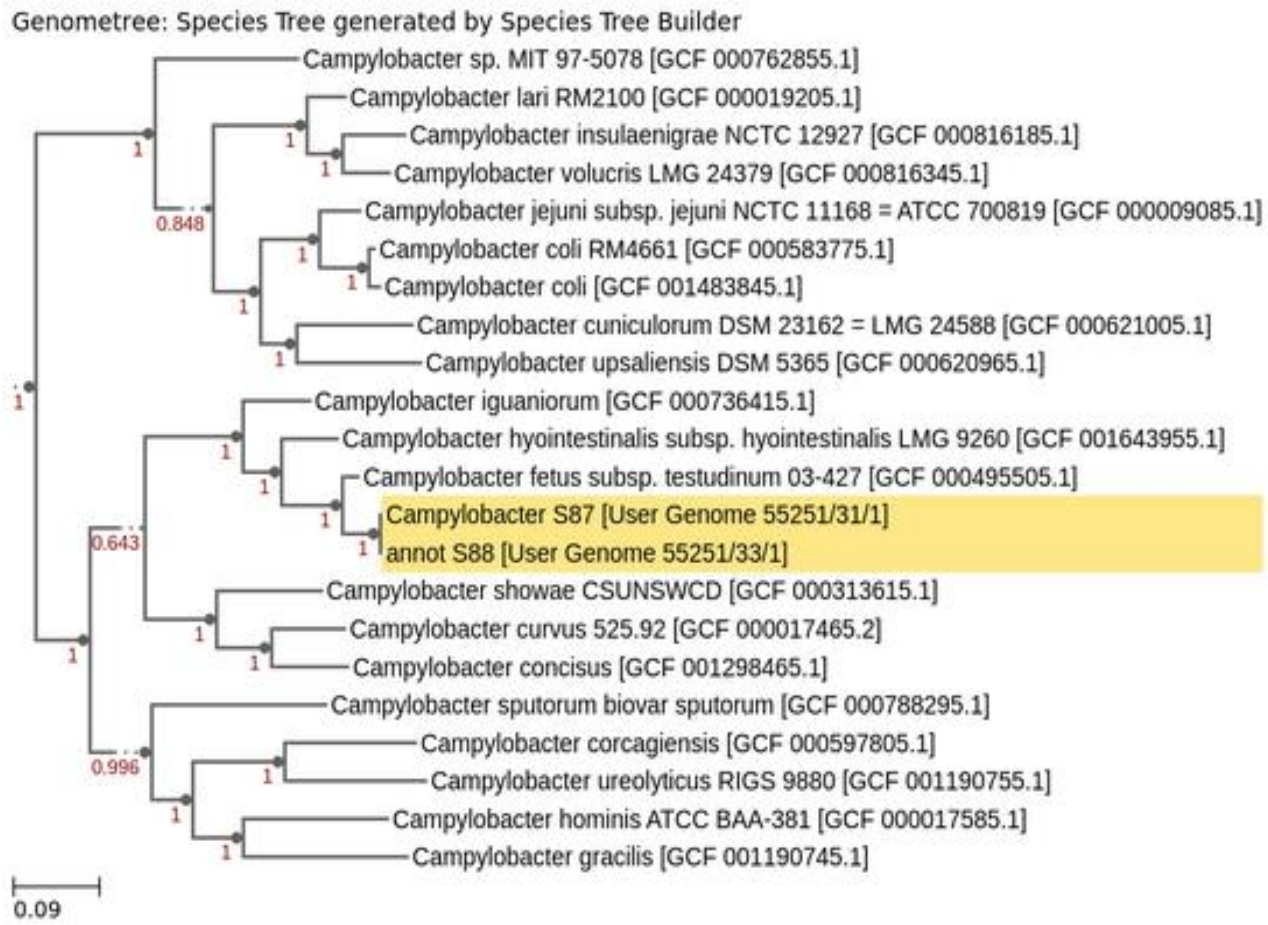

Figure 10. Whole-genome Neighbor joining phylogenetic tree. Phylogenetic tree of the complete genome of *Campylobacter fetus* subsp. *fetus* NW_ME1 and *venerealis* NW_ME2 align with 20 complete *Campylobacter* genomes; both *Campylobacter fetus* subspecies isolated in this study expressedmore significant homology. The digits indicated the distance or divergence between species (genome) used in the tree. The scale bar indicates 0.09 nucleotide substitutions per nucleotide position

DISCUSSION

The current study revealed that most of the *Campylobacter fetus* subspecies were multidrug-resistant to almost all the antibiotics tested against, as demonstrated in tables 2 and 3. The resistance of *Campylobacter fetus* subsp. *fetus* to different antibiotics was also reported in a study on the temporal prevalence of antimicrobial resistance in *Campylobacter* spp. from beef cattle in Alberta Feedlots in Canada. The researchers indicated that the *Campylobacter fetus* (39%) was resistant to doxycycline and tetracycline. The same study presented that isolated *Campylobacter fetus* (97%) were highly resistant to nalidixic acid (Tremblay et al., 2003). This corroborates with the findings of this study, that 85.2% of *Campylobacter fetus* subsp. *fetus* and 61.3% of *Campylobacter fetus* subsp. *venerealis* were observed to be highly resistant against nalidixic acid. The resistance of *Campylobacter fetus* subsp. *fetus* was also reported by Kenar and Gökçe (2019). The bacteria were isolated from the livers of slaughtered ruminants and aborted ovine fetuses. Their results, based on the disk diffusion method, revealed that the highest resistance was manifested against ciprofloxacin and tetracycline (45.45%) while the highest sensitivity was against gentamicin (90.91%) and chloramphenicol (100%) (Kenar and Gökçe, 2019).

These findings slightly differ from the results of the present study; though both studies used disc diffusion methods, it was observed that the resistance to ciprofloxacin and tetracycline was higher when compared with the previous study, respectively 63.0% and 59.3%. On the other hand, the susceptibility of gentamicin and chloramphenicol was very low, 29.6%, and 11.1%, respectively. Tremblay and Gaudreau (1998) evaluated the susceptibility profile for a total number of 59 *Campylobacter fetus* subsp. *fetus* against eight antimicrobial agents using the disc diffusion method, agar dilution, and E-test. The findings of their study concluded that *Campylobacter fetus* subsp. *fetus* was observed to be susceptible to meropenem, gentamicin, ampicillin, and imipenem. Further, the findings revealed that *Campylobacter fetus* subsp. *fetus* (27%) exhibited resistance to profile to tetracycline for both methods used disk diffusion and agar dilution (Tremblay and Gaudreau, 1998).

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The results of the current study were different from previous ones as isolated *Campylobacter fetus* were not all susceptible to ampicillin and gentamicin; furthermore, imipenem and meropenem were not used in this study; this difference might be related to the source of isolated bacteria. In the present study, the isolated bacteria were obtained from sheath wash, while the previous study was obtained from clinical samples. In this study, it was also observed the resistance of *Campylobacter fetus* subsp. *venerealis* to different antibiotics such as tetracycline, ampicillin, neomycin, streptomycin and other antimicrobial agents as presented in table 3.

The phenotypic antimicrobial resistance profile of *Campylobacter fetus* subsp. *fetus* and *Campylobacter fetus* subsp. *venerealis* have never yet been investigated and reported in South Africa; this was one of the primary reasons for this study to investigate the genes involved in the mechanism of resistance in these two subspecies. These results aligned with observation revealed by researchers in the past few decades. An increasing number of isolated *Campylobacter* spp. have developed resistance to fluoroquinolones and other antimicrobials such as macrolides, aminoglycosides, and beta-lactams. In the current study, resistance against these classes of antibiotics was observed among the isolated bacteria. There is strong evidence indicating that the spread and dissemination of antibiotic resistance observed in many organisms are linked to the use of the antimicrobial agent in animal production and veterinary medicine for growth promoter and diseases prevention, which is also observed in *Campylobacter* spp. in humans (Wieczorek and Osek, 2013). In this study, both *Campylobacter fetus* subsp. *fetus* and *venerealis* expressed resistance to nalidixic acid, which confirmed that resistance to nalidixic acid had been proved to be an important marker to distinguish between *C. jejuni* and *C. fetus* (Taylor et al., 1985).

The WGS analysis revealed that isolated *Campylobacter fetus* subsp. *fetus* and *venerealis* harbored 28 resistance genes and 39 virulence factors. Among the encoded resistance genes, some have never been reported in *Campylobacter fetus* subsp. *fetus* and *venerealis*, such as gidB, ykkc, ykkcd, rho, MacB, and MacA, suggesting a new upcoming mechanism of resistance in these subspecies. Among the genes encoded, some are genetic determinants for fluoroquinolone (gyrA and gryB), for chloramphenicol, and tetracycline (ykkc and ykkcd), for aminoglycoside (gidB). Additionally, multidrug resistance efflux pumps (CmeABC), Broad-specificity multidrug efflux pump (ykkc and ykkcd), efflux pump conferring antibiotic resistance (MacA and MacB), and protein-altering cell wall charge conferring antibiotic resistance (PgsA) were among important CDS encoded. In *Escherichia coli* the expression of ykkc or ykkd was observing to induce phenotypic multidrug-resistant profile in bacteria, including resistance to neutral drugs, cationic and anionic, as it is shown in figure 4 (Jack et al., 2000). Further, the overexpression of these efflux pumps contributes to reducing susceptibility by decreasing the intracellular concentration of the antimicrobial agents (Nikaido and Pagès, 2012). This finding corroborates with the result of the current study, which observed the expression of different efflux pumps as they have been presented in table 7. The study revealed that collaborating MacB, in concert with the adaptor MacA and the outer membrane exit duct, TolC, could underpin the efflux of various drugs and export of virulence factors from multiple Gram-negative bacterial species. Additionally, a study revealed that in Gram-negative bacteria such as *Stenotrophomonas maltophilia*, *Escherichia coli*, and *Salmonella* spp MacA, MacB together with Tolc conferred resistance to numerous classes of antibiotics respectively aminoglycosides, macrolides as well as polymyxins (Nishi et al., 2003; Lin et al., 2014). These findings correlated with the results of the current study because MacA, MacB, and Tolc were encoded in both *Campylobacter fetus* subsp. *fetus* and *venerealis*, and this clarified why our isolated bacteria presented a resistance profile to macrolides and aminoglycosides antibiotics. On the other hand, in this study, the multidrug resistance efflux pumps (CmeABC) were encoded in both isolated bacteria. The efflux pump (CmeABC) has been pointed out to have an important role in the emergence of fluoroquinolone-resistant *Campylobacter* (Yan et al., 2006). It has also been reported that CmeABC is an important multidrug efflux pump in *Campylobacter* species and contributes to its intrinsic and acquired resistance to a broad spectrum of antibiotics, including fluoroquinolone resistance (Lin et al., 2002; Pumbwe and Piddock, 2002; Lin et al., 2003). The CmeABC is distributed in tripartite multidrug efflux pumps, which consist of CmeC (outer membrane protein), *CmeA* (periplasmic fusion protein) and CmeB (inner membrane efflux transporter) figure 3 (Lin et al., 2002).

This finding aligned with the results of the present study, that different operon of the efflux pump were detected in *Campylobacter fetus* subsp. *venerealis* and *Campylobacter fetus* subsp. *fetus*.. It has also been reported that these three proteins (CmeA), (CmeB) and (CmeC) are encoded by a three-gene operon (cmeABC) and function together to form a membrane channel that extruded toxic substrates directly out of *Campylobacter* cells (Lin et al., 2002). The CmeABC contributed actively to the intrinsic and acquired resistance of *Campylobacter* species to structurally diverse antimicrobials (Lin et al., 2002; Pumbwe and Piddock, 2002; Luo et al., 2003).

Additionally, this multidrug efflux pump (CmeABC) is well known to confer resistance against bile. It is required for the growth of *Campylobacter* spp. in media containing bile that serves to colonize the intestinal tract in animals (Lin et al., 2003). Furthermore, In this study, the transcriptional repressor of CmeABC, operon *CmeR* was also detected in isolated bacteria subjected to the whole genome analysis. It has been revealed that *CmeR* interacts with the cmeABC promoter and modulated the expression of cmeABC. On the other hand, the CmeR is known to improve the production and expression of a multidrug efflux pump (Lin et al., 2005). In the present study, the isolated bacteria were

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phenotypically resistant to macrolide tested against erythromycin and azithromycin. The genome sequencing reveals the presence of MacB (macrolide export ATP binding permease protein) as well as MacA gene as it has been shown in figures 6 and 7.

It has been reported that MacB, along with its periplasmic adaptor protein MacA, was first identified in *Escherichia coli* transporter genes as providing resistance to macrolide drugs in a strain lacking the major RND efflux pump AcrAB (Kobayashi et al., 2001). It is also known that these genes MacA and MacB have never been reported in *Campylobacter fetus* subspecies around Sound Africa, and this might be the first report. Another study investigated the role of MacAB in Gram-negative species such as *Stenotrophomonas maltophilia* and found that MacA and B together with Tolc conferred resistance to a variety of macrolides, aminoglycosides, and polymyxins in Gram-negative species (Nishi et al., 2003; Lin et al., 2014).

These findings corroborated with the results of this study, in which MacA and B were detected in isolated bacteria, and phenotypic resistance to macrolide and aminoglycoside was also observed. The auxiliary studies of the MacAB-Tolc framework examined by the Zgurskaya research facility illustrated that the ATPase movement of reconstituted MacB is subordinate to intaglio MacA. Assist, the same consider affirmed that MacAB interceded antimicrobial resistance in vivo requires the nearness of the external layer efflux channel TolC (Tikhonova et al., 2007). Tikhonova and his co-authors corroborated with the findings of this study that the presence of outer membrane efflux channel Tolc was detected in the isolated bacteria and observed in this study that the presence of gene gidB encoded with methyltransferase. This gene has been pointed out as conferring high-level antimicrobial resistance in Gram-negative such as *salmonella* (Mikheil et al., 2012). The research found out that the cancellation of gidB conferred resistance to the aminoglycoside antimicrobials, such as neomycin and streptomycin, whereas gidB protein had a noteworthy part within the modification of antimicrobial susceptibility (Mikheil et al., 2012). This might be the only explanation related to the aminoglycoside observed in this study. Moreover, alteration inside gidB has been linked to a low level of streptomycin resistance in some bacterial species and tall recurrence of the development of streptomycin-resistant mutants, which was too detailed in *Campylobacter upsaliensis* (Nishimura et al., 2007; Okamoto et al., 2007; Olkkola et al., 2015).

It has been moreover detailed that in microorganisms, gidB influenced antimicrobial sensitivity by a process that includes a post-transcriptional alteration (Mikheil et al., 2012). In this study, Broad-specificity multidrug efflux pump *YkkCD* was encoded in isolated bacteria; the gene was reported in Gram-positive *Bacillus subtilis* and *Escherichia coli.* It has been reported that in *Escherichia coli* strain DH5α when the gene ykkc and ykkd were expressed, a broad-spectrum multidrug resistance phenotype was observed to different antimicrobial agents, such as chloramphenicol, streptomycin, and tetracycline. Further resistance was also observed to a broader range of toxic compounds (Jack et al., 2000). Considering different genes encoded in our isolated bacteria subjected to the WGS, it is possible to conclude that the multidrug resistance observed in this study might be linked to these genes.

Moreover, some genes found in this study, such as gibB, ykkc, ykkcd, MacA, and MacB, have never been reported in *Campylobacter fetus* subsp. *fetus* and *Campylobacter fetus* subsp. *venerealis* isolated from the sheath wash, their presence in this species might be considered as a new upcoming mechanism of resistance in *Campylobacter fetus* subspecies.

The WGS analysis also revealed presence of numerous genes (table 8) associated with bacterial motility and chemotaxis (fliI, flip, flhA, fliM, cheY, fliQ and fliN). Among them, FliM, FliG, and FliN are important proteins which form switch complex C ring. This complex interacts with the CheY and CheZ chemotaxis proteins, in addition to contact components of the motor that determine the direction of flagellar rotation. In the current study, flagellar motor rotation (MotA and B) were also detected. It has been reported that these flagella conferred swimming motility and distinctive darting motility, which was necessary for infection of humans to cause diarrheal disease and in animals to promote commensalism (Balaban and Hendrixson, 2011). The FliM and FliN proteins have also been reported in *Escherichia coli* and *Salmonella* spp, where they form complexes beneath FliG (Paul et al., 2011; McDowell et al., 2016). The middle domain of FliM contains a CheC-like domain that was not detected in the present study, which forms a continuous belt in the middle of the C ring (Park et al., 2006; Sircar et al., 2015).

In contrast, CheY, together with FliM impact the clockwise or counter-wise motor rotation for chemotaxis (Mathews et al., 1998; Szurmant et al., 2003; Ahn et al., 2013). It has also been reported that in bacterial FliG, FliM, and FliN proteins act as a molecular switch, enabling the motor to spin in both counterclockwise and clockwise directions (Morimoto and Minamino, 2014) while MotA and MotB act as a proton pathway to couple proton flow through the channel with torque generation (Blair and Berg, 1990). Another study reported that MotA/B complex of alkaliphilic *Bacillus clausii* could conduct both H^+ and Na^+ at different pH ranges (Terahara et al., 2008). Additionally study conducted on utilitarian bi-directional flagellar motor uncovered that the two rotor proteins, FliM and FliN, illustrated significantly enthusiastic turnovers between the motor and their cytoplasmic pools (Morimoto and Minamino, 2014). The FliM turnover played an important role in switching the direction of flagellar motor rotation (Delalez et al., 2010; Yuan et al., 2012). In the present study, the responsible gene for adhesion and colonization was identified as virB11. This gene has been detected in retail chicken breast and fecal samples obtained from chicken, suggesting that chicken is a

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potential factor that could invade human intestinal cells with the gene (Thakur et al., 2010; Lapierre et al., 2016). However, in this study, virB11 was detected from sheath wash obtained from the bull, which also suggested that cattle, in general, might constitute a risk factor in the transmission of these genes to humans. Present findings revealed that flagellar and adhesion genes were exceptionally preserved in *Campylobacter* species as suggested in other studies (Thakur et al., 2010; Koolman et al., 2015; Lapierre et al., 2016). In the current study, other virulence marker determinants, including Cytolethal Distending Toxin (A, B, and C), were present in the isolated bacteria. Other studies revealed that three subunits are required for full toxin activity (No et al., 2002; Lapierre et al., 2016). It has also been indicated that these genes played an important role in the host mucosal inflammatory response for Interleukin-8 (IL-8) released by intestinal cells (Perera et al., 2007). Moreover, these genes have been pointed to cause diarrhea by interfering with the division and differentiation of the intestinal crypt cells (Wieczorek et al., 2018). The ciaB marker, which plays a significant role both in the invasiveness and in the colonization of the epithelial cells, was also identified in *Campylobacter fetus* in this study. Moreover, this gene has been detailed to easier the conquering of epithelial cells of the gastrointestinal tract, where it delivered expanding harm to the columnar epithelial cells, which led to swelling and adjusting of attacked cells as a result of the cytotoxin and enterotoxin activities (Sopwith et al., 2006; Perera et al., 2007).

In this study, the type IV secretion system VirD4 was detected only in *Campylobacter fetus* subsp. *fetus* NW_ME1. The type IV translocation pilus is encoded by the gene VirB operon that consists of eleven genes (virB1-virB11); translocation is also dependent on an additional gene virD4, which encodes the type IV coupling protein T4CP (van der Graaf–van Bloois et al., 2016). These findings align with the results of the present study that virB5, virB1, virB9, virB8, virB4, virB3, and virD4 (like coupling protein) were detected in both isolated bacteria. In this study, fic domain protein was found in the isolated bacteria, and it has been reported that this gene had significant roles in multiple cellular processes, including disrupting the host cell processes that were important to pathogen survival and replication after transmission into eukaryotic cells (Worby et al., 2009). Furthermore, the surface layer protein was encoded in this study in both isolated bacteria, and it is well known that surface layer proteins are responsible for the pathogenicity of *Campylobacter fetus* subspecies (Blaser et al., 1987; Pei and Blaser, 1990). On the other hands, the study revealed that both the surface layer proteins and T4SS regions are suggested to have a role in the pathogenicity of *Campylobacter fetus* subspecies, and it might be possible that these features have a synergistic role in immune escape (van der Graaf–van Bloois et al., 2016). Among the important genes encoded in this study, it was also observed the presence of GidA tRNA uridine 5-carboxymethyl aminomethyl modification enzyme, which is presented in table 8. This gene has been considered to be part of the major virulence mechanism in Gram-positive and negative bacteria such as *Escherichia coli*, *Salmonella* spp and *staphylococcus* but never been reported in *Campylobacter fetus* subspecies (Shippy and Fadl, 2014).

CONCLUSION

The findings of this study indicated that *Campylobacter fetus* subsp. *fetus* and *Campylobacter fetus* subsp. *venerealis* subjected to antimicrobial tests were observed to be multidrug resistance to almost antibiotics tested against. The WGS analysis of *Campylobacter fetus* subsp. *fetus* NW_ME1 and *Campylobacter fetus* subsp. *venerealis* NW_ME2 revealed the presence of different genes involved in the mechanism of resistance of the isolated bacteria and virulence factors that are susceptible to initiate the disease. Furthermore, the study encoded genes that have never been reported in *Campylobacter fetus* subsp. *fetus* and *venerealis* such as Broad-specificity multidrug efflux pump (ykkc and ykkd), 16S rRNA (guanine^{527}-N^{7}) methyltransferase (gidB), efflux pump conferring antibiotic resistance (MacA and MacB) suggesting a new resistance mechanism among these subspecies.

DECLARATIONS

Acknowledgments

The author acknowledges the technical support and advice of various colleagues working in the diagnostic and molecular laboratory of the Animal Health Department.

Authors' contributions

Mulunda Mwanza provided the funds for the project, participated in the drafting of the research proposal, and participated in the revisions of the final draft of the manuscript. Lubanza Ngoma provided technical support in the laboratory. Mpinda Edoaurd Tshipamba, designed the project, elaborated its plan, collected the samples, conducted the laboratory work, analyzed the results, and wrote the final manuscript for the publication.

Competing interests

The authors of this study declared that there is no financial conflict related to this work, which can negatively impact its publication.

To cite this paper: Tshipamba ME, Lubanza N and Mwanza M (2021). Genome Analysis of Antimicrobial Resistance Genes and Virulence Factors in Multidrug-Resistant *Campylobacter fetus* Subspecies Isolated from Sheath Wash. *World Vet. J.*, 10 (4): 465-480. DOI: https://dx.doi.org/10.54203/scil.2020.wvj57

REFERENCES

Acke E, McGill K, Golden O, Jones B, Fanning, and Whyte P (2009). A comparison of different culture methods for the recovery of campylobacter species from pets. Zoonoses and public health, 56 (9-10): 490-495. DOI: https://doi.org/10.1111/j.1863-2378.2008.01205.x .

Ahn D-R, Song H, Kim J, Lee S, and Park SY (2013). The crystal structure of an activated thermotoga maritima chey with n-terminal region of flim. International journal of biological macromolecules, 54: 76-83. DOI: https://doi.org/10.1016/j.ijbiomac.2012.12.003 .

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, and Lipman DJ (1997). Gapped blast and psi-blast: A new generation of protein database search programs. Nucleic acids research, 25 (17): 3389-3402. DOI: https://doi.org/10.1093/nar/25.17.3389

Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S et al. (2018). Kbase: The united states department of energy systems biology knowledgebase. Nature biotechnology, 36 (7): 566. DOI: https://doi.org/ 10.1038/nbt.4163

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M et al. (2008). The rast server: Rapid annotations using subsystems technology. BMC genomics, 9 (1): 75. DOI: https://doi.org/10.1186/1471-2164-9-75 .

Balaban M, and Hendrixson DR (2011). Polar flagellar biosynthesis and a regulator of flagellar number influence spatial parameters of cell division in campylobacter jejuni. PLoS pathogens, 7 (12): e1002420. DOI: https://doi.org/10.1371/journal.ppat.1002420

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Max A, Pham S, Tesler G, Vyahhi N, Teslere G, et al. (2012). Spades: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of computational biology, 19 (5): 455-477. DOI: https//doi.org/ 10.1089/cmb.2012.0021 .

Bellows DS, Ott SL, and Bellows RA (2002). Cost of reproductive diseases and conditions in cattle. The Professional Animal Scientist, 18 (1): 26-32. DOI: https://doi.org/10.15232/S1080-7446(15)31480-7.

Blair DF, and Berg HC (1990). The mot A protein of e. Coli is a proton-conducting component of the flagellar motor. Cell, 60 (3): 439-449. DOI: https://doi.org/10.1016/0092-8674(90)90595-6

Blaser MJ, Smith PF, Hopkins JA, Heinzer I, Bryner JH, and Wang WL (1987). Pathogenesis of campylobacter fetus infections: Serum resistance associated with high-molecular-weight surface proteins. Journal of Infectious Diseases, 155(4): 696-706. DOI: https://doi.org/10.1093/infdis/155.4.696 .

Bolger AM, Lohse M and Usadel B (2014). Trimmomatic: A flexible trimmer for illumina sequence data. Bioinformatics, 30(15): 2114-2120. DOI: https://doi.org/10.1093/bioinformatics/btu170.

Delalez NJ, Wadhams GH, Rosser G, Xue Q, Brown MT, Dobbe LM, Berry RM, Leake MC, and Armitage JP (2010). Signal-dependent turnover of the bacterial flagellar switch protein flim. Proceedings of the national academy of sciences, 107(25): 11347-11351. DOI: https://doi.org/10.1073/pnas.1000284107

Di Giannatale E, Di Serafino G, Zilli K, Alessiani A, Sacchini L, Sacchini L, Garofolo G, Aprea G, and Marotto F (2014). Characterization of antimicrobial resistance patterns and detection of virulence genes in campylobacter isolates in italy. Sensors, 14 (2): 3308-3322. DOI: https://doi.org/ 10.3390/s140203308.

Erickson NE, Lanigan E, Waugh T, Gesy K, and Waldner C (2017). Evaluation of long-acting oxytetracycline and a commercial monovalent vaccine for the control of campylobacter fetus subsp. Venerealis infection in beef bulls. The Canadian Veterinary Journal, 58(10): 1051. PMID: 28966354.

Guerra AG, Chaban B, Hill JE, Waldner CL, and Hendrick SH (2014). Clinical sensitivity and specificity of a real-time pcr assay for campylobacter fetus subsp venerealis in preputial samples from bulls. American journal of veterinary research, 75 (9): 851-860.

DOI: https://doi.org/10.2460/ajvr.75.9.851 .

Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire M, Gonzalez et al. (2018). Refseq: An update on prokaryotic genome annotation and curation. Nucleic acids research, 46 (D1): D851-D860. DOI:https://doi.org/10.1093/nar/gkx1068 .

institute Cals (2013). Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement. Secondary title. Twenty-Third Informational Supplement ed. Available at : www.clsi.org

Jack DL, Storms ML, Tchieu JH, Paulsen IT, and Saier MH (2000). A broad-specificity multidrug efflux pump requiring a pair of homologous smr-type proteins. Journal of bacteriology 182 (8): 2311-2313. DOI: https://doi.org/10.1128/jb.182.8.2311-2313.2000.

BAKWS JC and Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 45: 493-496. PMID: 5908210.

KENAR B, and GÖKÇE M (2019). Antibiotic resistance to campylobacter spp. Isolated from the livers of slaughtered ruminants and aborted ovine fetuses. Kocatepe Veteriner Dergisi, 1-1. DOI: https://doi.org/10.30607/kvj.505685.

Kobayashi N, Nishino K, and Yamaguchi A (2001). Novel macrolide-specific abc-type efflux transporter in escherichia coli. Journal of bacteriology, 183(19): 5639-5644. DOI: https://doi.org/10.1128/JB.183.19.5639-5644.2001

Koolman L, Whyte P, Burgess C, and Bolton D (2015). Distribution of virulence-associated genes in a selection of campylobacter isolates. Foodborne pathogens and disease, 12(5): 424-432. DOI:https://doi.org/10.1089/fpd.2014.1883 .

Lapierre L, Gatica MA, Riquelme V, Vergara C, Yañez JM, Martin BS, Saenz L, Vidal M, Martinez MC, Araya P et al (2016). Characterization of antimicrobial susceptibility and its association with virulence genes related to adherence, invasion, and cytotoxicity in campylobacter jejuni and campylobacter coli isolates from animals, meat, and humans. Microbial Drug Resistance, 22(5): 432-444. DOI: https://doi.org/10.1089/mdr.2015.0055 .

To cite this paper: Tshipamba ME, Lubanza N and Mwanza M (2021). Genome Analysis of Antimicrobial Resistance Genes and Virulence Factors in Multidrug-Resistant *Campylobacter fetus* Subspecies Isolated from Sheath Wash. *World Vet. J.*, 10 (4): 465-480. DOI: https://dx.doi.org/10.54203/scil.2020.wvj57

Lin J, Akiba M, Sahin O, and Zhang Q (2005). Cmer functions as a transcriptional repressor for the multidrug efflux pump cmeabc in campylobacter jejuni. Antimicrobial agents and chemotherapy, 49 (3): 1067-1075. DOI: https://doi.org/10.1128/AAC.49.3.1067-1075.2005 .

Lin J, Michel LO, and Zhang Q (2002). Cmeabc functions as a multidrug efflux system in campylobacter jejuni. Antimicrobial agents and chemotherapy, 46 (7): 2124-2131. DOI: https://doi.org/10.1128/aac.46.7.2124-2131.2002 .

Lin J, Sahin O, Michel LO and Zhang Q (2003). Critical role of multidrug efflux pump cmeabc in bile resistance and in vivo colonization of campylobacter jejuni. Infection and immunity, 71 (8): 4250-4259. DOI: https://doi.org/10.1128/IAI.71.8.4250-4259.2003 .

Lin YT, Huang YW, Liou RS, Chang YC andYang TC (2014). Macabcsm, an abc-type tripartite efflux pump of stenotrophomonas maltophilia involved in drug resistance, oxidative and envelope stress tolerances and biofilm formation. Journal of Antimicrobial Chemotherapy, 69 (12): 3221-3226. DOI: https://doi.org/10.1093/jac/dku317 .

Luo N, Sahin O, Lin J, Michel LO, and Zhang Q (2003). In vivo selection of campylobacter isolates with high levels of fluoroquinolone resistance associated with gyra mutations and the function of the cmeabc efflux pump. Antimicrobial agents and chemotherapy, 47(1): 390-394. DOI: https://doi.org/10.1128/AAC.47.1.390-394.2003.

Mak S, Xu Y, and Nodwell JR (2014). The expression of antibiotic resistance genes in antibiotic-producing bacteria. Molecular microbiology, 93(3): 391-402. DOI: https://doi.org/10.1111/mmi.12689.

Mathews MA, Tang HL and Blair DF (1998). Domain analysis of the flim protein of escherichia coli. Journal of bacteriology, 180 (21): 5580-5590. DOI: https://doi.org/10.1128/JB.180.21.5580-5590.1998.

McCool C, Townsend M, Wolfe S, Simpson M, Olm T, Jayawardhana GA, and Carney JV (1988). Prevalence of bovine veneral disease in the victoria river district of the northern territory: Likely economic effects and practicable control measures. Australian veterinary journal, 65 (5): 153-156. DOI:https://doi.org/ 10.1111/j.1751-0813.1988.tb14445.x .

McDowell MA, Marcoux J, McVicker G, Johnson S, Fong YH, Stevens R, Bowman LA, Degiacomi MT, Yan J, Wise A et al (2016). Characterisation of shigella spa33 and thermotoga flim/n reveals a new model for c-ring assembly in t3ss. Molecular microbiology, 99 (4): 749-766. DOI: https://doi.org/ 10.1111/mmi.13267.

Mikheil DM, Shippy DC, Eakley NM, Okwumabua OE and Fadl AA (2012). Deletion of gene encoding methyltransferase (gidb) confers high-level antimicrobial resistance in salmonella. The Journal of antibiotics, 65 (4): 185-192. DOI:https://doi.org/ 10.1038/ja.2012.5 .

Morimoto YV, and Minamino T (2014). Structure and function of the bi-directional bacterial flagellar motor. Biomolecules, 4 (1): 217-234. DOI: https://doi.org/10.3390/biom4010217.

Morrell EL, Barbeito CG, Odeon C, Gimeno EJ, and Campero CM (2011). Histopathological, immunohistochemical, lectinhistochemical and molecular findings in spontaneous bovine abortions by campylobacter fetus. Reproduction in Domestic Animals, 46(2): 309-315. DOI: https://doi.org/10.1111/j.1439-0531.2010.01668.x.

Nikaido H, and Pagès JM (2012). Broad-specificity efflux pumps and their role in multidrug resistance of gram-negative bacteria. FEMS microbiology reviews, 36(2): 340-363. DOI: https://doi.org/10.1111/j.1574-6976.2011.00290.x .

Nishi J, Sheikh J, Mizuguchi K, Luisi B, Burland V, Boutin A, Rose DJ, Blattner FR, and Nataro JP (2003). The export of coat protein from enteroaggregative escherichia coli by a specific atp-binding cassette transporter system. Journal of Biological Chemistry, 278(46): 45680-45689. DOI: https://doi.org/10.1074/jbc.M306413200.

Nishimura K, Johansen SK, Inaoka T, Hosaka T, Tokuyama S, Tahara Y, Okamoto S, Kawamura F, Douthwaite S and Ochi K (2007). Identification of the rsmg methyltransferase target as 16s rrna nucleotide g527 and characterization of bacillus subtilis rsmg mutants. Journal of bacteriology, 189 (16): 6068-6073. DOI: https://doi.org/ 10.1128/JB.00558-07.

No HK, Park NY, Lee SH, and Meyers SP (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. International journal of food microbiology, 74 (1-2): 65-72. DOI: https://doi.org/10.1016/s0168-1605(01)00717-6.

Okamoto S, Tamaru A, Nakajima C, Nishimura K, Tanaka, Tokuyama S, Suzuki Y, and Ochi K (2007). Loss of a conserved 7-methylguanosine modification in 16s rrna confers low-level streptomycin resistance in bacteria. Molecular microbiology, 63(4): 1096-1106. DOI: https://doi.org/ 10.1111/j.1365-2958.2006.05585.x .

Olkkola S, Kovanen S, Roine J, Hänninen ML, Hielm-Björkman A, and Kvisto R (2015). Population genetics and antimicrobial susceptibility of canine campylobacter isolates collected before and after a raw feeding experiment. PloS one, 10(7): e0132660. DOI: https://doi.org/10.1371/journal.pone.0132660.

Park SY, Lowder B, Bilwes AM, Blair DF and Crane BR (2006). Structure of flim provides insight into assembly of the switch complex in the bacterial flagella motor. Proceedings of the national academy of sciences, 103 (32): 11886-11891. DOI:https://doi.org/ 10.1073/pnas.0602811103 .

Paul K, Gonzalez-Bonet G, Bilwes AM, Crane BR and Blair D (2011). Architecture of the flagellar rotor. The EMBO journal, 30(14): 2962-2971. DOI:https://doi.org/ 10.1038/emboj.2011.188.

Pei Z and Blaser MJ (1990). Pathogenesis of campylobacter fetus infections. Role of surface array proteins in virulence in a mouse model. The Journal of clinical investigation 85 (4): 1036-1043. DOI:https://doi.org/ 10.1172/JCI114533 .

Perera VN, Nachamkin I, Ung H, Patterson JH, McConville MJ, Coloe PJ and Fry BN (2007). Molecular mimicry in campylobacter jejuni: Role of the lipo-oligosaccharide core oligosaccharide in inducing anti-ganglioside antibodies. FEMS Immunology & Medical Microbiology, 50 (1): 27-36. DOI:https://doi.org/ 10.1111/j.1574-695X.2007.00225.x .

Pumbwe L, and Piddock LJ (2002). Identification and molecular characterisation of cmeb, a campylobacter jejuni multidrug efflux pump. FEMS Microbiology Letters, 206 (2): 185-189. DOI: https://doi.org/10.1111/j.1574-6968.2002.tb11007.x.

Schulze F, Bagon A, Müller W, and Hotzel H (2006). Identification of campylobacter fetus subspecies by phenotypic differentiation and pcr. Journal of clinical microbiology, 44 (6): 2019-2024. DOI:https://doi.org/ 10.1128/JCM.02566-05.

To cite this paper: Tshipamba ME, Lubanza N and Mwanza M (2021). Genome Analysis of Antimicrobial Resistance Genes and Virulence Factors in Multidrug-Resistant *Campylobacter fetus* Subspecies Isolated from Sheath Wash. *World Vet. J.*, 10 (4): 465-480. DOI: https://dx.doi.org/10.54203/scil.2020.wvj57

Shippy DC, and Fadl AA (2014). Trna modification enzymes gida and mnme: Potential role in virulence of bacterial pathogens. International journal of molecular sciences, 15(10): 18267-18280. DOI:https://doi.org/ 10.3390/ijms151018267 .

Sircar R, Borbat PP, Lynch MJ, Bhatnagar J, Beyersdorf MS, Halkides CJ, Freed JH, and Crane BR (2015). Assembly states of flim and flig within the flagellar switch complex. Journal of molecular biology, 427 (4): 867-886. DOI:https://doi.org/ 10.1016/j.jmb.2014.12.009.

Sopwith W, Birtles A, Matthews M, Fox A, Gee S, Painter M, Regan M, Syed Q and Bolton E (2006). Campylobacter jejuni multilocus sequence types in humans, northwest england, 2003–2004. Emerging infectious diseases, 12 (10): 1500. DOI:https://doi.org/ 10.3201/eid1210.060048.

Szurmant H, Bunn MW, Cannistraro VJ, and Ordal GW (2003). Bacillus subtilis hydrolyzes chey-p at the location of its action, the flagellar switch. Journal of Biological Chemistry, 278 (49): 48611-48616. DOI:https://doi.org/ 10.1074/jbc.M306180200.

Taylor D, Ng L, and Lior H (1985). Susceptibility of campylobacter species to nalidixic acid, enoxacin, and other DNA gyrase inhibitors. Antimicrobial agents and chemotherapy, 28 (5): 708-710. DOI:https://doi.org/ 10.1128/aac.28.5.708.

Terahara N, Krulwich TA, and Ito M (2008). Mutations alter the sodium versus proton use of a bacillus clausii flagellar motor and confer dual ion use on bacillus subtilis motors. Proceedings of the national academy of sciences, 105(38): 14359-14364. DOI: https://doi.org/ 10.1073/pnas.0802106105 .

Thakur S, Zhao S, McDermott PF, Harbottle H, Abbott J, English L, Gebreyes WA and White DG (2010). Antimicrobial resistance, virulence, and genotypic profile comparison of campylobacter jejuni and campylobacter coli isolated from humans and retail meats. Foodborne pathogens and disease, 7 (7): 835-844. DOI:https://doi.org/ 10.1089/fpd.2009.0487 .

Tikhonova EB, Devroy VK, Lau SYand Zgurskaya HI (2007). Reconstitution of the escherichia coli macrolide transporter: The periplasmic membrane fusion protein maca stimulates the atpase activity of macb. Molecular microbiology, 63(3): 895-910. DOI: https://doi.org/10.1111/j.1365-2958.2006.05549.x.

Tremblay C, and Gaudreau C (1998). Antimicrobial susceptibility testing of 59 strains of campylobacter fetus subsp. Fetus. Antimicrobial agents and chemotherapy, 42(7): 1847-1849. DOI: https://doi.org/ 10.1128/AAC.42.7.1847 .

Tremblay C, Gaudreau C and Lorange M (2003). Epidemiology and antimicrobial susceptibilities of 111 campylobacter fetus subsp. Fetus strains isolated in quebec, canada, from 1983 to 2000. Journal of clinical microbiology, 41 (1): 463-466. DOI: https://doi.org/ 10.1128/jcm.41.1.463-466.2003.

Truyers I, Luke T, Wilson D, and Sargison N (2014). Diagnosis and management of venereal campylobacteriosis in beef cattle. BMC veterinary research, 10(1): 280. DOI: https://doi.org/10.1186/s12917-014-0280-x.

van der Graaf–van Bloois L, Miller WG, Yee E, Gorkiewicz G, Forbes KJ, Zomer AL, Wagenaar JA and Duim B (2016). Campylobacter fetus subspecies contain conserved type iv secretion systems on multiple genomic islands and plasmids. PloS one, 11(4). DOI: https://doi.org/10.1371/journal.pone.0152832.

Vasquez L, Ball L, Bennett B, Rupp G, Ellis R, Olson JD, and Huffman MH (1983). Bovine genital campylobacteriosis (vibriosis): Vaccination of experimentally infected bulls. American journal of veterinary research, 44 (8): 1553-1557. Available at: https://europepmc.org/article/med/6194723.

Wangroongsarb P, Jittaprasatsin C, Suwannasing S, Suthivarakom K, and Khamthalang T (2011). Identification of genus campylobacter and four enteropathogenic campylobacter species by pcr. Journal of Tropical Medicine and Parasitology, 34: 17-29. Available at: www.ptat.thaigov.net.

Washington J, and Wood G (1995). Antimicroibial susceptibility tests: Dilution and disc diffusion methods. Manual of clinical microbiology.PP.1327-1331. Available at: https://pubmed.ncbi.nlm.nih.gov/5325707/.

Wieczorek K and Osek J (2013). Antimicrobial resistance mechanisms among campylobacter. BioMed research international, Article ID 340605, pp. 1-12. DOI: https://doi.org/10.1155/2013/340605.

Wieczorek K, Wołkowicz T and Osek J (2018). Antimicrobial resistance and virulence-associated traits of campylobacter jejuni isolated from poultry food chain and humans with diarrhea. Frontiers in microbiology, 9: 1508. DOI: https://doi.org/10.3389/fmicb.2018.01508.

Worby CA, Mattoo S, Kruger RP, Corbeil LB, Koller A, Zekarias B, Lazar C, and Dixon JE (2009). The fic domain: Regulation of cell signaling by adenylylation. Molecular cell, 34 (1): 93-103. DOI: https://doi.org/ 10.1016/j.molcel.2009.03.008 .

Yan M, Sahin O, Lin J, and Zhang Q (2006). Role of the cmeabc efflux pump in the emergence of fluoroquinolone-resistant campylobacter under selection pressure. Journal of Antimicrobial Chemotherapy, 58 (6): 1154-1159. DOI: https://doi.org/ 10.1093/jac/dkl412 .

Yuan J, Branch RW, Hosu BG, and Berg HC (2012). Adaptation at the output of the chemotaxis signalling pathway. Nature, 484 (7393): 233-236. DOI: https://doi.org/ 10.1038/nature10964.

To cite this paper: Tshipamba ME, Lubanza N and Mwanza M (2021). Genome Analysis of Antimicrobial Resistance Genes and Virulence Factors in Multidrug-Resistant *Campylobacter fetus* Subspecies Isolated from Sheath Wash. *World Vet. J.*, 10 (4): 465-480. DOI: https://dx.doi.org/10.54203/scil.2020.wvj57

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 481-491, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj58

Impact of In-Ovo Injection of Folic Acid and Glucose on Hatchability, and Post-hatching Performance of Broiler Chickens

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ORIGINAL ARTICLE
pii: S232245682000058-10
Received: 11 Oct 2020
Accepted: 26 Nov 2020

ABSTRACT

The present study was designed to investigate the impact of in-ovo injection of folic acid and glucose on hatching eggs from 55 weeks old broiler breeders. A total number of 900 hatching eggs were collected from Arbor Acres broiler breeders, then, eggs were divided into 6 groups including 1) Negative Control (non-injected, NC), 2) Dry Punch Control (pricked without injecting any solution, DPC), 3) Positive Control (eggs were injected with 0.5 mL normal saline, PC), 4) Folic Acid group (eggs were injected with 0.2 mg/ egg folic acid, FA), 5) Glucose group (eggs were injected with 125 mg/ egg glucose, Glu), and 6) Folic Acid with Glucose group (eggs were injected with 0.2 mg folic acid with 125 mg/ egg glucose, FA+Glu). Each treatment was divided into five replicates of 30 eggs each. Eggs were injected into the albumen under the air sac. After in-ovo injection, the eggs were stored for four days before hatching. After hatching, the chickens were reared in groups according to the treatments. All treatments were divided into 10 replications of 9 chickens in each. In-ovo injection with folic acid decreased the albumen pH significantly to 9.19 after 4 days of injection, while the negative control was 9.43. Hatching quality was severely affected by all in-ovo injection treatments, but no significant differences were found between the treatment groups concerning the hatchability of fertile eggs. Injection treatments had no significant effect on the growth rate or the production number in any of the weeks. Injection of folic acid and (FA+Glu) significantly increased chickens' body weight at two and four weeks of age. Also, the dressing percentage when using folic acid and (FA+Glu) was significantly increased to 72.1% and 72.5%, respectively, compared to the positive control group (68.3%). In conclusion, our data suggested that in-ovo injection with a mixture of folic acid and glucose (0.2 mg folic acid+ 125 mg/ egg glucose) could be used to enhance carcass characteristics. Further studies should be conducted to find the effects of in-ovo injection folic acid and glucose on different incubation days and at different sites of injection.

Keywords: Broilers, Folic Acid, Glucose, Hatchability, In- Ovo injection, Old breeders, Post-hatch

INTRODUCTION

A range of healthy chickens with high growth ability and viability is very important to the poultry industry. The age of broiler breeder is one of the most important factors affecting hatchability and chickens' quality. The eggs of old breeders have a lower fertility and hatchability than those of the young breeder (Elibol et al., 2002; Vieira et al., 2005; Iqbal et al., 2016). Previous studies showed that the low incidence of hatchability in eggs of older breeders was due to many contributing factors, such as a poorer eggshell quality due to the larger surface (Bennett, 1992), and the deterioration in the albumen quality (Tona et al., 2004).

Older breeders' eggs were associated with larger size and thinner shells with higher porosity, which had been associated with a higher percentage of egg moisture loss during incubation. This increased mortality in the early phase of embryogenesis due to dehydration (Peebles et al., 2001), led to poor hatching quality (Narushin and Romanov, 2002). With increasing age of the breed, the albumen pH increased at oviposition, which may be due to the faster release of CO_2 through the eggshell due to a higher eggshell porosity (Meijerhof, 1994). There seems to be an association between albumen pH before incubation and viability of embryo during early phase of embryogenesis, an albumen pH of 8.2 appears to be optimal for embryogenesis (Reijrink et al., 2008). Lapao et al. (1999) found that most of the rise in albumen pH occurred during the first four days of storage.

At hatching, older breeders were associated with an increase in day-old chicken weight and a decrease in chick quality (Koppenol et al., 2015). Also, the chickens from an old breeder were less feed efficient (higher FCR) than the chickens from the young breeder (Ulmer-Franco et al., 2010).

In recent years, much attention has been paid to the field of in-ovo injection. In-ovo technology is a method that can potentially enhance the hatchability and post-hatch performance of broiler chickens (Zhang et al., 2019). With In-ovo technology, various substances were injection into the air chamber or directly into the egg (Kucharska-Gaca et al., 2017). Various factors influenced the effectiveness of in-ovo injection, including the injection site, the stage of development of the embryo, the level of contamination in the hatchery, and the in-ovo injection equipment (Ricks et al.,

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1999). Various nutrients were examined for effectiveness in improving embryonic development, hatchability, and post-hatch performance including carbohydrates, amino acids, peptides, electrolytes and vitamins (Kucharska-Gaca et al., 2017). The effectiveness of in-ovo injection methodology on hatching quality is still under study due to the optimum site and time of injection and the suitable volume and nutrient of injected solution (Tasharofi et al., 2018). Folic acid is a critical vitamin during reproduction and the hatching requirement is higher compared to egg production (Vieira, 2007). Folate was essential for embryonic development, regardless of whether in-ovo feeding of folic acid could influence the growth performance (Li et al., 2016). Parnian et al. (2019) found that in-ovo injection of folic acid improvement the body weight of the chickens.

Embryonic blood plasma glucose concentrations reduced with increasing age of the breeder (Christensen et al., 1996). Glucose or dextrose is a simple sugar that is used by cells as an essential source of energy and a metabolic intermediate, and is the most important source of energy for embryonic development (Starck and Ricklefs, 1998). Therefore, the aim of this work was to investigate if the in-ovo injection of folic acid and glucose into eggs of old broiler breeders could be useful for improving hatchability and post-hatching productivity.

MATERIALS AND METHODS

Ethical approval

The present study has been conducted in accordance with the guidelines of the Ethics Committee of the Faculty of Agriculture of Cairo University. The experimental fieldwork was carried out in the Agriculture Experimental Station of the Faculty of Agriculture- Cairo University in Giza, Egypt.

Structure

A total number of 900 hatching eggs were collected from Arbor Acres broiler parents at 55 weeks of age. The eggs were prepared at El Ahlia hatchery, El Ahlia Poultry Company, Tanta city, Egypt. . The eggs were randomly divided into six treatments group (150 eggs each) four days before incubation; each group was divided into five replicates (30 eggs each). The treatments consisted of (1) Negative Control, in which no eggs were injected (NC), (2) Dry Punch Control, in which shell and shell membranes were pricked without injecting any solution (DPC), (3) Positive Control, in which eggs were injected with 0.5 mL normal saline (PC), (4) Folic Acid group, in which eggs were injected with 0.5 mL normal saline containing 0.2 mg folic acid (FA), (5) Glucose group, in which eggs were injected with 0.5 mL normal saline containing 125 mg glucose (Glu) and (6) Folic acid with Glucose group, in which eggs were injected with 0.5 mL normal saline contain 0.2 mg folic acid and 125 mg glucose (FA + Glu). The eggs were injected in the albumen under the air sac.

In-ovo injection

At the time of injection, the large ends of the freshly laid eggs were cleaned with ethyl alcohol (70%) and were penetrated by a pin, taking care not to injure the outer egg membrane. The solutions were injected into the albumen (0.5 mL/egg) at a depth of 12 mm (Akhlaghi et al., 2013), via a disposable syringe (1 ml syringe). The punched eggs were sealed with melted paraffin wax, then were given an identification number and stored for four days (18°C and 75% relative humidity (RH)) until the end of incubation. Two eggs from each replicate were randomly selected to evaluate the albumen pH before injection and after two to four days of injection. The pH of egg albumen was measured using a pH meter at room temperature. The measured pH of all solutions was 7.23, 3.98, 4.08, and 3.46 for sodium chloride, folic acid, glucose and the combination of folic acid and glucose, respectively.

General Management

All eggs were set in an incubator at average temperature of 37.5°C, and relative humidity of 56.5%. The eggs were turned hourly. At 444 hours of incubation, the eggs were transferred to a hatcher at an average temperature of 36.6°C, and a relative humidity of 61.2%. After hatching, all unhatched eggs were examined to calculate the embryonic mortality, which was classified as unfertile and early mortality (1-12 days) and late embryo mortality (13-21days). Total embryonic mortality was determined as the amount of the all dead embryos. Other calculated parameters consisted of fertility percentage, hatchability of total eggs and hatchability of fertile eggs. Fertility percentage was calculated as (number of fertile eggs divided by number of total eggs) multiply by 100. Hatchability of total eggs calculated as (number of hatched chicks divided by number of total eggs) multiply by 100. Hatchability of fertile eggs calculated as (number of hatched chicks divided by number of fertile eggs) multiply by 100

After hatching, 540 chickens were then transported to the farm of the Faculty of Agriculture Cairo University. Each treatment was divided into ten replicates (Nine chickens each). Each replicate was housed separately in several cages in semi-closed system house.

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Feed and water were available *ad libitum*. Chickens received a commercial broiler starter diet with 3025 kcal of ME/kg and 23% Crude Protein (CP) from first to 14th day. At the age of 14 to 28 days, the chickens were fed a grower diet with 3150 kcal of ME/kg and 21% CP. From 28 days to 35 days of age, the chickens were fed a finisher diet containing 3200 kcal of ME/kg and 19% CP. Starter feed was provided as crumbles, and subsequent feeds were provided as pellets.

Measurements

The live body weights of chickens were recorded individually weekly from day one to five weeks of age. The weekly feed intake per replicate (g) was calculated and then the feed intake for chickens was calculated as the feed intake for each replicate within a certain time interval divided by the number of chickens in the same replicate during the same time period. Then it was divided into seven parts to calculate the daily feed intake. Feed conversion ratio calculated as the average feed consumption (g) for each replicate with a time period divided by the average body weight gain (g) for the same replicate over the same period. Dead chickens were weighed to include their weight in the feed conversion estimates.

Growth rate (GR) was calculated according to the following:

GR = W2 –W1 / [(W1+W2) / 2]

Where, W1 refers to body weight at the beginning of a certain week and W2 to the body weight at the end of the same week.

The mortality rate was recorded daily and calculated as percentage for each replicate.

Production number: (Average kilograms of growth per day x (100 – mortality %) / FCR) x 100

To determine the carcass quality, ten chickens were randomly selected from each treatment at five weeks of age, weighed individually and slaughtered after eight hours fasting. After the blood was drawn, they were defeathered, processed, and eviscerated. Carcass yield (dressing, breast meat, and hind meat) was determined as a percent of the living body weight. Giblets (liver, heart, and gizzard), spleen, thymus, and bursa of fabricius were obtained.

Statistical analysis

The data were subjected to a one-way Analysis of Variance using the general linear model method of XLSTAT (2014) version 2014.5.03. In-ovo injection treatments were the main factor. Percentage data were subjected to arcsine transformation prior to the analysis. The mean values were compared using Duncan's multiple range test (Duncan, 1955) if there was a significant difference ($p \leq 0.05$). The model used was as follows: $Yjk = \mu + Hj + Ejk$

Where: Yjk = individual observation; μ = Overall mean; Hj = Effect of in-ovo injection treatments (j = 1, 2, 3, 4, 5, 6); Ejk = Residual error.

RESULTS AND DISCUSSION

Albumen pH

The effect of in-ovo injection treatments on albumen pH is presented in table 1. No significant differences in egg albumen pH were observed after two days post injection between any treatment groups. However, in all treatments, the albumen pH appeared to be too high when compared to previous researches. Akhlaghi et al. (2013) reported that the non-injected control was 8.67 after two days was 9.07 after four days of treatment. In our experiment, the albumen pH was 8.98 after two days and was 9.43 after four days. This may be due to the transport of eggs from the parents' farm to hatchery conditions was unsuitable. Four days after the injection, only folic acid could cause a significant ($p = 0.037$) decrease in the albumen pH from 9.43 in the negative control group to 9.19. This result may be due to the acidity of folic acid, folate pH < 5 (Combs and McClung, 2016), and the pH of the solution injected into eggs was 3.98. The recent results partly agreed with Ebrahimi et al. (2012), who found no differences in albumen pH due to injection of bicarbonate or phosphate buffer solutions injected into the albumen. Ebrahimi et al. (2012) stated that the volume of the buffer solutions, which had to optimally lower the albumen pH, was too large, which could potentially be detrimental to the embryo.

Embryonic mortality percentage

The effects of in-ovo injection treatments on embryonic mortality and hatchability are presented in table 2. All injection treatments increased early, late, and total embryonic mortality compared to the negative control, but the differences were not significant.

Fertility and Hatchability

There were no significant differences in the hatchability of fertile eggs between any of the injection groups. All treatments decreased either fertility or the hatchability of fertile eggs (Table 2). Hatchability of total eggs was also adversely affected by in-ovo injection, but the differences were only significant between the negative control and folic

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acid, glucose and the combination of folic acid and glucose. Folic acid, glucose and the combination of folic acid and glucose injection decreased the hatchability of fertile eggs to 76.4 %, 75.4 %, and 73.9%, respectively, compared to 91.0 % in the negative control. The results of the present study indicated that the albumen pH plays a minimal role in decreasing the hatchability. This is because the hatchability of the control eggs was high in eggs four days after injection where the albumen pH was high.

Table 1. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on albumen pH (means ± Standard Error)

Item	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
2 days post injection	8.98 ± 0.05	9.01 ± 0.05	8.94 ± 0.05	8.97 ± 0.05	8.94 ± 0.05	8.88 ± 0.05	0.4741
4 days post injection	9.43 ± 0.05[a]	9.35 ± 0.05[a]	9.36 ± 0.05[a]	9.19 ± 0.05[b]	9.36 ± 0.05[a]	9.31 ± 0.05[ab]	0.0367

[a,b;] Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

Table 2. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on embryonic mortality and hatchability (means ± Standard Error)

Item	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Non fertile (%)	12.1 ± 2.6	15.7 ± 2.6	18.6 ± 2.6	18.6 ± 2.6	18.6 ± 2.6	22.9 ± 2.6	0.1368
Early mortality (%)	2.9 ± 2.2	2.9 ± 2.2	9.3 ± 2.2	7.9 ± 2.2	10.0 ± 2.2	6.4 ± 2.2	0.1128
Late mortality (%)	5.0 ± 2.6	13.6 ± 2.6	8.6 ± 2.6	11.4 ± 2.6	10.0 ± 2.6	13.6 ± 2.6	0.1867
Total mortality (%)	7.9 ± 3.4	16.4 ± 3.4	17.9 ± 3.4	19.3 ± 3.4	20.0 ± 3.4	20.0 ± 3.4	0.1408
Apparent fertility (%)	87.9 ± 2.6	84.3 ± 2.6	81.4 ± 2.6	81.4 ± 2.6	81.4 ± 2.6	77.1 ± 2.6	0.1368
Hatchability of fertile eggs (%)	91.0 ± 4.1	80.3 ± 4.1	78.0 ± 4.1	76.4 ± 4.1	75.4 ± 4.1	73.9 ± 4.1	0.0811
Hatchability of total eggs (%)	80.0 ± 4.2[a]	67.9 ± 4.2[ab]	63.6 ± 4.2[b]	62.1 ± 4.2[b]	61.4 ± 4.2[b]	57.1 ± 4.2[b]	0.0144

[a,b;] Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

Ebrahimi et al. (2012), found that in-ovo injection of bicarbonate or phosphate buffer solutions before incubation decreased the hatchability of fertile eggs to 32.0% and 8.3% respectively, while the hatchability of fertile eggs in the controls was 87.5%. In the present results, injection of glucose decreased the hatchability of fertile eggs, but may not be significant due to the organogenesis of important segments of the chicken embryo occur in the first week of embryonic development. Based on this information, in-ovo injection of glucose prior to this critical phase can be an effective stimulator for optimal organ development (Bellairs and Osmond, 2005). Ebrahimi et al. (2012) stated that decreases in hatchability of fertile eggs were due to the injected active ingredients which adversely affect the environment for the embryo. The pH or osmolality of the solutions could adversely affect the surrounding microenvironment of the early embryo. This could rationalize the numerically increased apparent fertility of the negative control in the results of the present study. The results of the present study were in part with Zhai et al. (2011b), who reported that the hatchability of fertile eggs was lower in eggs injected with glucose, fructose, maltose, sucrose, or dextrin, compared to the control group without injected, dry punch, and saline-injected control groups. However, Zhai et al. (2011a) stated that the hatchability of fertile eggs was not impaired by any injection treatment with Glucose, sucrose, maltose, or dextrin. Salmanzadeh et al. (2012) found that in-ovo injection with 75 mg or 100 mg glucose, dissolved in 0.5 mL deionized water after seven days of incubation into the albumen led to less hatchability than the negative control treatment. They stated that the decrease in hatchability could be due to the injection into the albumin under the air sac, which stopped the respiration in the developing embryo. Tasharofi et al. (2018) explained the decrease in hatchability in eggs injected with dextrose due to an overload of the energy metabolism in embryos due to the injection of high carbohydrate levels, which adversely affected hatchability. However, Zhang et al. (2016) found that the hatchability of eggs injected with glucose was very close to that of the negative control group. The results of the present research did not agree with Li et al. (2016) and Liu et al. (2016) found that in-ovo injection of 100 µg or 150 µg of folic acid (the injection volume in each egg was 0.1 ml) into the yolk sac after 11 days at embryonic age increased the hatchability compared to the control treatment (0 µg of folic acid). This may be attributed to the time difference or to the injection site.

On the other hand, Nouri et al. (2018) revealed that there was no significant difference in hatchability for eggs in-ovo, which had been injected with folic acid (40, 80, and 120 µg) in albumen on day seven of incubation, compared to the negative control group and positive group (in-ovo injection of sterile water, 40 µg). Robel (2002) stated that hatchability in turkey eggs injected with folic acid at 25-day of incubation was not significantly affected compared to the

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negative control.

The discrepancy in the results may be due to the timing of the injection. The eggs were injected before incubation, so it was more susceptible to contamination. In-ovo injection, especially in early embryonic life, did not improve hatchability. It seems that in-ovo injection at the beginning of embryonic development could damage the internal environment of the egg and also have negative effects on hatchability (Salmanzadeh et al., 2012). The injection volume can also be too large. Zhai et al. (2011c) stated that the hatchability of fertile eggs was negatively related to the injection volume. Therefore, the in-ovo injection volume should be limited to prevent the embryo from becoming excessive hydrated and a subsequent decreasing hatchability. The effectiveness of in-ovo injection on hatchability is still not clear enough. The optimal site and time of injection as well as the volume of the injected solution and the appropriate nutrient have yet to be determined (Tasharofi et al., 2018).

Post-hatch performance

The in-ovo injection had no significant effect ($p > 0.05$) on the body weight of chicken at hatching at one, three and five weeks of age (Table 3). However, a significant effect of the in-ovo injection treatment on body weight of the chicken at two and four weeks of age was observed. The body weight of chickens in the group injected with the combination of FA and Glu was the highest compared to the other injection treatments. However, the difference was not significant compared to the negative control. In-ovo injection with FA, Glu, and FA + Glu had no significant influence on the body weight of the chickens at hatching.

Table 3. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on body weight (g) (means ± Standard Error) of broiler chickens

Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
One day	47.5 ± 0.4	47.7 ± 0.4	47.7 ± 0.4	48.6 ± 0.4	48.5 ± 0.4	48.2 ± 0.4	0.1671
Week 1	173 ± 1.9	173 ± 2.0	170 ± 2.1	168 ± 2.1	168 ± 2.1	171 ± 2.3	0.2760
Week 2	405 ± 7.3^{ab}	387 ± 7.8^{b}	406 ± 8.2^{ab}	394 ± 7.9^{b}	393 ± 8.3^{b}	425 ± 8.7^{a}	0.0233
Week 3	892 ± 13.8	854 ± 14.7	864 ± 15.4	857 ± 14.9	855 ± 15.6	908 ± 16.3	0.0587
Week 4	1527 ± 22.2^{a}	1447 ± 23.6^{b}	1456 ± 25.0^{b}	1461 ± 24.0^{b}	1454 ± 25.1^{b}	1537 ± 26.3^{a}	0.0184
Week 5	2177 ± 29.3	2122 ± 31.5	2100 ± 33.8	2103 ± 31.9	2123 ± 33.4	2157 ± 35.2	0.4381

$^{a,b;}$ Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

Liu et al. (2016) found that the in-ovo injection of 150 µg FA into the yolk sac after 11 days of embryonic age significantly increased the body weight of the one-day-old chickens. Liu et al. (2016) also reported that folic acid injection might up-regulate IGF2 expression, and they reported the genomic correlation between chickens' body weight and plasma IGF2 levels. The body weight of chickens in the (FA + Glu) group was significantly higher after two and four weeks than in the DPC group. These results partly agreed with the results reported by Nouri et al. (2018). They stated that body weight on day 21 was significantly improved in chickens that had been injected in-ovo with 120 µg folic acid in albumen on the seventh day of incubation. Li et al. (2016) reported that body weight was significantly increased by the age of 42 days. Salmanzadeh (2012), Kanagaraju and Rathnapraba (2019) also indicated that in-ovo injection with 0.5 ml of 25 % glucose (on day-7 of incubation in the albumen and on day-18 of incubation in the amnion, respectively) improved body weight. Zhai et al. (2011c) found that injected chicken embryo in the amnion on day 19 of incubation with 0.1, 0.4, 0.7, or 1.0 mL of various carbohydrates (Glucose, fructose, sucrose, maltose, and dextrin) associated with chicken's body weight. The recent results indicated that the combination of folic acid and glucose played an important role in poultry growth performance. An increases in the body weight of in-ovo-injected broiler embryos with (FA + Glu) could be viewed as a consequence of the improvement in enteric development and a subsequent enhancement in nutrient absorption (Zhai et al. 2011c), or as a good nutrient for better use of the energy by the embryos Uni et al. (2005).

Kanagaraju and Rathnapraba (2019) also stated that the in-ovo injection of 0.5 ml of 25 % glucose into the amnion on day 18 of incubation significantly improved duodenal, jejunal and ileal histomorphology (villi height, width, crypt depth, and villi surface area) of broilers, which led to the enhancement of digestion and absorption of nutrients. On other hand, Zhang et al. (2016) indicated that the individual injection of 0.4 mL glucose (25 mg) on day 18 of the incubation did not affect hatching weight and growth performance of the chickens during the first week of post-hatching. The difference between Zhang et al. (2016) results and previous studies was related to the carbohydrate type, injection dose, genetic strain, and egg size.

The in-ovo injection had no significant effect on the daily feed intake at the age of one, two, three, and five weeks, the average daily feed intake and the total feed intake (Table 4). The only significant difference was observed at four

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weeks of age between DPC treatment and (FA + Glu) treatment, which consumed more food than the first one. The results of the present study were agreed with Li et al. (2016), who stated that there were no significant differences in the average daily feed intake between control and folic acid injected treatment. However, recent results did not agree with Nouri et al. (2018), they found that the feed intake of in-ovo chickens that were injected with folic acid was significantly increased compared to the control group. Salmanzadeh (2012) reported that the in-ovo injection of 0.5 ml of 25 % glucose into the albumen on day seven of incubation did not affect feed intake. Kanagaraju and Rathnapraba (2019) found that the treatment of in-ovo injection of 0.5 ml of 25 % glucose on day 18 of incubation into the amnion significantly increased feed intake compared to the negative control and positive groups. This discrepancy in the results might be due to the differences in injection time. In-ovo injection (shortly before hatching) with carbohydrates enabled the early adaptation of the avian gastrointestinal tract during embryonic development (Kucharska-Gaca et al., 2017) and adapted them to their new diet after hatching (Cardeal et al., 2015) in more feed intake.

Table 5 present that the in-ovo injection had no significant effects on the Feed Conversion Ratio (FCR) in any of the age group examined. In general, in-ovo injection with FA improved FCR insignificantly compared to (Glu) and (FA + Glu) treatments. The present results were partly agreed with Li et al. (2016), who found that injection treatment with 100 and 150 µg folic acid significantly improved FCR. However, Nouri et al. (2018) stated that the FCR in broilers that had been injected with 120 µg folic acid in albumen on day 7 of incubation was significantly improved on 0–42 days compared to the control treatment. On the other hand, Salmanzadeh (2012), Kanagaraju and Rathnapraba (2019) found that chickens that were injected with glucose in-ovo had a higher FCR than chickens hatched from the control group and the positive group.

No significant effect on weekly chickens' mortality was observed (Table 7). Injection treatments had no significant effect ($p>0.05$) on the growth rate in all weeks (Table 6) and on the production number (Table 8). In-ovo injection of folic acid numerically increased the production number compared to the positive control.

Table 4. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on average daily feed intake (g) (means ± Standard Error) of broiler chickens.

Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Week 1	23.3 ± 0.7	24.0 ± 0.87	23.3 ± 0.9	23.0 ± 0.8	22.9 ± 0.8	23.9 ± 0.9	0.9194
Week 2	44.6 ± 2.3	42.5 ± 2.6	45.7 ± 2.6	41.4 ± 2.5	42.6 ± 2.5	44.7 ± 2.6	0.8282
Week 3	94.6 ± 2.3	90.0 ± 2.7	87.7 ± 2.7	87.0 ± 2.5	89.1 ± 2.5	89.8 ± 2.7	0.2927
Week 4	133.9 ± 3.7^{a}	116.5 ± 4.4^{b}	125.5 ± 4.4^{ab}	124.3 ± 4.1^{ab}	128.3 ± 4.1^{ab}	135.2 ± 4.4^{a}	0.0346
Week 5	156.9 ± 4.7	147.8 ± 5.6	139.7 ± 5.6	145.3 ± 5.2	156.1 ± 5.2	151.6 ± 5.6	0.1802
Average daily feed intake	90.7 ± 1.8	84.1 ± 2.2	84.4 ± 2.2	87.8 ± 2.0	84.2 ± 2.0	89.1 ± 2.2	0.0934
Total feed intake	3173 ± 64	2945 ± 76	2954 ± 76	2947 ± 71	3073 ± 71	3117 ± 76	0.0934

$^{a,b;}$ Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

Table 5. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on feed conversion (means ± Standard Error) of broiler chickens.

Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Week 1	1.31 ± 0.04	1.33 ± 0.04	1.34 ± 0.04	1.34 ± 0.04	1.35 ± 0.04	1.37 ± 0.04	0.9383
Week 2	1.31 ± 0.04	1.32 ± 0.04	1.33 ± 0.04	1.29 ± 0.04	1.35 ± 0.04	1.24 ± 0.04	0.5759
Week 3	1.40 ± 0.02	1.33 ± 0.03	1.33 ± 0.03	1.32 ± 0.03	1.35 ± 0.03	1.31 ± 0.03	0.1547
Week 4	1.46 ± 0.04	1.37 ± 0.05	1.51 ± 0.05	1.45 ± 0.04	1.51 ± 0.04	1.50 ± 0.04	0.3017
Week 5	1.67 ± 0.05	1.56 ± 0.06	1.52 ± 0.06	1.57 ± 0.06	1.62 ± 0.06	1.66 ± 0.06	0.4033
0 – 35 days	1.49 ± 0.03	1.42 ± 0.03	1.50 ± 0.03	1.42 ± 0.03	1.49 ± 0.03	1.51 ± 0.03	0.1419

No significant differences were observed. SE: Standard Error, FA: Folic Acid, Glu: Glucose

Table 6. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on growth rate (means ± Standard Error) of broiler chickens.

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Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Week 1	113.6 ± 1.2	113.6 ± 1.4	112.0 ± 1.4	110.2 ± 1.4	110.1 ± 1.4	111.9 ± 1.5	0.2695
Week 2	79.2 ± 2.9	75.2 ± 3.3	81.9 ± 3.3	79.6 ± 3.3	78.4 ± 3.3	84.6 ± 3.5	0.4989
Week 3	75.6 ± 1.5	75.5 ± 1.7	72.1 ± 1.7	74.1 ± 1.7	75.6 ± 1.7	72.7 ± 1.8	0.5210
Week 4	53.0 ± 1.0	51.8 ± 1.1	50.7 ± 1.1	52.1 ± 1.1	51.9 ± 1.1	51.7 ± 1.2	0.7972
Week 5	35.3 ± 1.6	37.6 ± 1.8	36.7 ± 1.8	37.1 ± 1.8	37.8 ± 1.8	33.4 ± 1.9	0.5453

No significant differences were observed. SE: Standard Error, FA: Folic Acid, Glu: Glucose

Table 7. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on weekly chick mortality (means ± Standard Error) of broiler chickens.

Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Week 1	0.76 ± 0.36	0.00 ± 0.41	0.00 ± 0.41	0.00 ± 0.41	0.00 ± 0.41	0.00 ± 0.44	0.6156
Week 2	1.95 ± 0.83	0.00 ± 0.96	0.00 ± 0.96	0.00 ± 0.96	2.35 ± 0.96	0.00 ± 1.02	0.2269
Week 3	1.04 ± 0.49	0.00 ± 0.56	0.00 ± 0.56	0.00 ± 0.56	0.00 ± 0.56	0.00 ± 0.60	0.6156
Week 4	0.00 ± 0.38	0.00 ± 0.44	1.11 ± 0.44	0.00 ± 0.44	0.00 ± 0.44	0.00 ± 0.47	0.3996
Week 5	0.00 ± 1.39	2.24 ± 1.60	4.69 ± 1.60	1.11 ± 1.60	1.23 ± 1.60	3.13 ± 1.70	0.3334
Sum	3.75 ± 1.65	2.24 ± 1.90	5.80 ± 1.90	1.11 ± 1.90	3.58 ± 1.90	3.13 ± 2.02	0.6313
Survival rate	96.3 ± 1.6	97.8 ± 1.9	94.2 ± 1.9	98.9 ± 1.9	96.4 ± 1.9	96.9 ± 2.0	0.6313

No significant differences were observed, SE: Standard Error, FA: Folic Acid, Glu: Glucose

Table 8. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on production number (means ± Standard Error) of broiler chickens.

Age	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
PN	397.1 ± 14.2	411.2 ± 16.7	371.0 ± 16.7	411.4 ± 15.7	386.4 ± 15.7	391.4 ± 16.7	0.4939

No significant differences were observed, SE: Standard Error, FA: Folic Acid, Glu: Glucose

Carcass characteristics

The effects of in-ovo injection treatments of hatching eggs on carcass characteristics are present in figure 1 and figure 2. Injection treatments had a significant effect on dressing percentage ($p > 0.05$). In-ovo injection of FA and (FA + Glu) resulted in a higher dressing percentage compared to the positive control group. Also, the in-ovo injection of (FA + Glu) resulted in a significantly higher percentage of front parts compared to the positive control. The percentage of hind parts was significantly higher due to the in-ovo injection of FA compared to the DPC treatment. No significant effects were observed on carcass meat percentage (Figure 2). The percentage of Liver, heart and total giblet was not significantly affected by any injection treatments (Table 9). However, the gizzard percentage was significantly lower in the negative control groups than in all other groups with the exception of the DPC. The in-ovo injection of (FA + Glu) resulted in a significantly higher in percentage of proventriculus than the negative control, the positive control and the folic acid injection group. Likewise, the intestinal diameter of the (FA + Glu) treatment was significantly wider than the rest of the treatments. Folic acid, glucose, and (FA + Glu) treatments had a significantly shorter intestine than the negative control group (Table 9). The percentage of all lymphoid organs (spleen, bursa, and thymus) was not significantly affected by in-ovo injection treatments (Table 10).

To cite this paper: Abdel-Halim, A., Mohamed FR, Elmenawey MA, Gharib HB (2021). Impact of In-Ovo Injection of Folic Acid and Glucose on Hatchability and Post-hatching Performance of Broiler Chickens. *World Vet. J.*, 10 (4): 481-491. DOI: https://dx.doi.org/10.54203/scil.2020.wvj58

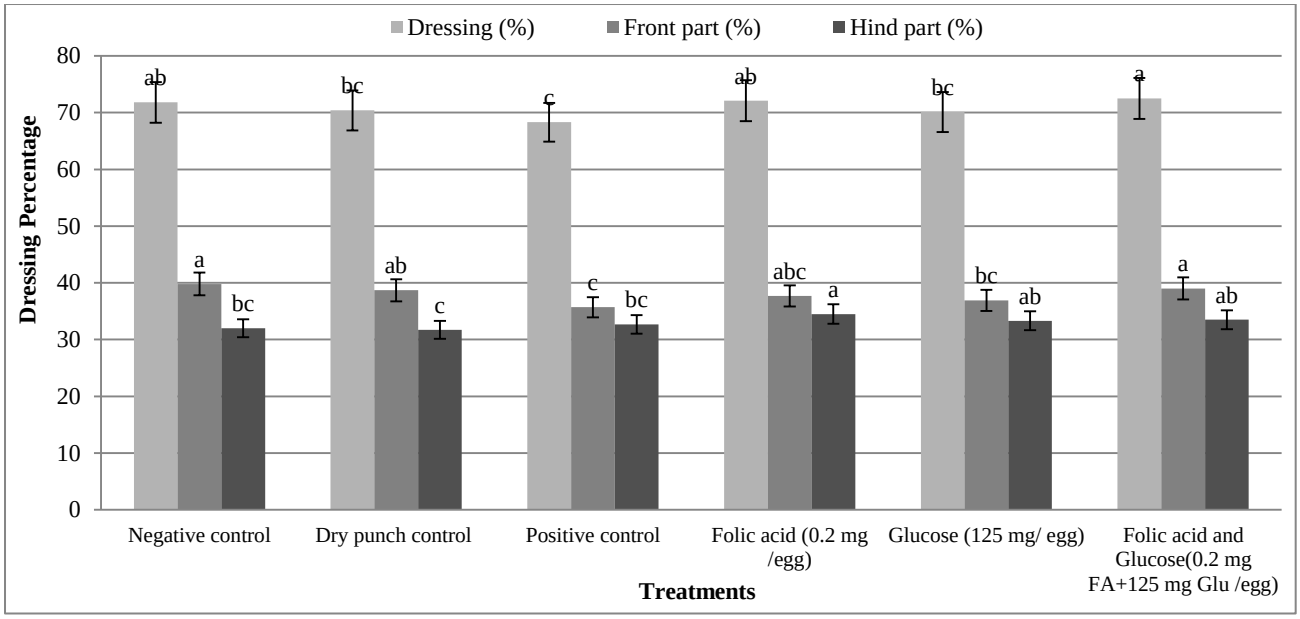

Figure 1. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler parents on carcass dressing as a percentage of living body weight of broiler chickens.

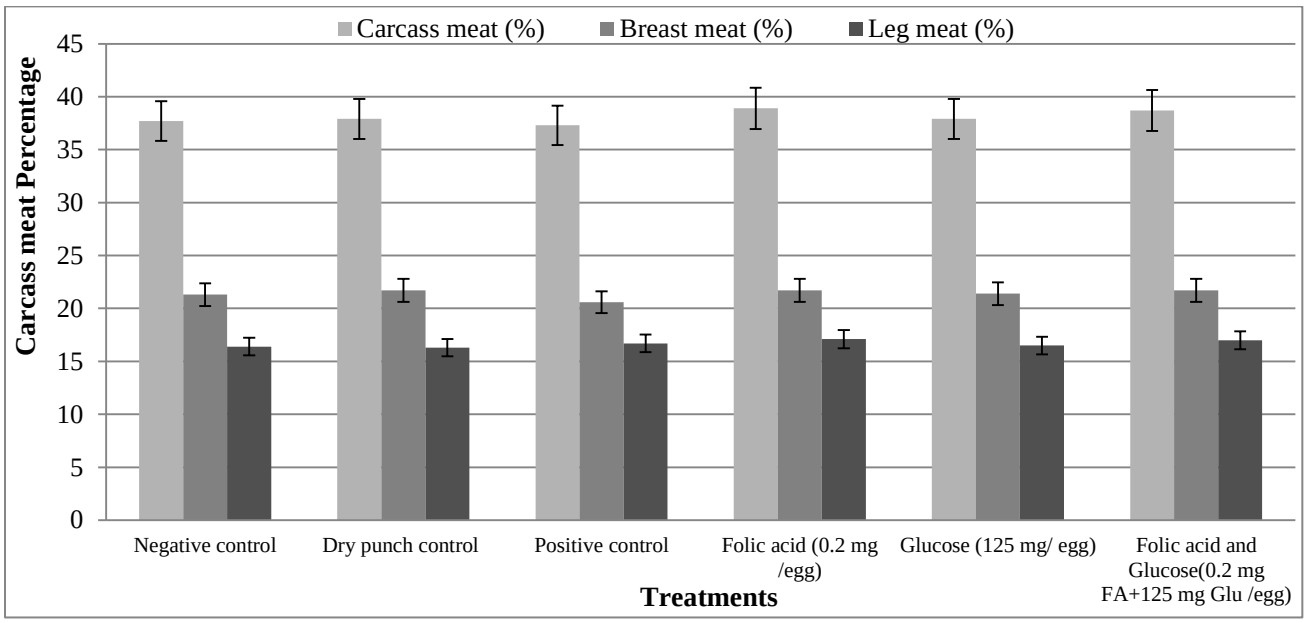

Figure 2. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on carcass dressing as percentage of living body weight of broiler chickens.

Table 9. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on giblets as percentage to live body weight (means ± Standard Error) of broiler chickens.

Items	**Negative control**	**Dry punch control**	**Positive control**	**Folic acid** (0.2 mg /egg)	**Glucose** (125 mg/ egg)	**Folic acid and Glucose** (0.2 mg FA+125 mg Glu /egg)	**p value**
Liver (%)	2.29 ± 0.08	2.44 ± 0.09	2.34 ± 0.09	2.12 ± 0.09	2.29 ± 0.08	2.35 ± 0.08	0.1868
Heart (%)	0.50 ± 0.02	0.58 ± 0.02	0.52 ± 0.02	0.57 ± 0.02	0.51 ± 0.02	0.55 ± 0.02	0.0765
Gizzard (%)	1.59 ± 0.11^{b}	1.91 ± 0.11^{ab}	2.12 ± 0.11^{a}	1.93 ± 0.11^{a}	2.09 ± 0.11^{a}	2.05 ± 0.11^{a}	0.0168
Giblet (%)	4.39 ± 0.17	4.92 ± 0.18	4.98 ± 0.18	4.62 ± 0.18	4.99 ± 0.17	4.95 ± 0.17	0.0695
Proventriculus (%)	0.45 ± 0.03^{b}	0.55 ± 0.03^{ab}	0.47 ± 0.03^{b}	0.46 ± 0.03^{b}	0.54 ± 0.03^{ab}	0.59 ± 0.03^{a}	0.0144
Length of intestine (cm)	189.8 ± 5.4^{a}	174.2 ± 5.7^{ab}	173.7 ± 5.7^{ab}	164.7 ± 5.7^{b}	168.3 ± 5.4^{b}	163.0 ± 5.4^{b}	0.0150
Diameter of intestine (cm)	1.13 ± 0.040^{b}	1.07 ± 0.042^{b}	1.16 ± 0.042^{b}	1.11 ± 0.042^{b}	1.10 ± 0.040^{b}	1.28 ± 0.040^{a}	0.0097

$^{a,b:}$ Means within a row followed by different superscripts differ significantly ($p \leq 0.05$). SE: Standard Error, FA: Folic Acid, Glu: Glucose

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Table 10. Impact of in-ovo injection of folic acid and glucose into albumen in freshly laid eggs from Arbor Acres broiler breeders on lymphoid organs as percentage to live body weight (means ± Standard Error) of broiler chickens.

Items	Negative control	Dry punch control	Positive control	Folic acid (0.2 mg /egg)	Glucose (125 mg/ egg)	Folic acid and Glucose (0.2 mg FA+125 mg Glu /egg)	p value
Spleen (%)	0.10 ± 0.01	0.14 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.1206
Bursa (%)	0.14 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	0.16 ± 0.02	0.0985
Thymus (%)	0.69 ± 0.03	0.71 ± 0.03	0.64 ± 0.03	0.62 ± 0.03	0.63 ± 0.03	0.66 ± 0.03	0.3642

No significant differences were observed. SE: Standard Error, FA: Folic Acid, Glu: Glucose

The present results agreed with Abd El-Azeem et al. (2014), who reported that in-ovo injection of FA significantly increased the carcass percentage of broilers, referring this effect to the anabolic effect of the treatments on muscle gain. Nouri et al. (2018) stated that there were no significant differences in the carcass characteristics of broilers in-ovo that were injected with FA (40, 80, and 120 µg) compared to the control group at the age of 42 days. Also, Abdel-Fattah and Shourrap (2012) reported that in-ovo injection of FA significantly increased the weight of the breast muscles of chickens at hatching and at 42 days of age. Furthermore, the positive effects of using FA on breast meat yield reflected its advantageous use in enhancement broilers carcass meat yield (Abdel-Fattah and Shourrap, 2012).

Previous studies indicated that in-ovo injection of carbohydrates enhanced muscle yield. The intra yolk sac injection of dextrose may have motivated the release of insulin, which might have increased the use of protein metabolically (Tasharofi et al., 2018). Salmanzadeh et al. (2011) found that in-ovo injection of glucose after seven days of incubation could improve the percentage of carcass and breast. Salmanzadeh et al. (2011) stated that in-ovo injection of glucose at 42 days of age had no significant effect on the liver, heart and gizzard of broiler chickens.

The results of current research revealed that the injection treatment of (FA + Glu) significantly increased the dressing percentage, front parts and hind parts. Previous studies demonstrated that the in-ovo injection of carbohydrate solutions or carbohydrates in combination with other nutrients (e.g., β-hydroxy-β-methylbutyrate) could increase breast muscle weight in broilers by six to eight percent up to 25 days post-hatching (Uni et al., 2005). Zhang et al. (2016) reported that the combined injection of six mg creatine monohydrate with 25 mg glucose on 18 day of incubation in the amnion had a synergistic effect on the enhancement of embryo energy status and the increase of the muscle creatine and phosphocreatine concentrations. Therefore, Zhang et al. (2016) concluded that it would be useful for improving embryonic development, and then improving chicken growth. Salmanzadeh et al. (2012) reported that glucose and glucose with magnesium in-ovo injection significantly increased the breast muscle size; they refer this effect to the hypothesis that an exogenous dietary supplement could substitute glucose with amino acids from the pectoral muscle. In other words, the exogenous supplies of nutrients increase protein deposition, probably by reducing muscle wasting. Salmanzadeh et al. (2012) also reported that the in-ovo injection of glucose and glucose with magnesium resulted in increased carcass and gizzard yields compared to the negative control and the positive control group. However, Salmanzadeh et al. (2012) noted that this treatment had no statistically significant effect on the weight of legs, wings, neck, liver and heart of broiler chickens (Salmanzadeh et al., 2012).

CONCLUSION

In conclusion, the in-ovo injection of folic acid decreases the albumen pH after four days of injection in the eggs from older breeder. The in-ovo injection of folic acid or glucose treatments had no significant effect on the hatchability of the fertile eggs. On the other hand, in-ovo injection with a combination of folic acid and glucose had significantly positive effects on chickens' body weight at two and four weeks of age and dressing percentage, without affecting the feed intake and feed conversion ratio. No significant differences were observed on chickens' mortality, growth rate and production number in all injection treatment groups. It could be concluded that studies on the best time, volume, and the site of folic acid and glucose injection are also needed.

DECLARATIONS

Authors' contribution

Fatma Rasmy Mohamed and Hassan Bayoumi Gharib designed the curriculum and facilitate the experimental work; Mohamed Abdel-Rahman El-menawey performed the experimental statistical analyzes. Amal Ahmed Abdel-Halim applied the practical part of the study, tabulated the experimental data and wrote the research article. All authors have revised the manuscript.

Competing interests

The authors have no competing interests. In addition, we have no authorship or article publication conflict.

To cite this paper: Abdel-Halim, A., Mohamed FR, Elmenawey MA, Gharib HB (2021). Impact of In-Ovo Injection of Folic Acid and Glucose on Hatchability and Post-hatching Performance of Broiler Chickens. *World Vet. J.*, 10 (4): 481-491. DOI: https://dx.doi.org/10.54203/scil.2020.wvj58

Consent to publish

All the authors approved and agreed to publish the manuscript.

REFERENCES

Abd El-Azeem, Nafisa A, Marwa Sh Abdo, Madkour M, and El-Wardany I (2014). Physiological and histological responses of broiler chicks to in ovo injection with folic acid or l-carnitine during embryogenesis. Global Veterinaria, 13(4): 544-551. DOI: https:// https://doi.org/10.5829/idosi.gv.2014.13.04.85231

Abdel-Fattah SA and Shourrap MI (2012). Physiological effects of in ovo L carnitine and embryonic thermal conditioning on pre and posthatch development of broiler chicks. In 3rd Mediterranean Poultry Summit and 6th international Poultry Conference, pp. 26-29. Available at: https://www.researchgate.net/profile/Mohamed_Shourrap/publication/316441817

Akhlaghi A, Ahangari YJ, Hashemi SR, Navidshad B, Pirsaraei ZA, Deldar H and Liang JB (2013). Prestorage in ovo injection of biological buffers: An approach to improve hatchability in long-term stored eggs. Poultry science, 92(4): 874-881. DOI: https://doi.org/10.3382/ps.2012-02610

Bellairs R, and Osmond M (2005). Atlas of chick development. Elsevier. Available at: https://books.google.com.eg/books?hl=en&lr=&id=UDCUi8cHWEwC&oi

Bennett CD (1992). The influence of shell thickness on hatchability in commercial broiler breeder flocks. Journal of Applied Poultry Research, 1(1): 61-65. DOI: https://doi.org/10.1093/japr/1.1.61

Cardeal PC, Caldas EOL, Lara LJC, Rocha JSR, Baiao NC, Vaz DP and da Silva Martins NR (2015). In ovo feeding and its effects on performance of newly-hatched chicks. World's Poultry Science Journal, 71(4): 655-662. DOI: https://doi.org/10.1017/S0043933915002445

Christensen VL, Donaldson WE and McMurtry JP (1996). Physiological differences in late embryos from turkey breeders at different ages. Poultry science, 75(2): 172-178. DOI: https://doi.org/10.3382/ps.0750172

Combs JrGF and McClung JP (2016). The vitamins: fundamental aspects in nutrition and health. Academic press. Available at: https://books.google.com.eg/books?hl=en&lr=&id=UEy0DAAAQBAJ&oi

Duncan DB (1955). Multiple ranges, and multiple F tests. Biometrics, 11: 1-42. Available at: https://www.jstor.org/stable/3001478?seq=1

Ebrahimi MR, Ahangari YJ, Zamiri MJ, Akhlaghi A, and Atashi H (2012). Does preincubational in ovo injection of buffers or antioxidants improve the quality and hatchability in long-term stored eggs? Poultry science, 91(11): 2970-2976. DOI: https://doi.org/10.3382/ps.2012-02246

Elibol O, Peak SD, and Brake J (2002). Effect of flock age, length of egg storage, and frequency of turning during storage on hatchability of broiler hatching eggs. Poultry science, 81(7): 945-950. DOI: https://doi.org/10.1093/ps/81.7.945

Iqbal J, Khan SH, Mukhtar N, Ahmed T, and Pasha RA (2016). Effects of egg size (weight) and age on hatching performance and chick quality of broiler breeder. Journal of applied animal research, 44(1): 54-64. DOI: https://doi.org/10.1080/09712119.2014.987294

Kanagaraju P, and Rathnapraba S (2019). Effect of in-ovo injection of glucose and egg white protein on the production performance and gut histomorphometry of broiler chicken. Indian Journal of Animal Research, 53(5): 675-679. DOI: http://dx.doi.org/10.18805/ijar.B-3555

Koppenol A, Delezie E, Wang Y, Franssens L, Willems E, Ampe B, and Everaert N (2015). Effects of maternal dietary EPA and DHA supplementation and breeder age on embryonic and post-hatch performance of broiler offspring: Age and n-3 pufa affect embryonic and post-hatch performance. Journal of animal physiology and animal nutrition, 99: 36-47. DOI: https://doi.org/10.1111/jpn.12308

Kucharska-Gaca J, Kowalska E, and Dębowska M (2017). In ovo feeding–technology of the future–a review. Annals of Animal Science, 17(4): 979-992. DOI: https://doi.org/10.1515/aoas-2017-0004

Lapao C, Gama LT, and Soares MC (1999). Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. Poultry science, 78(5): 640-645. DOI: https://doi.org/10.1093/ps/78.5.640

Li S, Zhi L, Liu Y, Shen J, Liu L, Yao J, and Yang X (2016). Effect of in ovo feeding of folic acid on the folate metabolism, immune function and epigenetic modification of immune effector molecules of broiler. British Journal of Nutrition, 115(3): 411-421. DOI: https://doi.org/10.1017/S0007114515004511

Liu Y, Zhi L, Shen J, Li S, Yao J, and Yang X (2016). Effect of in ovo folic acid injection on hepatic IGF2 expression and embryo growth of broilers. Journal of animal science and biotechnology, 7(1): 40. DOI: https://doi.org/10.1186/s40104-016-0099-3

Meijerhof R (1994). Theoretical and empirical studies on temperature and moisture loss of hatching eggs during the pre-incubation period. Available at: https://library.wur.nl/WebQuery/wurpubs/fulltext/205157

Narushin VA, and Romanov MN (2002). Egg physical characteristics and hatchability. World's Poultry Science Journal, 58(3): 297-303. DOI: https://doi.org/10.1079/WPS20020023

Nouri S, Ghalehkandi JG, Hassanpour S, and Aghdam-Shahryar H (2018). Effect of in ovo feeding of folic acid on subsequent growth performance and blood constituents' levels in broilers. International Journal of Peptide Research and Therapeutics, 24(3): 463-470. DOI: https://doi.org/10.1007/s10989-017-9629-x

Parnian A, Navidshad B, Mirzaei F, Behmaram R, and Deldar H (2019). Effect of in ovo injection of nicotonic acid, pantothenic acid or folic acid on immune system and growth of broiler chickens. Iranian Journal of Veterinary Medicine, 13(4): 411-420. DOI: https://dx.doi.org/10.22059/ijvm.2019.278345.1004976

Peebles ED, Doyle SM, Zumwalt CD, Gerard PD, Latour MA, Boyle CR, and Smith TW (2001). Breeder age influences embryogenesis in broiler hatching eggs. Poultry Science, 80(3): 272-277. DOI: https://doi.org/10.1093/ps/80.3.272

Reijrink IAM, Meijerhof R, Kemp B, and Van Den Brand H (2008). The chicken embryo and its micro environment during egg storage and early incubation. World's Poultry Science Journal, 64(4): 581-598. DOI: https://doi.org/10.1017/S0043933908000214

Ricks CA, Avakian A, Bryan T, Gildersleeve R, Haddad E, Ilich R, and Whitfill C (1999). In ovo vaccination technology. Advances in veterinary medicine, 41: 495-515. Available at: https://pubmed.ncbi.nlm.nih.gov/9890038/

Robel EJ (2002). Assessment of dietary and egg injected d-biotin, pyridoxine and folic acid on turkey hatchability: folic acid and poultry weight. World's Poultry Science Journal, 58(3): 305-315. DOI: https://doi.org/10.1079/WPS20020024

Salmanzadeh M, Nezhad YE, Shahryar HA, Ashrafi S, Moghaddam PP, and Lotfi A (2011). The effects of in ovo administration of glucose on carcass characterizes of broiler chickens. Global Veterinária, 6(5): 429-432. Available at: http://www.idosi.org/gv/gv6(5)11/1.pdf

Salmanzadeh M, Ebrahimnezhad Y, Shahryar HA, and Beheshti R (2012). The effects of in ovo injection of glucose and magnesium in broiler breeder eggs on hatching traits, performance, carcass characteristics and blood parameters of broiler chickens. Arch. Geflugelkunde, 76: 277-284. Available at: https://www.cabdirect.org/cabdirect/abstract/20123387531

Salmanzadeh M (2012). The effects of in-ovo injection of glucose on hatchability, hatching weight and subsequent performance of newly-hatched

chicks. Brazilian journal of poultry science, 14(2): 137-140. DOI: https://doi.org/10.1590/S1516-635X2012000200008

Starck JM and Ricklefs RE (Eds.) (1998). Avian growth and development: evolution within the altricial-precocial spectrum (No. 8). Oxford University Press on Demand, Available at: https://books.google.com.eg/books?hl=en&lr=&id=A0HB7Mq4lOYC&oi

Tasharofi S, Mohammadi F, Amiri N, and Nazem MN (2018). Effects of intra-yolk-sac injection of dextrose and albumin on performance, jejunum morphology, liver and pectoral muscle glycogen and some serum metabolites of broilers. Journal of animal physiology and animal nutrition, 102(4): 917-923. DOI: https://doi.org/10.1111/jpn.12882

Tona K, Onagbesan O, De Ketelaere B, Decuypere E, and Bruggeman V (2004). Effects of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and chick posthatch growth to forty-two days. Journal of Applied Poultry Research, 13(1): 10-18. DOI: https://doi.org/10.1093/japr/13.1.10

Ulmer-Franco AM, Fasenko GM, and Christopher EEOD (2010). Hatching egg characteristics, chick quality, and broiler performance at 2 breeder flock ages and from 3 egg weights. Poultry science, 89(12): 2735-2742. DOI: https://doi.org/10.3382/ps.2009-00403

Uni Z, Ferket PR, Tako E, and Kedar O (2005). In ovo feeding improves energy status of late-term chicken embryos. Poultry Science, 84(5): 764-770. DOI: https://doi.org/10.1093/ps/84.5.764

Vieira AR, Murray JC, Trembath D, Orioli IM, Castilla EE, Cooper ME, and Speer M (2005). Studies of reduced folate carrier 1 (RFC1) A80G and 5, 10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms with neural tube and orofacial cleft defects. American Journal of Medical Genetics Part A, 135(2): 220-223. DOI: https://doi.org/10.1002/ajmg.a.30705

Vieira SL (2007). Chicken embryo utilization of egg micronutrients. Brazilian Journal of Poultry Science, 9(1): 1-8. DOI: https://doi.org/10.1590/S1516-635X2007000100001

XLSTAT (2014). Statistical software for MS Excel. statiscal and data analysis with MS Excel Addinsoft 224 Centre Street, 3rd floor New York, Ny10013 USA. Available at: https://www.xlstat.com/en/news/xlstat-2014-5

Zhai W, Bennett LW, Gerard PD, Pulikanti R and Peebles ED (2011a). Effects of in ovo injection of carbohydrates on somatic characteristics and liver nutrient profiles of broiler embryos and hatchlings. Poultry science, 90(12): 2681-2688. DOI: https://doi.org/10.3382/ps.2011-01532

Zhai W, Gerard PD, Pulikanti R and Peebles ED (2011b). Effects of in ovo injection of carbohydrates on embryonic metabolism, hatchability, and subsequent somatic characteristics of broiler hatchlings. Poultry science, 90(10): 2134-2143. DOI: https://doi.org/10.3382/ps.2011-01418

Zhai W, Rowe DE and Peebles ED (2011c). Effects of commercial in ovo injection of carbohydrates on broiler embryogenesis. Poultry science, 90(6): 1295-1301. DOI: https://doi.org/10.3382/ps.2010-01130

Zhang L, Zhu XD, Wang XF, Li JL, Gao F, and Zhou GH (2016). Individual and combined effects of in-ovo injection of creatine monohydrate and glucose on somatic characteristics, energy status, and post-hatch performance of broiler embryos and hatchlings. Poultry science, 95(10): 2352-2359. DOI: https://doi.org/10.3382/ps/pew130

Zhang H, Elliott KEC, Durojaye OA, Fatemi SA, Schilling MW, and Peebles ED (2019). Effects of in ovo injection of L-ascorbic acid on growth performance, carcass composition, plasma antioxidant capacity, and meat quality in broiler chickens. Poultry science, 98(9): 3617-3625. DOI: http://dx.doi.org/10.3382/ps/pez173

To cite this paper: Abdel-Halim, A., Mohamed FR, Elmenawey MA, Gharib HB (2021). Impact of In-Ovo Injection of Folic Acid and Glucose on Hatchability and Post-hatching Performance of Broiler Chickens. *World Vet. J.,* 10 (4): 481-491. DOI: https://dx.doi.org/10.54203/scil.2020.wvj58

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 492-498, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj59

Peculiarities of Mineral Metabolism of Holstein Heifers' Diet Supplemented with Copper Nanopowders

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ORIGINAL ARTICLE
pii: S232245682000059-10
Received: 04 Nov 2020
Accepted: 11 Dec 2020

ABSTRACT

The current study aimed to investigate the effect of copper nanopowder on physiological and mineral metabolism indicators of Holstein cattle during the growth process of the animal. There were two experimental groups (control and treatment) and each one included seven Holstein heifers. From the first month of each heifer in the experimental group, a suspension of copper nanoparticles in a dose of 0.04 mg/kg was orally administered daily to the usual diet. The biologically active additive was administered to the animal's diet at intervals. The weight of the animals was measured monthly, a clinical blood test was performed, the mineral composition of the blood was studied, the mineral composition of animal hair was also examined. The findings indicated a positive dynamics in the increase of body weight in the treatment group, compared to the control group. As a result of clinical blood tests, it was noted that the number of erythrocytes, the level of hemoglobin, and hematocrit increased in the experimental animals due to copper nanoparticles compared to these parameters in these animals at the beginning of the tests. Mineral analysis of blood samples in the treatment group presented an increase in the levels of copper, potassium, iron, zinc, and manganese while in animals of the control groups there was an increase in sodium, calcium, and phosphorus. According to the blood serum and hair analysis, copper in the nanodispersed state indicated antagonistic effects on boron, silicon, antimony, molybdenum of the treatment group and there was a synergist in aluminum, titanium, manganese, cobalt, iron, and potassium levels compared to the beginning of the experiment. The obtained results indicated that the addition of copper nanopowder to the diet of experimental animals increased the growth, stimulated the function of hematopoiesis, and improved the characteristics of mineral metabolism of the Holstein heifers.

Keywords: Copper nanopowder, Cattle, Mineral metabolism, Physiological characteristics

INTRODUCTION

One of the main tasks in feeding farm animals is the development of the physiological and biological mechanisms of their high productivity. The basis of modern livestock production and biotechnology includes the determination of factors affecting the quantity and quality of products as well as the investigation of the necessary conditions for the maximum manifestation of the genetic potential by changing the compositions of the diet (Arsanukaev, 2005). At the present stage of animal husbandry development, new biologically active additives are developed and introduced into the diet of animals to enhance physiological processes and improve the quality of products (Natyrov and Arilov, 2002; Arsanukaev, 2005; Zaynalabdieva et al., 2014).

Among the substances that play an important role in farm animal nutrition, a significant place is taken by micronutrients, which are necessary for growth and reproduction (Myazin et al., 2006). The main source of micronutrients for animals is feed. However, the mineral composition of the feed depends on the type of soil, climatic conditions, and agrochemical measures (Myazin et al., 2006; Klyshevskaya, 2010; Dubovik and Dubovik, 2016).

Therefore, a lack or excess of some elements in a diet can lead to a decrease in productivity, deterioration in product quality, and the efficiency of feed use (Demidyuk, 1984). In zootechnical practice, inorganic salt types, such as sulfate, carbonate, chloride, and phosphate, are used to fill the deficit of mineral substances in the diet of farm animals. The zootechnical practice has several shortcomings in satisfying the physiological and biochemical criteria for the compatibility and combining ability of bioelements at different stages of metabolism. Some of shortcomings can be inorganic salts which are toxic and poorly absorbed by the digestion system. (Demidyuk, 1984; Antonovich et al., 2005).

Extensive research has been carried out to improve the technology of micronutrient in farm animal nutrition, especially on the integration of micronutrients complexes from natural, synthetic, and microbial origins into the diet (Bogoslovskaya et al., 2009; Timasheva et al., 2014; Chernova et al., 2015).

To cite this paper: Stepanova IA, Nazarova AA and Arisov MV (2021). Peculiarities of Mineral Metabolism of Holstein Heifers' Diet Supplemented with Copper Nanopowders. *World Vet. J.*, 10 (4): 492-498. DOI: https://dx.doi.org/10.54203/scil.2020.wvj59

An alternative to traditional forms of micronutrients can be nanopreparations containing metals (iron, cobult and copper) in the form of nanosized particles. The Scientific and Educational Center "Nano- and Biotechnologies" at Ryazan State Agrotechnological University, Russia, is studying the biological activity of metal nanopowders in various agricultural sectors (Polischuk et al., 2015a; Polischuk et al., 2018; Churilov et al., 2019), including the feeding of highly productive animals with the ability to catalyze biochemical processes in the body and increase metabolism (Nazarova et al., 2014; Miroshnikova et al., 2015; Sizova et al., 2016). With this background in mind, The aim of current study was to avaluate the effect of copper nanopowder on the mineral metabolism indicators during growth process of Holstein heifers.

MATERIALS AND METHODS

Ethical approval

The present study study used the following methods to reduce the pain and suffering of the experimental animals. The most humane and sparing methods and procedures were used towards animals to prevent their pain and suffering. Any painful procedures with animals were carried out according to the guidelines and standards, such as European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123). Strasbourg, 1986. Directive of the European Parliament and the Council of the EU 2010/63/EU of September 22, 2010, on the protection of animals used for scientific research.

Experimental groups

The current research was conducted during 2016-2017 and placed at the Ryazan State Agrotechnological University named P.A. Kostychev and the livestocks were farm «Rassvet» in the Ryazan region, Russia. The samples of the study included a total number of 14 heifers of Holstein breed with black and white coats. The experimental animals were selected according to the principle of balanced analog groups by considering gender, age (1 month), breed and weight (57.5 – 60.2 kg). The samples were kept in similar conditions of feeding and management. The general research scheme is presented in Table 1. At the beginning of the research, the main diet consisted of cow's milk, after the age of 3 month, the basic diet of animals consisted of a combined type of feeding including grass, corn silage, haylage, compound feed and metal salts. The diet corresponded to the needs of the animals and physiological norms. The blood samples were subjected to the blood analysis at SHI "Ryazan Vet Laboratory". The analysis of the hair was carried out in the laboratory of the National Agency of Clinical Pharmacology and Pharmacy LLC, Moscow, Russia. The blood tests were performed before the study and 12 months after the study, the content of minerals in the blood serum of the animals was studied before the study and 6 months after it, the mineral composition of the wool was studied before the study and 40 days after it. The animals were weighed before the study (aged 1 month) and at the age of 2, 3, 4, 5, 7 and 11 month.

The employed experimental cuprum nanopowder (NP Cu) were produced at NITU MISiS with characteristics including finely-divided, homogeneous, dark-red powder and without any foreign particles, 99.98 % pure. The average size of the particles were 20-40 nm. The metal suspension was treated with ultrasound in aqueous media to create a biologically active ultradispersed system. Holstein heifers of Control and treatment groups were kept in separate houses (Figures 1 and 2).

Table 1. Experimental groups, duration and conditions

Groups	Number of Animals	Experiment Duration	Experimental Conditions
Control	7	12 months	Basic Diet (BD)
Treatment	7	12 months	BD + NP of cuprum (0.04 mg/kg of live weight a day in 2, 4, 6, 9 months of life)

Figure 1. A Holstein heifer in control group did not receive cuprum nanopowders at the the first month of the experiment.

Figure 2. A Holstein heifer in treatment group received cuprum nanopowders at the first month of the experiment.

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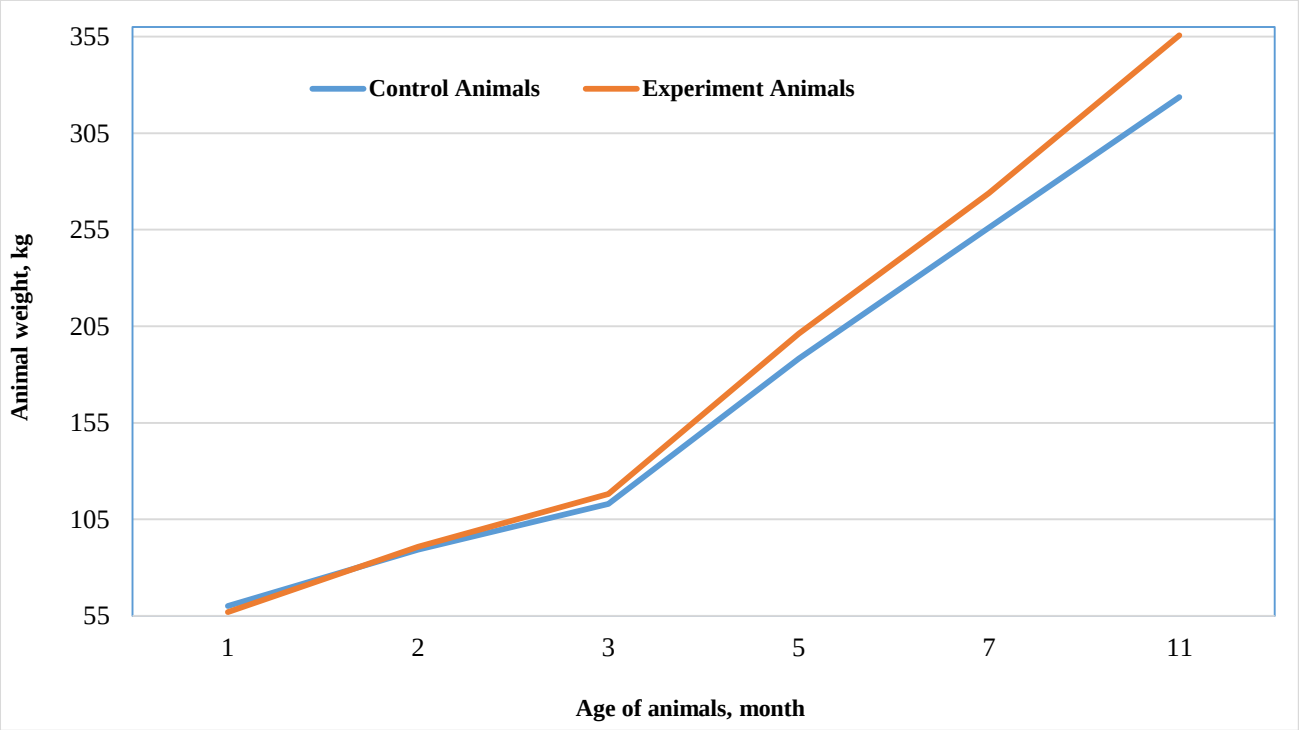
Experimental procedure

For animals from the age of 1 month, an aqueous suspension of a dietary copper nanoparticles supplement in a dose of 0.04 mg/kg was orally administered on daily basis by considering the live weight. The suspension was administered during the second, fourth, sixth, ninth, and eleventh month of age, due to the high chemical activity of the nanoparticles and prolonged action, confirmed by previous studies (Polischuk et al., 2015b; Makarov et al., 2017; Nazarova et al., 2019). The weight of the control and treatment groups was measured in kg every month (Graph 1).



Graph 1. The weight of Holstein heifers of the control and treatment groups during experimental period.

Statistycal analysis

The statistical presecure of current study included processing the experimental data with the ranging method (ranging of odd quantitative parameters) for balanced analogues-groups by K. White ($p \leq 0.05$).

RESULTS AND DISCUSSION

The obtained results indicated that the diet of young Holstein heifers supplemented with copper nanoparticles had a significant effect ($p \leq 0.05$) on the mass weight of experimental animals.In the present study, an increase in live weight gain was observed in heifers treated with copper nanoparticles. Four months after the initiation of the study, the increase in live weight was 5.8%, and by the 11th month, it was 9.8%, compared to the control group. The reason for this was the influence of copper on the absorption of calcium and phosphorus since there was active development of the skeleton during this period. Therefore, the use of the drug with copper nanoparticles ensured stable and intensive growth of animals.

Grozhevskaya (1973) indicated that under the influence of feeding copper sulfate to cows, a direct relationship was observed between an increase in the level of red blood cells and the amount of total protein and carotene. The obtained results indicated a simultaneous decrease in iodine and phosphorus with an increase in red blood cells and glucose in the blood samples of experimental animals of the treatment group. It is important to note that even a general increase in blood glucose of heifers led to a decrease in the level of ketone bodies. It is known that copper in the presence of iron is involved in the formation of hemoglobin in the blood and contributes to the transit of iron into the bone marrow (Overton and Yasui, 2014). Copper stimulates the formation of ossein, contributes to the normal development of bone tissue, and also affects the deposition of calcium and phosphorus, and the metabolism of carbohydrates, lipids, proteins, and minerals (Overton and Yasui, 2014). Glucose is a source of energy for almost all crucial physiological processes. Due to lack of glucose the animal body tries to compensate the energy deficit by transforming body fat into fatty acids. Decreasing of total number of blood protein is accompanied by a decrease in the body weight of cows and their reproductive abilities (Skopichev and Yakovlev, 2008).

In the course of the experiment, the main paraclinical scores of experimental heifers' blood samples were determined (Table 2). In control group, by the 12th month of the experiment, there was a slight increase in the level of

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red blood cells, hemoglobin, and platelets, which was associated with the natural processes of growth and development of young animals. The copper nanopowder treatmen group presented an increase in red blood cells (a significant increase of 14.7%) and hemoglobin (by 11.2%) ($p \leq 0.05$), compared with the beginning of the experiment, which in turn, affected the increase in hematocrit (a significant increase of 3.8%, compared to the beginning of the experiment) ($p \leq 0.05$). This finding was associated with the fact that copper nanoparticles are able to activate intracellular biochemical processes leading to requirement of a higher supply of oxygen for the tissues. The platelet content increased slightly, but this was observed both in the control and treatment groups, which was associated with the growth of animals and was independent of the introduction of nanoparticles. At the next stage of the study, the effect of copper nanopowder on the characteristics of the mineral metabolism of Holstein heifers breed during the growth of animals was studied. The blood mineral composition of the control and experimental animals was estimated before the start of the experiment and after six months (Table 3).

Table 2. Paraclinical scores of experimental Holstein heifers' blood samples

Scores	Control group		Treatment group (copper nanopowder)	
	At the beginning of the experiment	After 12 months	At the beginning of the experiment	After 12 months
White blood cells, 10^9 / l	7.9±0.1	7.5±0.4	7.5±0.5	7.9±0.4
Erythrocytes, 10^{12} / l	10.5±0.4	11.3±0.3	9.5±0.5	11.8±0.3**
Hemoglobin, g / l	105±2	115±5	107.5±3.3	119.9±5.1
Hematocrit, %	31.3±0.4	34.2±0.6	30.9±0.3	34.7±0.6***
The number of platelets, mln / l	310.7±7.8	327.0±6.5	317.5±5.8	333.7±10.2

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$

Table 3. The content of minerals and alkaline phosphatase in the blood serum of experimental Holstein heifers

Blood characteristics	**Control group**		**Treatment group (copper nanopowder)**	
	Before the experiment	After 6 months	Before the experiment	After 6 months
Calcium. mmol / L	2.80 ± 0.04	2.95 ± 0.05	2.96 ± 0.08	2.93 ± 0.05
Phosphorus. mol / L	1.75 ± 0.04	1.90 ± 0.03*	2.31 ± 0.02	2.28 ± 0.01
Alkaline phosphatase. IU/L	104 ± 3.6	118 ± 3.4*	173.3 ± 3.9	211.6 ± 5.0***
Copper. mmol / L	0.51 ± 0.002	0.42 ± 0.008	0.57 ± 0.011	0.72 ± 0.010***
Potassium. mmol / L	3.9 ± 0.04	3.1 ± 0.01***	4.08 ± 0.14	4.29 ± 0.15
Sodium. mmol / L	130.5 ± 4.4	151.8 ± 3.1**	155.6 ± 4.6	145.9 ± 1.3
Iron. mmol / L	25.9 ± 0.4	20.3 ± 0.7***	18.3 ± 0.3	29.1 ± 0.5***
Magnesium. mmol / L	0.86 ±0.005	0.75 ± 0.003***	0.88 ± 0.002	0.85 ± 0.001***
Zinc. micromol / l	19.8 ± 0.7	16.5 ± 0.4**	11.6 ± 0.3	15.4 ± 0.9**
Manganese. mmol / L	0.043±0.007	0.038±0.002	0.03±0.001	0.05±0.004**
Chlorides. mmol / L	101.1 ± 2.5	112.4 ± 1.3**	99.6 ± 1.7	119.4 ± 2.1***

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$

Six months after the start of the experiment, an increase in the content of calcium (+ 5.4%) and phosphorus (+ 8.6%) was observed in control group, which was primarily associated with intensive growth of heifers in the first month of age. The content of sodium (+ 16.3%) and chloride ions (+ 11.2%) also increased. There was more copper in the tissues of young animals than in the tissues of adult animals, for example, there was 14 mg/kg copper content in the muscles of calves, but 0.5 mg/kg in colostrum and 0.05 m/kg in milk of adult animals. The need for copper in cattle is higher than in other animal species, especially in range management. Copper deficiency causes growth retardation and decreased productivity. Moreover, it has a significant effect on the color of the coat, which can transform gray or brown, or become dirty yellow, and it increases the fragility of bones (Bolotnov, 2002). Excessive consumption of copper would manifest itself in the form of lipase taste of milk when 80 mg of copper was included per 1 kg of dry matter of the diet (Bolotnov, 2002). Several other changes were observed in the blood samples of copper nanopowder treatment group. About six month after the start of the experiment, an increase in the level of alkaline phosphatase (a significant increase of 22.1% compared from the beginning of the experiment), copper (+ 26.3%), potassium (+ 5.1%), iron (+ 59%), zinc (+ 32.7%), and manganese (+ 66%) were observed ($p \leq 0.05$). The sodium content decreased by 6.2%, the magnesium content did not change, which supported the data from the changes in the mineral composition of the hair. Before the start of the experiment, hair samples were taken from control and treatment group. For about 40 days after adding nanocopper in the diet, re-sampling of hair was carried out to analyze its mineral composition. A tendency of changes in

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the content of elements was also observed in the analysis of the mineral composition of Holstein heifer's hair which was similar to the changes in the blood (Table 4).

Table 4. Mineral composition of experimental Holstein heifers' hair (μg / l)

Elements	Control		Copper nanopowder	
	Before the experiment	After 40 days	Before the experiment	After 40 days
Lithium	0.158±0.0022	0.040±0.0006***	0.047±0.0007	0.007±0.0001***
Boron	2.342±0.033	2.670±0.038***	2.413±0.034	0.141±0.002***
Aluminum	14.247±1.213	9.369±1.133***	11.907±0.170	15.537±0.221***
Silicon	14.460±1.207	32.570±2.465***	22.700±3.324	13.900±1.198*
Titanium	0.008±0.000001	0.008±0.000002	0.006±0.00008	0.009±0.0001***
Manganese	1.139±0.016	1.112±0.015	0.751±0.010	2.084±0.029***
Chromium	0.858±0.0022	0.874±0.0124	0.689±0.009	0.763±0.010**
Cobalt	0.021±0.0003	0.027±0.0004***	0.016±0.0002	0.158±0.0022***
Nickel	0.354±0.005	0.183±0.002***	0.154±0.002	0.187±0.003***
Arsenic	0.034±0.001	0.037±0.003	0.029±0.0004	0.038±0.0005***
Selenium	0.939±0.013	0.957±0.017	0.803±0.011	0.820±0.012
Cadmium	0.003±0.00004	0.003±0.00006	0.001±0.0001	0.005±0.0007***
Antimony	0.005±0.0007	0.024±0.0030***	0.008±0.0001	0.006±0.00008
Mercury	0.115±0.0016	0.043±0.0006	0.036±0.0005	0.061±0.0009
Lead	0.261±0.003	0.401±0.006	0.241±0.003	0.289±0.004
Copper	18.700±0.267	12.870±0.183	11.440±0.163	11.900±0.170
Iron	27.600±0.394	21.900±0.312	18.700±0.267	33.500±0.478
Zinc	135.120±1.930	122.100±1.744	118.320±1.690	125.040±1.786
Sodium	645.700±9.224	794.600±11.351	500.000±7.142	449.100±6.415
Magnesium	32.550±0.465	29.767±0.425	35.083±0.501	30.550±0.437
Potassium	1244.020±17.771	1162.390±16.605	718.950±10.270	801.170±11.445
Calcium	846.000±12.085	999.000±14.271	1034.000±14.771	779.00±11.128
Molybdenum	0.090±0.001	0.127±0.002	0.094±0.001	0.134±0.002

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$

The following changes occurred in the hair of control animals during the experiment: there was a decrease in lithium (-74.7%), aluminum (-34.2%), nickel (-48.3%), mercury (-62.6%), copper (-31.2%), iron (-20.6%), zinc (-9.6%), magnesium (-8.5%), and potassium (-6.6%). At the same time, there was an increase in the content of the following elements: silicon (+125.2%), cobalt (+28.6%), antimony (+380%), lead (+53.6%), sodium (+23.1 %), calcium (+18.1%), and molybdenum (+41.1%).

Considering the fact that the diet of control and treatment groups was the same, except for the addition of copper nanopowder, then changes in the mineral composition of the hair coat and significant differences ($p \leq 0.05$) in the content of blood minerals were considered solely as a result of exposure to copper nanoparticles. General changes in the hair of control and treatment group were observed with a decrease in lithium levels and an increase in silicon and cobalt. Compared to the control group, nanopowder of copper treatment group presented antagonistic action with respect to boron (-94.2%), silicon (-38.8%), antimony (-25%), sodium (-10.2%), molybdenum (-86.7%) in comparison to the data from beginning of the experiment. The decrease in molybdenum was consistent with the antagonistic effect of copper in ionic form.

Furthermore, copper nanopowder contributed to an increase in the content of aluminum (+30.5%), titanium (+ 50%), manganese (+177.5%), cobalt (9 times), nickel (+21.4%), mercury (+69.4%), copper (+4%), iron (+79.1%), and potassium (+11.4%), compared to the beginning of the experiment. The nanoscale effect of copper was observed by an increase in the uptake of cadmium (4 times) and zinc (+5.7%). It is known that copper in ionic form was an antagonist of these metals. Copper and calcium in the form of salts were neutral to each other, and copper nanoparticles reduced calcium absorption by 24.7%.

Copper nanoparticles affected the increase of iron and cobalt content in the blood of animals. The reason is that these metals are synergistic in ionic form, but their combined use does not lead to such a significant increase in the assimilation and accumulation of each other in animals (Voynar, 1960).

The main place for copper absorption in animals is the small intestine and stomach. The copper absorbtion does not occur only as a result of simple diffusion but also by actively transferring the micronutrient through the intestinal wall and the active transferring increases significantly with deficiency of micronutrient (Kuznetsov and Kuznetsov, 2003). The copper in combination with aminoacids, dipeptides and polypeptides is absorbed better than in the form of sulfate and while the molecular weight of the complexes increases then the absorption decreases (Kuznetsov and Kuznetsov,

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2003). The mediator for absorption of copper, as well as zinc and cadmium, is a low molecular weight protein of the intestinal wall called metallothionein, which increases absorption in a passive way, linking the element to SH-groups and preparing it for further transfer. In addition, it can block absorption and protect the body from reaching toxic levels of metal (Kuznetsov and Kuznetsov, 2003). The absorption of copper is influenced by many feed factors, specifically protein. An increase in the protein level of the diet reduces its accumulation in the liver. Some heavy metals (such as lead, cadmium, mercury, silver, zinc, and arsenic) compete with copper upon absorptionwhich leaded to insufficiency of copper (Kuznetsov and Kuznetsov, 2003).

In general, the obtained results indicated that copper particles in the nanodispersed state activated the mineral metabolism in the body of Hostein heifers, contributing to the assimilation and accumulation of mineral substances. It should be noted that the effect of copper nanoparticles were significantly different from that copper in ionic form, which was associated with the size of the particles, their charge, the concentration, and the method of preparation.

CONCLUSION

According to the results of the current study, it can be concluded that the addition of copper nanopowder to the cattle diet stimulated the blood formation function of the body, which was manifested in an increase in red blood cells by 14.7% and hemoglobin content by 11.2%. Based on the blood serum and hair analysis and considering the theory of antagonistic and synergistic interaction of micronutrients, it was indicated that the characteristics of the mineral metabolism of Holstein cattle became significantly more active under the influence of copper nanoparticles. The analysis of the mineral composition of animal hair revealed that the addition of copper nanopowder led to an antagonistic effect on boron, silicon, antimony, and molybdenum. In addition, copper in the nanodispersed state presented itself as a synergist of aluminum, titanium, manganese, cobalt, iron, and potassium, compared to the beginning of the experiment. Additionally it can be concluded that copper nanopowder can be used as a biologically active additive in the diet of young cattle, which improves the general physiological state and as a stimulator of mineral metabolism.

DECLARATIONS

Authors' contribution

Anna A. Nazarova and Irina A. Stepanova collected data and designed the study. Anna A. Nazarova, Irina A. Stepanova, and Mikhail V. Arisov analyzed data and wrote the draft of manuscript. All authors read and approved the final manuscript.

Competing interests

The authors did not have any conflict of interests.

Acknowledgements

The authors are grateful to SHI "Ryazan Vet Laboratory", laboratory of the National Agency of Clinical Pharmacology and Pharmacy LLC, NITU MISiS for their assistance in data collection.

Consent to publish

The authors approved and agreed to publish the manuscript

REFERENCES

Antonovich EA, Podushnyak AE, and Schutskaya TA (2005). Toxicity of copper and its compounds. Institute of Environmental Hygiene and Toxicology. L.I. Medveda, Kiev., p. 28. Available at: http://www.medved.kiev.ua/arhiv_mg/stat_99/99_3_1.htm

Arsanukaev D (2005). Efficiency of microelement nutrition of male calves. Dairy and beef cattle breeding, 8: 12–14. Available at: http://foliant.ru/catalog/psulibr?SHOW_ONE_BOOK+21F354

Bogoslovskaya OA, Sizova EA, Polyakova VS, Miroshnikov SA, Leipunsky IO, Olkhovskaya IP and Glushchenko NN (2009). Studying the safety of administering copper nanoparticles with various physicochemical characteristics into the animal organism. Herald of the Orenburg State University, 2: 124−127. Available at: https://elibrary.ru/item.asp?id=13009569

Bolotnov L (2002). Minerals and vitamins in the diets of lactating cows. Compound feed, 4: 52-53. Available at: https://elibrary.ru/title_about.asp?id=9589

Chernova EL, Yastrebova OL and Chernov IS (2015). The effect of organic salts of biometals on cicatricial digestion and milk production of cows. Scientific notes of the Kazan State Academy of Veterinary Medicine named after N.E. Bauman, 221 (1): 246-249. Available at: https://elibrary.ru/item.asp?id=23221254

Churilov GI, Churilov DG, Nazarova AA, Polischuk SD, Churilova VV, Borychev SN, and Byshov NV (2019). Dinamics of accumulating pollutants and essential elements in the process of plant growth and development. International Journal Nanotechnology, 16 (1/2/3): 42-59. DOI: http://doi.org./10.1504/IJNT.2019.102391

Demidyuk AA (1984). Influence of Cuprum, Cobalt and Iodine Compensation in Diets on Metabolic Processes and Cows' Milk Productivity. Candidate of Biological Science Dissertation: 03.00.04, Lvov. DOI: http://doi.org./10.15389/ agrobiology.2016.6.903rus

Dubovik DV, and Dubovik EV (2016). The effect of mineral fertilizers on the content of heavy metals in the soil on the slopes. Bulletin of the Kursk State Agricultural Academy, 4: 60-62. Available at: https://elibrary.ru/download/elibrary_26642836_10621774.pdf

Grozhevskaya SB (1973). The biological significance of copper for the body of cattle. Issues of feeding agricultural animals. Proceedings of the Kirov and Perm Agricultural Institute, pp.12-19.

To cite this paper: Stepanova IA, Nazarova AA and Arisov MV (2021). Peculiarities of Mineral Metabolism of Holstein Heifers' Diet Supplemented with Copper Nanopowders. *World Vet. J.*, 10 (4): 492-498. DOI: https://dx.doi.org/10.54203/scil.2020.wvj59

Klyshevskaya SV (2010). Change in the content of micronutrients in soils during land reclamation. Bulletin of KrasGAU, 10: 45-48. Available at: https://elibrary.ru/item.asp?id=15486476

Kuznetsov S, and Kuznetsov A (2003). Micronutrients in animal feeding. Zhivotnovodstvo Rossii, 3: 16-18. Available at: https://fermer.ru/sovet/zhivotnovodstvo/80766

Makarov PM, Stepanova IA, Nazarova AA, Polischuk SD, and Churilov GI (2017). Physiological and biochemical parameters of holstein heifers when adding to their diet bio-drugs containing cuprum and cobalt nanoparticles. Nano Hybrids and Compos, 13: 123-129. DOI: http://doi.org./10.4028/www.scientific.net/NHC.13.123

Miroshnikova EP, Arinzhanov A, Kilyakova T, Sizova EA, and Miroshnikov SA (2015). Antagonism metal alloy nanoparticles of iron and cobalt: impact on micronutrient metabolism in carp and chicken. Human & Veterinary Medicine, 7 (4): 253-259. Available at: http://www.hvm.bioflux.com.ro/docs/2015.253-259.pdf

Myazin NG, Pavlov RA, and Sheina VV (2006). The effect of fertilizers on the accumulation of nitrates and heavy metals in soil and plants and on the productivity of a unit of grain-crop crop rotation. Agrochemistry, 2:22-29. Available at: https://www.elibrary.ru/item.asp?id=9217543

Natyrov AK, and Arilov AN (2002). Rationing of minerals in the diets of meat-breed male calves. Zootechnics, 5: 19–20. Available at: https://www.elibrary.ru/item.asp?id=9124894

Nazarova AA, Polishchuk SD, Stepanova IA, Churilov GI, Nguyen HC, and Ngo QB (2014). Biosafety of the application of biogenic nanometal powders in husbandry. Advances in Natural Sciences: Nanoscience and Nanotechnology, 5(1): 13-15. Available at: DOI: http://doi.org./10.1088/2043- 6262/5/1/015013

Nazarova AA, Stepanova IA, Churilov GI, Polischuk SD, Churilova VV, and Churilov DG (2019). Influence of copper nanopowder on parameters of carbohydrate and lipid metabolism of Holstein heifers. International Journal Nanotechnology, 16: 122-132. DOI: http://doi.org./10.1504/IJNT.2019.102399

Overton TR, and Yasui T (2014). Practical applications of trace minerals for dairy cattle. Journal of Animal Science, 92(2): 416-426. DOI: http://doi.org./10.2527/jas.2013-7145

Polischuk SD, Churilov GI, Borychev SN, Byshov NV, and Nazarova AA (2018). Nanopowders of cuprum, cobalt and their oxides used in the intensive technology for growing cucumbers. International Journal Nanotechnology, 15: 352-369. DOI: http://doi.org./10.1504/IJNT.2018.094792

Polischuk SD, Nazarova AA, and Stepanova IA (2015a). Toxicological characterization of bio-active drugs on basis of Iron Fe, Co, and Copper Cu nanopowders. IOP Conference Series.: Materials Science and Engineering,98: 012037. DOI: http://doi.org./10.1088/1757-899X/98/1/012037

Polischuk SD, Nazarova AA, Kutskir MV, Churilov DG, Ivanycheva YN, Kiryshin VA, and Churilov GI (2015b). Ecologic-biological effects of cobalt, cuprum, copper oxide nano-powders and humic acids on wheat seeds. Modern Applied Science, 9 (6): 354–364. DOI: http://dx.doi.org/10.5539/mas.v9n6p354

Sizova EA, Korolev VL, Makaev ShA, Miroshnikova EP, and Shakhov BA (2016). Morpho-biochemical parameters of broilers' blood when correcting the diet with salts and nanoparticles of Cu. Agricultural Biology, 51(6): 903-911. DOI: http://dx.doi.org/10.15389/agrobiology.2016.6.903rus

Skopichev VG, and Yakovlev VI (2008). Special physiology. Part 2. Food-producing animals physiology. Moscow, Russia. P. 555. Available at: https://www.elibrary.ru/item.asp?id=19511352

Voynar AI (1960). Biological role of micronutrients in animals and humans. Publishing house 2nd, Moscow. Available at: https://rusneb.ru/catalog/002072_000044_ARONB-RU_%D0%90%D1%80%D1%85%D0%B0%D0%BD%D0%B3%D0%B5%D0%BB%D1%8C%D1%81%D0%BA%D0%B0%D1%8F%20%D0%9E%D0%9D%D0%91_DOLIB_-080502/

Zaynalabdieva KhM, Arsanukaev DL, and Alekseeva LV (2014). Influence of Microelements on Hematological Parameters of Pigs. Izvestiya of Orenburg State Agrarian University, 2: 189-190. Available at: https://www.elibrary.ru/item.asp?id=21646802

Timasheva AB, Miroshnikov SV, Notova SV, and Lebedev SV (2014). Influence of Asparginat and Cuprum Nanoparticles in Biotic Dose on Element Status of LabAnimals. Microelements in Medicine, 15(2): 29-33. Available at: https://www.elibrary.ru/item.asp?id=21692640

To cite this paper: Stepanova IA, Nazarova AA and Arisov MV (2021). Peculiarities of Mineral Metabolism of Holstein Heifers' Diet Supplemented with Copper Nanopowders. *World Vet. J.*, 10 (4): 492-498. DOI: https://dx.doi.org/10.54203/scil.2020.wvj59

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 499-507, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj60

Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits

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REVIEW ARTICLE
pii: S232245682000060-10
Received: 04 Nov 2020
Accepted: 20 Dec 2020

ABSTRACT

Rabbits are considered an important and healthy source of animal protein all over the world. They are susceptible to important diseases that can reduce their productivity, causing severe economic losses. Coccidiosis is one of the important protozoon diseases caused by *Eimeria* species. Rabbits are highly susceptible to coccidiosis, especially after weaning time. Coccidiosis in rabbits has two forms, namely hepatic and intestinal. Affected animals indicated the symptoms of diarrhea, reduced appetite, dehydration, and weight loss as well as liver and intestinal lesions. Diagnosis is based on the detection of the infective stages of the protozoon in feces or affected tissues. Prevention and control are achieved by adopting hygienic measures and using different anticoccidial drugs. The use of natural alternatives for the prophylaxis of coccidiosis in rabbits indicated promising results. Vaccine production trials are still under investigation. Accordingly, this review article aims to shed light on coccidiosis in rabbits considering pathology, diagnosis, and control.

Keywords: *Eimeria*, Intestine, Liver, Rabbits, Treatment

INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) are regarded as a potential source of animal protein for human consumption. The meat of rabbits is recommended for human consumption more than other sources of proteins due to its high nutritious protein, calcium, phosphorus, and linoleic acid, with low fat and cholesterol contents (Nistor et al., 2013). In addition to the commercial use of rabbits, they can be used for wool production and in medical research as laboratory animals, and they are raised as pets for hobby purposes (Al-Mathal, 2008).

Rabbits are susceptible to dangerous viral, bacterial, and parasitic diseases that drastically affect their production. Coccidiosis or eimeriosis is one of the most important and widely distributed parasitic diseases in rabbits (Grès et al., 2003; El-Shahawi et al., 2012; Okumu et al., 2014; Yin et al., 2016; Bachene et al., 2019; Hamid et al., 2019). The disease affects the intestine, liver, and bile duct of the animal and is associated with digestive disorders (Manjunatha et al., 2019). Coccidiosis is a highly contagious disease that has negative impacts on the domestic rabbit industry worldwide (Pakandl, 2009; Geru et al., 2017). It seriously impairs growth performance parameters, causes high morbidity and mortality rates (Abdel-Megeed et al., 2005), and reduces the carcass weight by more than 23% (Barriga and Arnoni, 1981). Coccidiosis causes annual losses in the rabbit industry in the USA amounting to 127 million dollars, and similar losses may happen worldwide (Chapman, 2009). The prevalence of coccidiosis in rabbits is varied and influenced by geographical location, season, as well as management factors like housing and rearing conditions, and the use of preventive coccidiostats (Chowdhury and Fraser, 2008). Furthermore, the prevalence of coccidiosis in weaned rabbits is higher than that in growing and adult ones (El-Ghoneimy and El-Shahawy, 2017). Duszynski and Couch (2013) stated that the rate of coccidial infection ranged from 64% to 100% all over the world.

The disease is caused by an intracellular ubiquitous protozoon parasite of the genus *Eimeria* (Jing et al., 2012; Mäkitaipale et al., 2017). Mixed infection with more than one *Eimeria* spp. is common (Jithendran and Bhat, 1996). There are 15 *Eimeria* spp. that affect the intestinal tract and one species (*E. stidea)* affecting the liver and bile duct (Li and Ooi, 2009). *E. intestinalis*, *E. magna*, *E. piriformis*, *E. perforans*, *E. media*, *E. agnotsa*, *E. exigua*, *E. flavescens*, *E. irresidua*, *E. coecicola*, *E. vejdovskyi*, *E. roobroucki*, *E. oryctolagi*, *E. nagpurensis*, and *E. matsubayashi* invade the small intestine (Soulsby, 1968). The highly pathogenic *Eimeria* spp. in rabbits are *E. intestinalis* and *E. flavescens*, the moderately pathogenic spp. are *E. magna*, *E. irresidua*, and *E. piriformis*, and the low pathogenic or nonpathogenic spp. are *E. exigua*, *E. media*, *E. coecicola*, and *E. perforans* (Jithendran, 1995). The rapid spread of infection, due to the direct and short life cycle of the parasite, is characteristic of coccidiosis (García-Rubio et al., 2017). The severity of coccidial infection depends on the number of ingested oocysts, age, and the immune status of the animal (Miller and Fowler, 2003). In the early stage of infection with coccidiosis, sudden diarrhea, or death may occur without any clinical signs.

To cite this paper: Abd El-Ghany WA (2021). Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits. *World Vet. J.*, 10 (4): 499-507. DOI: https://dx.doi.org/10.54203/scil.2020.wvj60

Clinical infection is characterized by apathy, diarrhea, dehydration, reduced appetite, and weight loss resulting in death (Ogolla et al., 2018).

Prevention and control of coccidiosis in rabbits are achieved through careful management, enhancing rabbits' immunity, vaccination, and using synthetic anticoccidial drugs. Housing in large, dry, sunny, and disinfected rooms is very essential to fight coccidial infection (Pilarczyk et al., 2020). Although synthetic anticoccidials were effective, their hazardous use in the field resulted in some drawbacks. Therefore, there is a shift toward using novel approaches that pose a minimal risk to human or animal health. Different kinds of herbal extracts (Indrasanti et al., 2017; Sorour et al., 2018; Rivero-Perez et al., 2019) and acidifiers (Shkromada et al., 2019) showed successful preliminary results in treating coccidiosis in rabbits. Efficient vaccines are very important for the prevention of rabbit coccidiosis (Song et al., 2017), but so far, no vaccine is available for rabbits.

Given the above information, the aim of the present review is to shed light on coccidiosis in rabbits considering pathology, diagnosis, and control.

Parasite life cycle

Infection usually occurs through the ingestion of contaminated feed and water containing sporulated oocysts (i.e., infective oocysts consist of four sporocysts, each containing two sporozoites). Upon entering the gut, due to the effects of gastric and pancreatic juices, the oocysts walls rupture and the sporozoites invade the intestinal epithelial cells and then pass via the mesenteric lymph nodes and hepatic portal circulation to the liver where they enter the epithelial cells of the bile duct becoming trophozoites and then schizonts. The recognition and invasion processes may occur via the action of sugar residues in *Eimeria* sporozoites (John et al., 1999), followed by the release of merozoites (merogony stage) that form four generations in the asexual stage of the parasite. Lastly, male (micro) and female (macro) gametocytes combine sexually (gametogony stage) to form zygotes that develop into non-sporulated oocysts. The non-sporulated oocysts pass in the bile and are shed in the feces of the infected rabbits about 18 days after infection. Under favorable environmental conditions, sporulated oocysts are formed within three days (Gardiner et al., 1998).

Susceptibility

All breeds of domestic are highly susceptible to coccidiosis, and those of 1-4 months old more susceptible than adults (González-Redondo et al., 2008; Papeschi et al., 2013; Bachene et al., 2018). Suckling rabbits cannot be infected with coccidiosis before three weeks of age. It has been found that the production of *E. flavescens* and *E. intestinalis* oocysts in suckling rabbits increases with age (Pakandl and Hlásková, 2007). More susceptibility in young rabbits may be due to reduced immunity resulting from weaning stress, feeding and reproductive status (Drouet-viard et al., 1997a; Al-Mathal, 2008). Also, it was found that female rabbits were more infected than males (Faraj, 2017).

Types of rabbit coccidiosis

Hepatic coccidiosis

Hepatic coccidiosis involves the liver and bile ducts of rabbits causing cirrhosis and cholestasis (Singla et al., 2000). Hepatic coccidiosis is associated with severe economic losses in rabbitries and is caused by *E. stiedae* that is one of the most pathogenic species of *Eimeria* (Xin et al., 2016; Al-Taee and Al-Zubaidi, 2017). The oocysts of this species of *Eimeria* were first detected in the bile of infected rabbits in 1674 (Duszynski and Couch, 2013). The most adverse effects of hepatic coccidiosis are reduced growth and feed utilization and increased mortality rate in young rabbits (Hanptman et al., 2001). Like other *Eimeria* spp., *E. stiedae* penetrates the intestinal wall and migrates to the bile ducts where it reproduces (Kraus et al., 1984). Hepatic coccidiosis is mostly chronic and subclinical infection specifically in adults who are carriers and sources of infection (Barriga and Arnoni, 1981; Al-Mathal, 2008; Pakandl, 2009). Some of the affected animals displayed symptoms of anorexia, polydipsia, brown watery diarrhea, dehydration, icteric membranes, poor feed conversion, growth retardation, wasting of the back and hindquarters, coarse hair, abdominal distension, and even death especially in young rabbits with severe infection (Erdogmus and Eroksuz, 2006; Lakshmanan et al., 2011; Al-Saeed et al., 2017). High mortality results from high doses of oocysts, while morbidity results from diarrhea and reduction in body weight (Renaux et al., 2003). Several studies have described the post-mortem findings of hepatic coccidiosis. On gross examination, enlarged and cirrhosed liver with multiple distributed whitish nodules containing creamy thick exudate, and distended gall bladder has been reported. Moreover, histologic findings included cholangitis, bile duct hyperplasia, hemorrhage, dilatation and congestion of the central veins as well as necrosis of hepatocytes and hepatic fibrosis (Sanyal and Sharma, 1990; Cam et al., 2008; AL-Naimi et al., 2012; Sorour et al., 2018).

Intestinal coccidiosis

There are about 15 species of *Eimeria* that are known to induce pathology in the intestine of rabbits causing intestinal coccidiosis. The most common *Eimeria* spp. that cause intestinal coccidiosis in rabbits are *E. magna*, *E.*

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irresidua, *E. media*, and *E. perforans* (Fox, 1984). The parasite colonizes distinct parts of the intestine and the mucosa at different depths (Pakandl, 2009). Intestinal coccidiosis often is observed in rabbits aged from six weeks to five months old. Older animals can acquire immunity after recovery and become carriers (Kulisic et al., 2006). This type of coccidial infection results in atrophy of the intestinal villi, malabsorption of nutrients, hypoproteinemia, electrolyte imbalance, dehydration, anemia (Dakshinkar and Dharmadhikari, 1985; Hana et al., 2011) and is manifested by diarrhea, weight loss, and mortality (Lebas et al., 1986). Affected rabbits suffer from weakness, gnashing teeth, dirty anus, weight loss, soft to watery hemorrhagic diarrhea, dehydration, and thirst (Fioramonti et al., 1982). Inflammation and edema in the ileum and jejunum associated with mucosal bleeding and ulcerations were observed (Coudert et al., 1995; Oncel et al., 2011). There are differences in pathogenicity among intestinal coccidial spp. (Jithendran, 1995). These differences in pathogenicity can be determined through the experimental infection of animals with different *Eimeria* species.

Laboratory diagnosis

Laboratory diagnosis of hepatic and intestinal coccidiosis depends on the analysis of feces of suspected rabbits. Microscopic identification of *Eimeria* spp. oocysts through the fecal analysis of suspected animals is very important (Pakandl et al., 2008). Developmental stages of *E. stiedae* have been detected in stained impression smears from the liver (Al-Rukibat et al., 2001; Sivajothi et al., 2016). Histopathological examination of the liver tissues, bile duct, or intestine is also used for the detection of different developmental stages of the parasite (Sivajothi et al., 2016). The oocyte detection site under a microscope is a guide to determining *Eimeria* species. Immuno-diagnosis of *E. stiedae* was investigated in previous studies (Zayed and Kutkat, 1998; Kandil et al., 2000). Serological diagnosis of *E. stiedae* using ELISA was reported (Abu-El-Ezz et al., 2010; Wei et al., 2020). Identification of *Eimeria* spp. using molecular assays such as multiplex PCR assay was reported (Oliveira et al., 2011; Yan et al., 2013). Hassan et al. (2015) detected *E. stiedae* schizonts using PCR 12 days after the experimental infection of rabbits, and this occurred prior to the development of lesions or shedding of the oocysts in feces.

It should be noted that the presence of oocysts in fecal samples does not confirm the presence of clinical disease. It has been reported that rabbits with high-intensity infection showed no clinical symptoms of coccidiosis (Pilarczyk et al., 2020). The induction of symptoms may depend on the virulence and pathogenicity of the infecting *Eimeria* spp. (Pakandl, 2009).

Prevention and treatment

Management practices

Coccidiosis in rabbits is aggravated by poor hygienic conditions and high stocking densities that encourage the spread of protozoa (González-Redondo et al., 2008). Rabbits raised in groups are more affected than those kept alone (Sharma et al., 2016). Accordingly, the first steps for preventing the occurrence and spread of coccidiosis in a rabbitry are proper hygiene and husbandry practices as well as strict biosecurity measures (Pakandl et al., 2008; Schlolaut et al., 2013). Control of coccidial infection using common disinfectants is difficult as oocysts have a remarkable ability to survive under exogenous environmental conditions (Chapman et al., 2013).

Anticoccidial drugs

Prevention of coccidiosis in rabbits using coccidiostats is regulated by Regulation (EC) No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition. Globally, synthetic anticoccidial drugs, either ionophores or synthetic chemicals, remain the mainstream pharmaceuticals that are used for the control of rabbit coccidiosis (Pakandl, 2009). Anticoccidial drugs should be broad-spectrum, highly effective with a good therapeutic index, and easily administered for short time. Coccidiostats in rabbits prevent the developmental stages (schizogony and gamogony stages) of the parasite inside the host. Coccidiostats are usually added to the feed of animals; however, coccidiocidal drugs are added to water. It is preferable to prevent coccidial infection before its occurrence as the treatment is usually not very successful when clinical signs of coccidiosis appear (Pakandl, 2009). A previous study by Peeters et al. (1981) showed that concordant infection with hepatic and intestinal *Eimeria* spp. could be treated with narasin, while hepatic coccidiosis could be prevented by clopidol/methylbenzoquate, robenidine, and salinomycin (Peeters et al., 1982). Several reports recommended the use of toltrazuril for either prevention or treatment of coccidiosis in rabbits (Mikhail et al., 1981; Redrobe et al., 2010; Qamar et al., 2013; El-Ghoneimy and El-Shahawy, 2017). In a study by Vereecken et al. (2012), treatment with diclazuril, salinomycin, and robenidine showed significant improvement in both growth performance and parasitological parameters in infected rabbits compared to non-treated animals. However, using 25 ppm toltrazuril/liter of drinking water for two days was successful in treating the clinical hepatic coccidiosis (Singla et al., 2000), increasing body weight and lowering the mortality rate in rabbits infected with mixed intestinal and hepatic *Eimeria* spp. compared to control animals (Balicka-Ramisz et al., 2014). Treatment with toltrazuril immediately reduced signs and oocysts shedding, allowing the development of immunity against reinfection (Peeters and

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Greeroms, 1986). Treatment using diclazuril is recommended for eliminating oocyst shedding in rabbits worldwide (Vanparijs et al., 1989a, b; Peeters and Geeroms, 1989; Polozowski, 1993; Vereecken et al., 2012). Diclazuril and sulfachlorpyridazine were efficacious in treating rabbit coccidiosis (Ogolla et al., 2018). Studies presented variable results following the prophylactic and therapeutic use of sulphonamides in treating coccidiosis (Joyner et al., 1983; Polozowski, 1993; Redrobe et al., 2010; Qamar et al., 2013). Kolabskii et al. (1973) and Ogolla et al. (2018) reported the effectiveness of sulphachloropyrazine in controlling clinical coccidiosis in rabbits. Successful control of hepatic coccidiosis using sulphaquinoxaline was also reported (Magray et al., 2010). Amprosol, bifuran, and sulpha-based drugs have been used for the prevention of rabbit coccidiosis (Bhat et al., 2010). Trimethoprim-sulfamethoxazole showed moderate to satisfactory efficacious results in the treatment of field infection (Ogolla et al., 2018). It has been documented that sulfonamides protected against experimental *E. magna* and *E. media* infections better than colistin and trimethoprim (Bachene et al., 2019). Amprolium could not treat intestinal and hepatic coccidiosis (Laha et al., 1999; Ogolla et al., 2018). On the other hand, superior effects have been demonstrated following the prophylactic use of amprolium in intestinal coccidiosis especially when applied concurrently with other anticoccidials (Qamar et al., 2013; Laha et al., 2015; El-Ghoneimy and El-Shahawy, 2017). Some anticoccidials such as sulphonamides, salinomycin, and robenidine were toxic for pregnant does and kids, while few others used for poultry were recommended for rabbits (Ogolla et al., 2018).

Alternatives to anticoccidial drugs

Anticoccidial drugs and are relatively inexpensive and showed successful results. However, increase in consumer demand for the production of organic products, the potential development of resistant strains of parasites toward drugs (Pakandl, 2009), and the presence of antibiotic residues in meat created a potential need for searching for natural and safe alternatives to anticoccidial chemicals. Hence, several studies investigated the effects of natural alternatives such as sulfur and sulfates (including copper sulfate), tannic acid, bismuth compounds, thymol, camphor, alum, volatile oils, and garlic, oregano, sage, caraway, cinnamon, basil, and rosemary on rabbit coccidiosis (Kowalska et al., 2012). Oral prophylactic administration of garlic was effective in the amelioration of *E. stiedae* infection (Toulah and Al-Raw, 2007; Abu-Akkada et al., 2010; Indrasanti et al., 2017). Adding of a mixture of garlic oil and oregano to rabbit feed can also help in disease prevention (Kowalska et al., 2012; Nosal et al., 2014). Other plant extracts such as banana stem extract could decrease the number of *E. stiedae* oocysts *in vitro* (Indrasanti et al., 2015). However, Matekaire et al. (2005) stated that both banana root (*Musa paradisiaca*) and sulphadimidine sodium treatments caused a significant decrease in oocyst excretion in rabbits. Neem extract improved body weight and liver function in experimentally infected rabbits with *E. stiedae*, and these results were similar to those in the toltrazuril treated group (Ahmed et al., 2014). Cervantes-Valencia et al. (2015) demonstrated that the hydroalcoholic extract of *Curcuma longa* at doses of 25 and 40 mg/kg body weight reduced the fecal *Eimeria* sp. excretion in naturally infected rabbits. Artemisinin liquid extract, cinnamon, and clove essential oils could also be used for protection against *E. stiedae* infection in rabbits compared to toltrazuril (Sorour et al., 2018). Furthermore, it has been demonstrated that 25 and 50 mg *Salix babylonica* hydroalcoholic extract per kg of body weight successfully reduced the intestinal *Eimeria* oocyst count per gram of feces in rabbits (Rivero-Perez et al., 2019). The effects of herbal antioxidants (*Psidium guajava*) as inhibitors of *E. intestinalis*, *E. magna*, *E. flavescens*, and *E. stiedae* sporozoites were evaluated *in vitro*, and preliminary results showed that these herbs were the best substitutes to chemical anticoccidials (Cedric et al., 2017). A dose of 20 g *Calotropis procera* dried leaves powder per kg pelleted ration of rabbits reduced the number of oocysts in feces with the absence of alterations in the intestine and liver tissues of rabbits with mixed infections (Seddek et al., 2015). Aloe vera and liquid paraffin showed varied efficacy in the treatment of rabbit coccidiosis (Ogolla et al., 2017). Shkromada et al. (2019) proved that water treatment of rabbits with an acidifier concentrate (a mixture of benzoic, acetic, and propionic acids) reduced *Eimeria* invasion, increased body weight, and improved feed conversion. In addition, compared to anticoccidial drugs, this composition was not toxic, improved digestion processes, and inhibited the development of conventionally pathogenic microflora.

Vaccines

Vaccinations using oral or spray dispersion of precocious live lines of *E. magna* oocysts in the nest boxes (Drouet-Viard et al., 1997a, b; Licois, 2004), and vaccination against *E. magna* and *E. media* (Akpo et al., 2012) showed satisfactory preliminary results. Some efforts have been directed toward the production of recombinant vaccines against coccidiosis in rabbits (Hanada et al., 2003; Abdel-Megeed et al., 2005; Song et al., 2010). A recent trial for the preparation of a vaccine against hepatic coccidiosis was carried out. Using immuno-proteomic analysis of sporozoite proteins of *E. stiedae*, the immuno-reactive proteins were recognized by the sera of infected rabbits, which may be helpful for the production of a vaccine (Song et al., 2017). However, production of a vaccine on a commercial and field scale is still distant and limited, may be due to the high cost of production and the time required for the processes of

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optimization, registration, safety assessment, and distribution to customers (Song et al., 2017). Vaccination is promising and still requires extensive research and development to be applied in the near future.

CONCLUSION

As coccidiosis is considered a very important parasitic disease in rabbits, future studies should focus on finding novel approaches for the prevention and control of such a significant threat.

DECLARATIONS

Competing interests

The author has no conflict of interest.

REFERENCES

Abdel-Megeed KN, Abu El-Ezz NM and Abdel-Rahman EH (2005). Protective effect of *Eimeria stiedae* coproantigen against hepatic coccidiosis in rabbits. Journal of the Egyptian Society of Parasitology, 35: 581-595. Available at: https://pubmed.ncbi.nlm.nih.gov/16083069/

Abu-Akkada SS, Samah SO and Ashmawy KI (2010). Garlic and hepatic coccidiosis: prophylaxis or treatment? Tropical Animal Health and Production, 4: 1337-1343. DOI: https://www.doi.org/10.1007/s11250-010-9590-6

Abu-El-Ezz NMT, Megeed KNA, Mahdy OA and Hassan SE (2010). ELISA assessment in the diagnosis of hepatic coccidiosis in experimentally infected rabbits. Global Veterinaria, 9: 517-523. Available at: DOI: https://www.dx.doi.org/10.5829/idosi.gv.2012.9.5.6620

Ahmed SE, Abdel Razek MSZ, Ramadan ME and Esmail EM (2014). Hepatic coccidiosis in rabbits and comparative study on treatment with herbal drug. Zagazig Veterinary Journal, 42: 41-50. DOI: https://www.dx.doi.org/10.21608/zvjz.2014.59960

Akpo Y, Kpodekon MT, Djago Y, Licois D and Youssao IA (2012). Vaccination of rabbits against coccidiosis using precocious lines of *Eimeria magna* and *Eimeria media* in Benin. Veterinary Parasitology, 184: 73-76. DOI: https://www.doi.org/10.1016/j.vetpar.2011.08.012

Al-Mathal IM (2008). Hepatic coccidiosis of the domestic rabbit *Oryctolagus cuniculus domesticus* L. in Saudi Arabia. World Journal of Zoology, 3: 30-35. Available at: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.506.3784&rep=rep1&type=pdf

AL-Naimi RAS, Khalaf OH, Tano SY and Al-Taee EH (2012). Pathological study of hepatic coccidiosis in naturally infected rabbits. AL-Qadisiya Journal of Veterinary Medical Science, 11: 63-69. Available at: https://www.iasj.net/iasj?func=fulltext&aId=80344

Al-Rukibat RK, Irizarry AR, Lacey JK, Kazacos KR, Storandt ST and DeNicola DB (2001). Impression smear of liver tissue from a rabbit. Veterinary Clinical Pathology, 30: 57-61. DOI: https://www.doi.org/10.1111/j.1939-165X.2001.tb00259.x

Al-Saeed MH, Al Saeed AH and Jori MM (2017). Study of physiological and histological changes in rabbits induced with hepatic coccidiosis. Journal University of Kerbala, 15: 217-228. Available at: https://kj.uokerbala.edu.iq/article_123448.html

Al-Taee MNK and Al-Zubaidi MTS (2017). Protection against *Eimeria stiedae* in rabbits by using sonicated sporulated oocyst vaccine. Journal of Entomology and Zoology Studies, 205: 579-585. Available at: https://www.entomoljournal.com/archives/?year=2017&vol=5&issue=4&ArticleId=2110

Bachene MS, Temim S, Ainbaziz H and Bachene A (2019). Prevalence of rabbit coccidia in Medea Province, Algeria. World's Veterinary Journal, 9: 123-128. DOI: https://www.dx.doi.org/10.36380/scil.2019.wvj16

Bachene MS, Temim S, Ainbaziz H, Bachene A and Suo X (2018). A vaccination trial with a precocious line of *Eimeria magna* in Algerian local rabbits *Oryctolagus cuniculus*. Veterinary Parasitology, 261: 73-76. DOI: https://www.doi.org/10.1016/j.vetpar.2018.08.013

Balicka-Ramisz A, Wróbel M and Adadyńska K (2014). Epidemiology and economic benefits of treating rabbits coccidiosis in small farms from West Pomerania province, Poland. Annals of Parasitology, 60: 247-251. PMID: 25706421. Available at: https://pubmed.ncbi.nlm.nih.gov/25706421/

Barriga OO and Arnoni JV (1981). Pathophysiology of hepatic coccidiosis in rabbits. Veterinary Parasitology, 8: 201-210. DOI: https://www.doi.org/10.1016/0304-4017(81)90051-0

Bhat TK, Jlthendran KP and Kurade NP (2010). Rabbit coccidiosis and its control: a review. World Rabbit Science, 4: 37-41. DOI: https://www.doi.org/10.4995/wrs.1996.269

Cam Y, Alasever A, Eraslan G, Kibar M, Atalay O, Beyaz L, Inci A and Liman BC (2008). *Eimeria stiedae*: Experimental infection in rabbits and the effect of treatment with toltrazuril and ivermectin. Experimental Parasitology, 119: 164-171. DOI: https://www.doi.org/10.1016/j.exppara.2008.01.005

Cedric Y, Payne VK, Nadia NAC, Kodjio N, Kollins E, Megwi L, Kuiate JR and Mbida M (2017). *In vitro* anticoccidial, antioxidant activities and cytotoxicity of *Psidium guajava* extracts. Journal of Parasitic Diseases: Diagnosis and Therapy, 2: 14-24. Available at: https://www.alliedacademies.org/articles/in-vitro-anticoccidial-antioxidant-activities-and-cytotoxicity-of-psidium-guajava-extracts-8865.html

Cervantes-Valencia ME, Alcala-Canto Y, Salem AZM, Kholif AE, Ducoing-Watty AM, Bernad-Bernad MJ and Gutiérrez-Olvera C (2015). Influence of curcumin (*Curcuma longa*) as a natural anticoccidial alternative in adult rabbits: first results. Italian Journal of Animal Science, 14: 3838. DOI: https://www.doi.org/10.4081/ijas.2015.3838

To cite this paper: Abd El-Ghany WA (2021). Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits. *World Vet. J.*, 10 (4): 499-507. DOI: https://dx.doi.org/10.54203/scil.2020.wvj60

Chapman HD (2009). A landmark contribution to poultry science prophylactic control of coccidiosis in poultry. Poultry Science, 88: 813-815. DOI: https://www.doi.org/10.3382/ps.2008-00316

Chapman HD, Barta JR, Blake D, Gruber A, Jenkins M, Smith NC, Suo X and Tomley FM (2013). A selective review of advances in coccidiosis research. Advances of Parasitology, 83: 93-171. DOI: https://www.doi.org/10.1016/b978-0-12-407705-8.00002-1

Chowdhury AA and Fraser GC (2008). Coccidia (*Eimeria* spp.) of domestic rabbits in New South Wales. Australian Veterinary Journal, 86: 365-366. DOI: https://www.doi.org/10.1111/j.1751-0813.2008.00340.x

Coudert P, Licois D, Provot F and Drouet-Viard F (1995). *Eimeria* species and strains of rabbits. In: Eckert J, Braun R, Shirley MW, Coudert P, Editors: Guidelines on techniques in coccidiosis research. COST 89/820: Biotechnology. European Commission, Luxembourg, 52–73.

Dakshinkar NP and Dharmadhikari DN (1985). Haematological observations in intestinal coccidiosis during clinical outbreak. Poultry Adviser, 18: 55-56. Available at: http://cehea.org/wp-content/uploads/2016/08/59-.pdf

Drouet-viard F, Coudert P, Licois P and Boivin M (1997a). Acquired protection of the rabbit (*Oryctolagus cuniculus*) against coccidiosis using a precocious line of *Eimeria magna*: effect of vaccine dose and age at vaccination. Veterinary Parasitology, 69: 197-201. DOI: https://www.doi.org/10.1016/S0304-4017(96)01133-8

Drouet-viard F, Coudert P, Licois D and Boivin M (1997b). Vaccination against *Eimeria magna* coccidiosis using spray dispersion of precocious line oocysts in the nest box. Veterinary Parasitology, 70: 61-66. DOI: https://www.doi.org/10.1016/S0304-4017(96)01134-X

Duszynski DW and Couch L (2013). The Biology and Identification of the Coccidia (Apicomplexa) of Rabbits of the World. San Diego, California, USA. Academic Press, pp. 4-5.

El-Ghoneimy A and El-Shahawy I (2017). Evaluation of amprolium and toltrazuril efficacy in controlling natural intestinal rabbit coccidiosis. Iranian Journal of Veterinary Research, 18: 164-169. DOI: https://www.ncbi.nlm.nih.gov/pubmed/29163644

El-Shahawi GA, El-Fayomi HM and Abdel-Haleem HM (2012). Coccidiosis of domestic rabbit (*Oryctolagus cuniculus*) in Egypt: Light microscopic study. Parasitology Research, 110: 251-258. DOI: https://www.doi.org/10.1007/s00436-011-2479-0

Erdogmus ZS and Eroksuz Y (2006). DS hepatic coccidiosis in Angora rabbits. Journal of Animal and Veterinary Advances, 5: 462-463. DOI: https://medwelljournals.com/abstract/?doi=javaa.2006.462.463

Faraj AA (2017). Molecular diagnosis of natural infection with *Eimeria stiedae* in domestic rabbits in Baghdad city-Iraq. Journal of Entomology and Zoological Studies, 5: 1438-1440. Available at: https://www.entomoljournal.com/archives/?year=2017&vol=5&issue=3&ArticleId=1974

Fioramonti J, Sorraing JM, Licois D and Bueno L (1982). Intestinal motor and transit disturbances associated with experimental coccidiosis (*Eimeria magna*) in the rabbit. Annals of Veterinary Research, 12: 413-420. Available at: https://pubmed.ncbi.nlm.nih.gov/7137853/

Fox JG (1984). Laboratory Animal Medicine. Academic Press Inc.: New York, 1984.

García-Rubio V, Bautista-Gómez LG, Martínez-Castañeda JS and Romero-Núñez C (2017). Multicausal etiology of the enteric syndrome in rabbits from Mexico. Revista Argentina de Microbiologia, 49: 132-138. DOI: https://www.doi.org/10.1016/j.ram.2017.03.001

Gardiner GH, Fayer R and Dubey JP (1998). Apicomplexa. In: An Atlas of Protozoan Parasites in Animal Tissues, 2nd edition, Armed Forces Institute of Pathology, Washington, DC., 20-30.

Geru T, Wang Y, Li C, Gu X, Cui P, Fang S, Suo X and Liu X (2017). High pathogenicity and strong immunogenicity of a Chinese isolate of *Eimeria magna* Pérard, 1925. Parasitology International, 66: 207-209. DOI: https://www.doi.org/10.1016/j.parint.2017.01.014

González-Redondo P, Finzi A, Negretti P and Micci M (2008). Incidence of coccidiosis in different rabbit keeping systems. Brazilian Archive of Veterinary Medicine and Animal Science, 60: 1267-1270. DOI: https://www.doi.org/10.1590/S0102-09352008000500034

Grès V, Voza T, Chabaud A and Landau I (2003). Coccidiosis of the wild rabbit (*Oryctolagus cuniculus*) in France. Parasite, 10: 51-57. DOI: https://www.doi.org/10.1051/parasite/2003101p51

Hamid PH, Prastowo S and Kristianingrum Y (2019). Intestinal and hepatic coccidiosis among rabbits in Yogyakarta, Indonesia. Veterinary World, 12: 1256-1260. DOI: https://www.ncbi.nlm.nih.gov/pubmed/31641305

Hana A, Salasia SIO, Mangkoewidjojo S and Kusindarto DL (2011). Blood profile of rabbits infected with *Eimeria magna*. Animal Production, 13: 185-190. Available at: http://animalproduction.net/index.php/JAP/article/view/333

Hanada S, Umemoto Y, Omata Y, Koyama T, Nishiyama K, Kobayashi Y, Furuoka H, Matsui T, Maeda R and Saito A (2003). *Eimeria stiedae* merozoite 49-kDa soluble antigen induces protection against infection. Journal of Parasitology, 89: 613-617. DOI: https://www.doi.org/10.1645/0022-3395(2003)089[0613:esmksa]2.0.co;2

Hanptman K, Tichy F and Knotek Z (2001). Clinical diagnostics of hepatopathies in small mammals: Evaluation of importance of individual method. Acta Veterinaria Brno, 70: 297-311. DOI: https://www.doi.org/10.2754/avb200170030297

Hassan KM, El-Askalany MA, Mousa WM, Shokier KAM, Arafa WM and Aboelhadid SM (2015). Molecular diagnosis of *Eimeria stiedae* in hepatic tissue of experimentally infected rabbits in comparison with traditional methods. Egyptian Veterinary Medical Society of Parasitology Journal, 11: 51-57. Available at: https://www.researchgate.net/deref/http%3A%2F%2Fdx.doi.org%2F10.21608%2Fevmspj.2015.37917?_sg%

Indrasanti D, Indradji M, Hastuti S, Fatikha AE and Rosyadi KA (2017). The administration of garlic extract on *Eimeria stiedai* oocysts and the hematological profile of the coccidia infected rabbits. Media Peternakan, 40: 158-164. DOI: https://www.doi.org/10.5398/medpet.2017.40.3.158

To cite this paper: Abd El-Ghany WA (2021). Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits. *World Vet. J.*, 10 (4): 499-507. DOI: https://dx.doi.org/10.54203/scil.2020.wvj60

Indrasanti D, Indradji M, Hastuti S, Wihadmadyatami H and Ismoyowati A (2015). The efficacies of banana stem extract as a candidate of coccidiostat against rabbit *Eimeria stiedai* oocysts: An *in vitro* analysis. Animal Production 17: 161-168. DOI: https://www.dx.doi.org/10.20884/1.anprod.2015.17.3.503

Jing F, Yin G, Liu X, Suo X and Qin Y (2012). Large-scale survey of the prevalence of *Eimeria* infections in domestic rabbits in China. Parasitology Research, 110: 1495-1500. DOI: https://www.doi.org/10.1007/s00436-011-2653-4

Jithendran KP (1995). Clinical coccidiosis in Angora rabbits. Veterinary Revista Kathmandu, 10: 21-22.

Jithendran KP and Bhat T (1996). Subclinical coccidiosis in angora rabbits, a field survey in Himachal Pradesh, India. World Rabbit Science, 4: 29-32. DOI: https://www.doi.org/10.4995/wrs.1996.267

John NM, Zea ME, Kawano T, Omata Y, Saito A, Toyoda Y and Milkano T (1999). Identification of carbohydrates on *Eimeria stiedae* sporozoites and their role in the invasion of cultured cells *in vitro*. Veterinary Parasitology, 81: 99-105. DOI: https://www.doi.org/10.1016/s0304-4017(98)00239-8

Joyner LP, Catchpole J and Berrett S (1983). *Eimeria stiedai* in rabbits: The demonstration of responses to Chemotherapy. Research in Veterinary Science, 34: 64-67. DOI: https://www.doi.org/10.1016/S0034-5288(18)32285-9

Kandil OM, Mahdy OA, Mousa WM and Derbala AA (2000). Evaluation of two *E. stiedae* antigens for serodiagnosis of hepatic coccidiosis in experimentally infected rabbits. Journal of Egyptian Veterinary Medical Association, 60: 83-91. Available at: https://scholar.cu.edu.eg/?q=drolfat/publications&sort=year&order=asc

Kolabskii NA, Dubovoi BL and Vergerenko LV (1973). Effectiveness of sulfachlorpyrazine in coccidiosis of rabbits. Veterinariia, 4: 71-73. Available at: https://www.hindawi.com/journals/jvm/2018/5402469/

Kowalska D, Bielanski P, Nosel P and Kowal J (2012). Natural alternatives to coccidiostats in rabbit nutrition. Annals of Animal Science, 12: 561-574. DOI: http://www.izoo.krakow.pl/en/periodicals

Kraus AL, Weisbroth SH, Flatt RE and Brewer N (1984). Biology and diseases of rabbits. In: laboratory animal medicine Fox, J.G. (ed.), Pp: 270-240. Academic Press, Orlando, USA.

Kulisic Z, Tambur Z, Malicevic Z, Aleksic-Bakrac N and Misic Z (2006). White blood cell differential count in rabbits artificially infected with intestinal coccidia. Journal of Protozoology Research, 16: 42-50. DOI: https://www.doi.org/10.32268/jprotozoolres.16.3-4_42

Laha R, Das M and Goswami A (2015). Coccidiosis in rabbits in a subtropical hilly region. Indian Journal of Animal Research, 49: 231-233. DOI: https://arccjournals.com/journal/indian-journal-of-animal-research/B-2614

Laha R, Dey HA and Harbola PC (1999). Comparative efficacy of sulphadimidine and combination of amprolium, sulphaquinoxaline in the control of natural coccidial infection in rabbits. Indian Veterinary Journal, 76: 1013-1015. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6218750/

Lakshmanan B, Ravindran R, Vasudeven VN and Devada K (2011). Hepatic coccidiosis in rabbits in Kerala. Journal of Indian Veterinary Association, Kerala, 9: 56-57. DOI: http://jivaonline.net/jivaonline/index.php

Lebas F, Coudert P, Rouvier R and De Rochambeau H (1986). The rabbit husbandry, health and production. Animal Production and Health, no. 21, FAO, Rome, Italy.

Li M and Ooi H (2009). Fecal occult blood manifestation of intestinal *Eimeria* spp. infection in rabbit. Veterinary Parasitology, 161: 327-329. DOI: https://www.doi.org/10.1016/j.vetpar.2009.01.009

Licois D (2004). Domestic rabbit enteropathies. Proc. 8th World Rabbit Congress, invited paper. Puebla, Mexico, pp. 385-403.

Magray A, Khan AR and Rashid M (2010). Study on fatal hepatic coccidiosis in rabbits of Kashmir Valley. Journal of Environment and Biological Sciences, 24: 243-245. Available at: https://zvjz.journals.ekb.eg/article_59960_2c1fcb70729e80575ebc5585fa4eaf1d.pdf

Mäkitaipale J, Karvinen I, Virtala AMK and Näreaho A (2017). Prevalence of intestinal parasites and risk factor analysis for *Eimeria* infections in Finnish pet rabbits. Veterinary Parasitology, Regional Studies and Reports, 9: 34-40. DOI: https://www.doi.org/10.1016/j.vprsr.2017.04.005

Manjunatha V, Rout M, Sujay CS, Jaisingh N, Salin N and Byregowda SM (2019). Clinico-pathologic observations of spontaneous hepatic coccidiosis in broiler rabbits maintained in Bannerghatta Biological Park in Karnataka state of India. Indian Journal of Animal Research, 53: 528-532. DOI: https://www.doi.org/10.18805/ijar.B-3436

Matekaire T, Mupangwa JF and Kanyamura EF (2005). The efficacy of banana plant (*Musa paradisiaca*) as a coccidiostat in rabbits. International Journal of Applied Research in Veterinary Medicine, 3: 326-331. Available at: arvm.com/articles/Vol3Iss4/MATEKAIRE%20IJARVM%20V3N4W.pdf

Mikhail EG, Sabet S, El-Boulaqi HA, Zaki IE and Gaber A (1981). Treatment of hepatic coccidiosis in rabbits by tinidazole. Journal of the Egyptian Society of Parasitology, 1: 389-397. PMID: 7299175. Available at: https://europepmc.org/article/med/7299175

Miller RE and Fowler ME (2003). Zoology and Wild Animal Science. McGraw-Hill: London.

Nistor E, Bampidis VA, Pacala N, Pentea M, Tozer J and Prundeanu A (2013). Nutrient content of rabbit meat as compared to chicken, beef and pork meat. Journal of Animal Production Advances, 3: 172-176. DOI: https://www.doi.org/10.5455/JAPA.20130411110313

Nosal P, Kowalska D, Bielanski P, Korwal J and Kornas S (2014). Herbal formulations as feed additives in the course of rabbi subclinical coccidiosis. Annals of Parasitology, 60: 65-69. PMID: 24930248. Available at: https://pubmed.ncbi.nlm.nih.gov/24930248/

Ogolla KO, Chebet J and Gathumbi PK (2017). Farmer practices that influence risk factors, prevalence and control strategies of rabbit coccidiosis in Central Kenya. Livestock Research for Rural Development, p. 29. DOI: http://www.lrrd.org/lrrd29/7/koko29134.html

To cite this paper: Abd El-Ghany WA (2021). Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits. *World Vet. J.*, 10 (4): 499-507. DOI: https://dx.doi.org/10.54203/scil.2020.wvj60

Ogolla KO, Gathumbi PK, Waruiru RM, Okumu PO, Kitala PM and Chebet J (2018). Efficacy of sulphachloropyrazine, amprolium hydrochloride, trimethoprim-sulphamethoxazole, and diclazuril against experimental and natural rabbit coccidiosis. Journal of Veterinary Medicine: 5402469. DOI: https://www.dx.doi.org/10.1155%2F2018%2F5402469

Okumu PO, Gathumbi PK, Karanja DN, Mande JD, Wanyoike MM, Gachuiri CK, Kiarie N, Mwanza RN and Borter DK (2014). Prevalence, pathology and risk factors for coccidiosis in domestic rabbits (*Oryctolagus cuniculus*) in selected regions in Kenya. Veterinary Quarterly, 34: 205-210. DOI: https://www.doi.org/10.1080/01652176.2014.978044

Oliveira UC, Fraga JS, Licois D, Pakandl M and Gruber A (2011). Development of molecular assays for the identification of the 11 *Eimeria* species of the domestic rabbit (*Oryctolagus cuniculus*). Veterinary Parasitology, 176: 275-280. DOI: https://www.doi.org/10.1080/01652176.2014.978044

Oncel T, Gulegen E, Senlik B and Bakirci S (2011). Intestinal coccidiosis in Angora rabbits (*Oryctolagus cuniculus*) caused by *Eimeria intestinalis*, *Eimeria perforans* and *Eimeria coecicola*. YYU Veteriner Fakultesi Dergisi, 22: 27-29. Available at: http://vfdergi.yyu.edu.tr/archive/2011/22-1/2011_22_(1)_27-29.pdf

Pakandl M (2009). Coccidia of rabbit: A review. Folia Parasitologica, 56: 153-166. DOI: https://www.doi.org/10.14411/fp.2009.019

Pakandl M and Hlásková L (2007). The reproduction of *Eimeria flavescens* and *Eimeria intestinalis* in suckling rabbits. Parasitology Research, 101: 1435-1437. DOI: https://www.doi.org/10.1007/s00436-007-0646-0

Pakandl M, HlaskovaL, Poplstein M, Chroma M, Vodicka T, Salat J and Mucksova J (2008). Dependence of the immune response to coccidiosis on the age of rabbit suckling. Parasitology Research, 103: 1265-1271. DOI: https://www.doi.org/10.1007/s00436-008-1123-0

Papeschi C, Fichi G and Perrucci S (2013). Oocyst excretion pattern of three intestinal *Eimeria* species in female rabbits. World Rabbit Science, 21: 77-83. DOI: https://www.doi.org/10.4995/wrs.2013.1235

Peeters JE and Geeroms R (1986). Efficacy of toltrazuril against intestinal and hepatic coccidiosis in rabbits. Veterinary Parasitology, 22: 21-35. DOI: https://www.doi.org/10.1016/0304-4017(86)90004-X

Peeters JE and Geeroms R (1989). Efficacy of diclazuril against robenidine resistant *Eimeria magna* in rabbits. The Veterinary Record, 124: 589-590. DOI: https://www.doi.org/10.1136/vr.124.22.589

Peeters JE, Geeroms R, Antoine O, Mammerickx M and Halen P (1981). Efficacy of narasin against hepatic and intestinal coccidiosis in rabbits. Parasitology, 83, 293-301. DOI: https://www.doi.org/10.1017/s0031182000085309

Peeters JE, Geeroms R, Molderez J and Halen P (1982). Activity of clopidol/methylbenzoquate, robenidine and salinomycin against hepatic coccidiosis in rabbits. Reihe B. Journal of Veterinary medicine. Series B, 29: 207-218. DOI: https://www.doi.org/10.1111/j.1439-0450.1982.tb01217.x

Pilarczyk B, Tomza-Marciniak A, Pilarczyk R, Januś E, Stanek P, Seremak B and Sablik P (2020). The effect of the sex, age, and breed of farmed rabbits and the choice of management system on the extensity and intensity of *Eimeria* infection. Veterinary World, 13: 1654-1660. DOI: https://www.dx.doi.org/10.14202%2Fvetworld.2020.1654-1660

Polozowski A (1993). Coccidiosis of rabbits and its control. Wiadomosci parazytologiczne, 39: 13-28. PMID: 8346656. Available at: https://pubmed.ncbi.nlm.nih.gov/8346656/

Qamar F, Sharif R, Qamar MM and Basharat A (2013). Comparative efficacy of sulphadimidine sodium, toltrazuril and amprolium for coccidiosis in rabbits. Science International (Lahore), 25: 295-298. Available at: https://www.researchgate.net/publication/316692292_Comparative_efficacy_of_sulphadimidine_sodium_toltrazuril_and_amprolium_for_Coccidiosis_in_Rabbits

Redrobe SP, Gakos G, Elliot SC, Saunders R, Martin S and Morgan ER (2010). Comparison of toltrazuril and sulphadimethoxine in the treatment of intestinal coccidiosis in pet rabbits. The Veterinary Record, 167: 287- 290. DOI: https://www.doi.org/10.1136/vr.c3453

Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition/ OJ L 268: 29-43. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R1831&rid=10

Renaux S, Quéré P, Buzoni-Gatel D, Sewald B, Le Vern Y, Coudert P and Drouetviard F (2003). Dynamics and responsiveness of T lymphocytes in secondary lymphoid organs of rabbits developing immunity to *Eimeria intestinalis*. Veterinary Parasitology, 110: 181-195. DOI: https://www.doi.org/10.1016/S0304-4017(02)00305-9

Rivero-Perez N, Herna´ndez-Alvarado JL, Valladares-Carranza B, Delgadillo-Ruiz L, Ojeda-Ramı´rez D, Sosa-Gutie´rrez CG, Morales-Ubaldo AL, Vega-Sanchez V and Zaragoza-Bastida A (2019). *Salix babylonica* L. as a natural anticoccidial alternative in growing rabbits. Evidence Based Complementary Alternative Medicine, pp. 1-8. DOI: https://www.doi.org/10.1155/2019/2107231

Sanyal PK and Sharma SC (1990). Clinicopathology of hepatic coccidiosis in rabbits. Indian Journal of Animal Science, 60: 924-928. Available at: https://arccjournals.com/journal/indian-journal-of-animal-research/B-3436

Schlolaut W, Hudson R and Rödel HG (2013). Impact of rearing management on health in domestic rabbits: A review. World Rabbit Science, 21: 145-159. DOI: https://www.doi.org/10.4995/wrs.2013.1029

Seddek ASh, El-Ghoneimy AA, Shibat El-hamd DW and Mahmoud EG (2015). Effect of *Calotropis procera* on rabbits coccidiosis. Egyptian Journal of Chemical and Environmental Health, 1: 768-784. Available at: http://cehea.org/wp-content/uploads/2016/08/59-.pdf

Sharma KG, Vidyarthi VK, Archana K and Zuyie R (2016). Probiotic supplementation in the diet of rabbits a review. Livestock Research International, 4: 1-10. Available at: http://jakraya.com/journal/pdf/11-lriArticle_1.pdf

Shkromada O, Skliar O, Paliy A, Ulko L, Suprun Y, Naumenko O, Ishchenko K, Kysterna O, Musiienko O and Paliy A (2019). Development of preventing means for rabbits' coccidiosis. Eureka: Health Sciences, 3: 58-68. DOI: https://www.dx.doi.org/10.21303/2504-5679.2019.00914

To cite this paper: Abd El-Ghany WA (2021). Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits. *World Vet. J.*, 10 (4): 499-507. DOI: https://dx.doi.org/10.54203/scil.2020.wvj60

Singla LD, Juyal PD and Sandhu BS (2000). Pathology and therapy in naturally *Eimeria stiedae*-infected rabbits. Journal of Protozoology Research, 10: 185-191. Available at: https://www.semanticscholar.org/paper/Pathology-

Sivajothi S, Reddy BS and Rayulu VC (2016). Study on impression smears of hepatic coccidiosis in rabbits. Journal of Parasitic Diseases, 40: 906-909. DOI: https://www.dx.doi.org/10.1007%2Fs12639-014-0602-8

Song H, Yan R, Xu L, Song X, Shah MAA, Zhu H and Li X (2010). Efficacy of DNA vaccines carrying *Eimeria acervulina* lactate dehydrogenase antigen gene against coccidiosis. Experimental Parasitology, 126: 224-231. DOI: https://www.doi.org/10.1016/j.exppara.2010.05.015

Song H, Dong R, Qiu B, Jing J, Zhu S, Liu C, Jiang Y, Wu L, Wang S, Miao J and Shao Y (2017). Potential vaccine targets against rabbit coccidiosis by immunoproteomic analysis. The Korean Journal of Parasitology, 55: 15-20. DOI: https://www.dx.doi.org/10.3347%2Fkjp.2017.55.1.15

Sorour SS, Abou-Asa S, Elhawary NM, Ghazy EW, Abd-El-Latif A, El-Abasy MA and Khalifa HO (2018). Anticoccidial and hepatoprotective effects of artemisinin liquid extract, cinnamon essential oil and clove essential oil against *Eimeria stiedae* infection in rabbits. Tropical Biomedicine, 35: 926-943. DOI: http://msptm.org/files/Vol35No4/926-943-Hazim-O-Khalifa.pdf

Soulsby EJL (1968). Helminths, Arthropods and Protozoa of Domesticated Animals. 6th ed. Bailliere Tindall, London, 676-682.

Toulah FH and Al-Raw MM (2007). Efficacy of garlic extract on hepatic coccidiosis in infected rabbits (*Oryctolagus cuniculus*): histological and biochemical studies. Journal of Egyptian Society of Parasitology, 37: 957-968. Available at: https://pubmed.ncbi.nlm.nih.gov/18383795/

Vanparijs O, Desplenter L and Marsboom R (1989a). Efficacy of diclazuril in the control of intestinal coccidiosis in rabbits. Veterinary Parasitology, 34: 185-190. DOI: https://www.doi.org/10.1016/0304-4017(89)90049-6

Vanparijs O, Hermans L, Van Der Flaes L and Marsboom R (1989b). Efficacy of diclazuril in the prevention and cure of intestinal and hepatic coccidiosis in rabbits. Veterinary Parasitology, 32: 109-117. DOI: https://www.doi.org/10.1016/0304-4017(89)90111-8

Vereecken D, Lavazza A, De Gussem K, Chiari M, Tittarelli C, Zuffellato A and Maertens L (2012). Activity of diclazuril against coccidiosis in growing rabbits: experimental and field experiences. World Rabbit Science, 20: 223-230. DOI: https://www.doi.org/10.4995/wrs.2012.1232

Wei W, Shen N, Xiao J, Tao Y, Luo Y, Angel C, Gu X, Xie Y, He R, Jing B, Peng X and Yang G (2020). Expression analysis and serodiagnostic potential of microneme proteins 1 and 3 in *Eimeria stiedai*. Genes, 11: 725. DOI: https://www.doi.org/10.3390/genes11070725

Xin C, Wu B, Li J, Gong P, Yang J, Li H, Cai X and Zhang X (2016). Complete genome sequence and evolution analysis of *Eimeria stiedai* RNA virus 1, a novel member of the family Totiviridae. Archive of Virology, 161: 3571-3576. DOI: https://www.doi.org/10.1007/s00705-016-3020-7

Yan W, Wang W, Wang T, Suo X, Qian W, Wang S and Fan D (2013). Simultaneous identification of three highly pathogenic *Eimeria* species in rabbits using a multiplex PCR diagnostic assay based on ITS1- 5.8S rRNA-ITS2 fragments. Veterinary Parasitology, 193: 284-288. DOI: https://www.doi.org/10.1016/j.vetpar.2012.11.013

Yin G, Goraya MU, Huang J, Suo X, Huang Z and Liu X (2016). Survey of coccidial infection of rabbits in Sichuan Province, Southwest China. Springer Plus, 5: 870. DOI: https://www.doi.org/10.1186/s40064-016-2586-6

Zayed AA and Kutkat MA (1998). SDS-Page and immunoelectrophoretic evaluation of sporulated oocysts antigens of *E. stiedae* infecting domestic rabbits. Alexandria Journal of Science, 14: 1-9. Available at: https://www.idosi.org/gv/GV9(5)12/3.pdf

To cite this paper: Abd El-Ghany WA (2021). Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits. *World Vet. J.*, 10 (4): 499-507. DOI: https://dx.doi.org/10.54203/scil.2020.wvj60

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 508-513, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj61

Multidrug-Resistant *Salmonella* spp. Isolated from Apparently Healthy Pigeons in a Live Bird Market in Chattogram, Bangladesh

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ORIGINAL ARTICLE
pii: S232245682000061-10
Received: 09 Oct 2020
Accepted: 29 Nov 2020

ABSTRACT

Multidrug-resistant *Salmonella* could pose a severe public health threat. The current study aimed to investigate the prevalence of antibiotic resistance and some antibiotic-resistant genes in *Salmonella* spp. isolated from pigeons in a live bird market, Chattogram, Bangladesh. A total of 100 cloacal swab samples were collected aseptically from apparently healthy pigeons in the live bird market, namely Riazuddin Bazar in Chattogram city, Bangladesh. Different bacteriological and biochemical tests were used for the isolation and identification of *Salmonella* spp. The susceptibility test of *Salmonella* isolates to different antibiotics was performed by the disk diffusion method. PCR assay using specific primers was used for antibiotic resistance genes detection. The results indicated that the prevalence of *Salmonella* spp. was 29% in sampled birds. The highest antibiotic resistance rate was found to be ampicillin (93.1%), followed by both sulfamethoxazole-trimethoprim and tetracycline (86.2%). In contrast, 65.5% of isolates were found sensitive to ciprofloxacin, followed by colistin (62.1%), kanamycin (55.2%), and gentamicin (48.3%). 96.6% of *Salmonella* isolates were classified as multidrug-resistant and harbored *bla*TEM, *tet*A, *sul*1, and *sul*2 genes. In conclusion, pigeons as carriers of antibiotic-resistant *Salmonella* spp. may pose a health risk to other birds and humans.

Keywords: Antibiogram, Antibiotic resistance genes, Pigeons, Prevalence, *Salmonella*

INTRODUCTION

Food animals have been recognized as a reservoir of resistant bacteria and a source of foodborne infections for humans (Szmolka and Nagy, 2013). Food chain cycle act as a vehicle to transmit antibiotic-resistant infectious agents from farm animals to humans (Molbak et al., 2002).

Pigeons (*Columbia livia*) have an important role in dispersing the bacterial agents to free-range poultry and have been considered a fecal contaminator of drinking water sources and rural harvests (Lillehaug et al., 2005). These birds are in contact with humans at home, farms, and live bird markets (LBM), and are responsible for the transmission of several diseases through their droppings (Weber, 1979). Several pathogenic microbes such as *E. coli*, *Salmonella* spp., *Cryptococcus* spp., and *Chlamydia* spp. are carried by pigeons (Tanaka et al., 2005).

LBMs are the most significant terminal hub of the poultry business in Asian countries, where individuals purchase live or freshly butchered poultry (Sarker et al., 2019a). Pigeons in LBMs are originated from various sources and territories and stocked in confined spaces at high densities. Moreover, at LBMs, customers come in close and direct interaction with live or processed poultry. Therefore, unhygienic conditions in LBMs may provoke the dissemination of infectious agents from pigeon to pigeon and pigeon to human. In the farms and LBMs, the apparently healthy pigeons are one of the sources of human salmonellosis (Hosain et al., 2012). Therefore, LBMs have an important role in the transmission of *Salmonella* in human food chain. To date, very little work on prevalence of antibiotic-resistant *Salmonella* in pigeons has been conducted in Bangladesh. Therefore, the present study aimed to determine the prevalence of antibiotic resistance and some resistance genes in *Salmonella* isolated from pigeons in LBM in Chattogram, Bangladesh.

To cite this paper: Bupasha ZB, Begum R, Karmakar S, Akter R, Bayzid M, Ahad A and Sarker MS (2021). Multidrug-Resistant *Salmonella* spp. Isolated from Apparently Healthy Pigeons in a Live Bird Market in Chattogram, Bangladesh. *World Vet. J.*, 10 (4): 508-513. DOI: https://dx.doi.org/10.54203/scil.2020.wvj61

MATERIALS AND METHODS

Ethical approval

The study protocol was approved by the Institutional Animal Ethics Committee, Chattogram Veterinary and Animal Sciences University, Bangladesh.

Samples collection

A total of 100 cloacal swab samples were aseptically collected from 100 apparently healthy pigeons in a LBM in Chattogram, Bangladesh during the period from February to April 2018. Using a simple random technique, 10 samples were collected from each shop. The swab samples were transferred to Falcon tubes containing 5 ml of buffered peptone water (BPW) (Oxoid, UK) and immediately transported to the laboratory in an icebox.

Isolation and identification of *Salmonella*

Samples were incubated overnight in BPW at 37 °C for enrichment. For selective enrichment, 100 µl of the pre-enriched sample was transferred to Rappaport-Vassiliadis (RV) medium (Oxoid, UK), incubated at 41.5 °C for 24 hours. A loopful of positive enrichment in RV was streaked onto Salmonella-Shigella (SS) agar (Oxoid, UK) and xylose lysine deoxycholate (XLD) agar (Oxoid, UK), incubated at 37°C for 24 hours. At least two single typical *Salmonella* colonies were randomly picked up and subjected to biochemical tests (triple sugar iron [TSI], indole, urease, oxidase, and catalase tests) (Begum et al., 2018). Positive *Salmonella* isolates were preserved into brain heart infusion (BHI) broth (Oxoid, UK) with 15% glycerol at -80 °C.

Extraction of chromosomal DNA

For the PCR, total DNA was extracted from the isolated bacterial agents using the boiling method (Sánchez et al., 2010). In brief, 2-3 pure cultured colonies were mixed with 200 µl of deionized water into 1.5 ml sterile Eppendorf tube, followed by boiling for 15 min. After boiling, it was kept on ice immediately for 10 min, centrifuged for 2 min at 15000 rpm. Finally, the collected supernatant was used as a DNA template for PCR.

Molecular detection of *Salmonella*

Genotypic confirmation of *Salmonella* was done by targeting the *sdi*A gene as previously described by Halatsi et al. (2006). The sequence of the specific primer pair for the *sdi*A gene is presented in Table 1. PCR amplification was accomplished with a 25 µl reaction mixture containing deionized water (10.5 µl), GoTaq master mix (Promega, USA) (12.5 µl), forward and reverse primers (0.5 µl each), and DNA template (1 µl). The thermal profile consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 50 °C for 1 min, elongation at 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR products were then electrophoresed by running in 1.5% agarose gel stained with ethidium bromide (Sigma-Aldrich, USA).

Table 1. Oligonucleotide primers used in the study

Target genes	Primers sequence (5´-3´)	Amplicon size (base pair)	References
*bla*TEM	F: TACGATACGGGAGGGCTTAC R: TTCCTGTTTTTGCTCACCCA	716	Belaaouaj et al. (1994)
*tet*A	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	Karczmarczyk et al. (2011)
*sul*1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	433	Sunde (2005)
*sul*2	F: CGGCATCGTCAACATAACCT R: TGTGCGGATGAAGTCAGCTC	721	Lanz et al. (2003)
*sdi*A (*Salmonella*)	F: AATATCGCTTCGTACCAC R: GTAGGTAAACGAGGAGCAG	274	Halatsi et al. (2006)

F: forward, R: reverse

Antibiotic susceptibility test

To assess the antibiotic susceptibility of *Salmonella* isolates, the disk diffusion method was performed on Mueller-Hinton agar (Oxoid, UK) plates as described by CLSI (2012). The isolates were tested against 10 commonly used antibiotics using antibiotic disks including ampicillin (10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), tetracycline (30 µg), sulfamethoxazole-trimethoprim (25 µg), colistin sulfate (10 µg), chloramphenicol (30 µg) and nalidixic acid (30 µg) (Oxoid, UK). The sensitivity results were interpreted according to CLSI (2012). Multidrug-resistant (MDR) was defined as isolate being resistant to at least three antimicrobial agents from different classes (Tenover, 2006).

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Detection of antibiotic resistance genes

All of the phenotypically resistant *Salmonella* isolates were subjected to PCR to detect the ampicillin resistance gene (*bla*TEM), tetracycline resistance gene (*tet*A), and sulfonamide resistance gene (*sul*1 and *sul*2) according to the previously published study (Sarker et al., 2019b). The sequence of primers used for target gene amplification is presented in Table 1.

Statistical analysis

Data were entered into an Excel spreadsheet (Microsoft Corporation, USA). Descriptive statistics were used to analyze the data by an online epidemiological calculator (Sergeant, ESG, 2019).

RESULTS

Prevalence and characteristic of *Salmonella*

The prevalence of *Salmonella* in the collected samples was 29% (29/100) (95% CI: 21.01-38.54). Distinctive *Salmonella* colonies on XLD agar were pink color with black centered, and on SS agar produced small, smooth, round, and black centered colonies. *Salmonella* isolates were positive to TSI and negative to indole, urease, oxidase, and catalase test.

Antibiotic resistance patterns

Antibiogram study of *Salmonella* showed that the isolates were highly resistant to ampicillin (93.1%, 27/29), tetracycline (86.2%, 25/29) and sulfamethoxazole-trimethoprim (86.2%, 25/29), followed by nalidixic acid (72.4%, 21/29), chloramphenicol (51.7%, 15/29) and ceftriaxone (48.28%, 14/29). To the contrary, the highest susceptibility rate was found against ciprofloxacin (65.5%, 19/29), followed by colistin (62.1%, 18/29) and kanamycin (55.2%, 16/29) (Figure 1). Of the 29 *Salmonella* isolates, 28 (96.6%) disclosed the MDR patterns (Table 2).

Table 2. Antibiotic resistance patterns and distribution of targeted resistant genes among 29 *Salmonella* isolates recovered from cloacal swab samples of pigeons in a live bird market in Chattogram, Bangladesh

Phenotypic pattern of antibiotic resistance	MDR isolate	Genotypic pattern of antibiotic resistance			
		*bla*TEM	*tet*A	*sul*1	*sul*2
AMP-SXT	-	+	-	+	+
AMP-TE-SXT	+	+	+	-	-
AMP-TE-CT-C	+	-	-	-	-
AMP-TE-SXT-NA	+	+	+	+	+
AMP-SXT-C-K	+	+	-	-	-
AMP-SXT-CIP-NA	+	+	-	-	+
AMP-SXT-CN-C-NA	+	-	-	+	-
AMP-CRO-TE-SXT-NA	+	+	+	-	-
AMP-TE-SXT-CT-C	+	+	+	-	+
CRO-TE-SXT-CN-NA	+	-	+	+	-
AMP-TE-SXT-CN-NA	+	+	-	-	+
AMP-TE-SXT-CN-CT-CIP	+	+	+	+	-
AMP-TE-SXT-C-NA-K	+	-	-	-	+
TE-SXT-CN-C-CIP-K	+	-	+	-	-
AMP-CRO-TE-SXT-CT-NA	+	+	-	+	+
AMP-TE-CN-CT-C-K	+	+	+	-	-
AMP-CRO-TE-SXT-C-NA	+	+	+	-	+
AMP-CRO-TE-SXT-CIP-NA	+	-	-	+	+
AMP-TE-SXT-CN-C-NA	+	+	+	-	-
AMP-CRO-TE-SXT-NA-K	+	+	-	-	+
AMP-CRO-TE-SXT-CN-C-NA	+	-	+	+	+
AMP-CRO-TE-SXT-CIP-NA-K	+	+	-	-	-
AMP-CRO-TE-SXT-C-NA-K	+	+	+	+	-
AMP-TE-SXT-CN-CT-CIP-NA	+	+	+	-	+
AMP-CRO-TE-SXT-CT-CIP-NA	+	-	+	+	-
AMP-CRO-TE-CN-CT-C-NA-K	+	+	-	-	-
AMP-CRO-TE-CT-C-CIP-NA-K	+	+	+	-	-
AMP-CRO-TE-SXT-C-CIP-NA-K	+	+	-	+	+
AMP-CRO-TE-SXT-CN-C-CIP-NA-K	+	+	+	+	+
Total	29	21	16	12	14

AMP: ampicillin, SXT: sulfamethoxazole-trimethoprim, TE: tetracycline, CT: colistin sulfate, C: chloramphenicol, NA: nalidixic acid, K: kanamycin, CIP: ciprofloxacin, CN: gentamicin, CRO: ceftriaxone, MDR: multidrug-resistant, *bla*TEM: ampicillin resistance gene, *tet*A: tetracycline resistance gene, *sul*1 and *sul*2: sulfa drug resistance genes.

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Antibiotic resistance genes

Out of 27 ampicillin-resistant isolates, 77.8% (21/27) isolates carried ampicillin resistance gene *bla*TEM. The prevalence of *tet*A in *Salmonella* isolates that were phenotypically resistant to tetracycline was 64% (16/25). Among isolates that were phenotypically resistant to sulfamethoxazole-trimethoprim, the prevalence of *sul*1, and *sul*2 genes were 48% (12/25), and 56% (14/25), respectively. The distribution of resistance genes along with resistance patterns of *Salmonella* isolates are presented in Table 2.

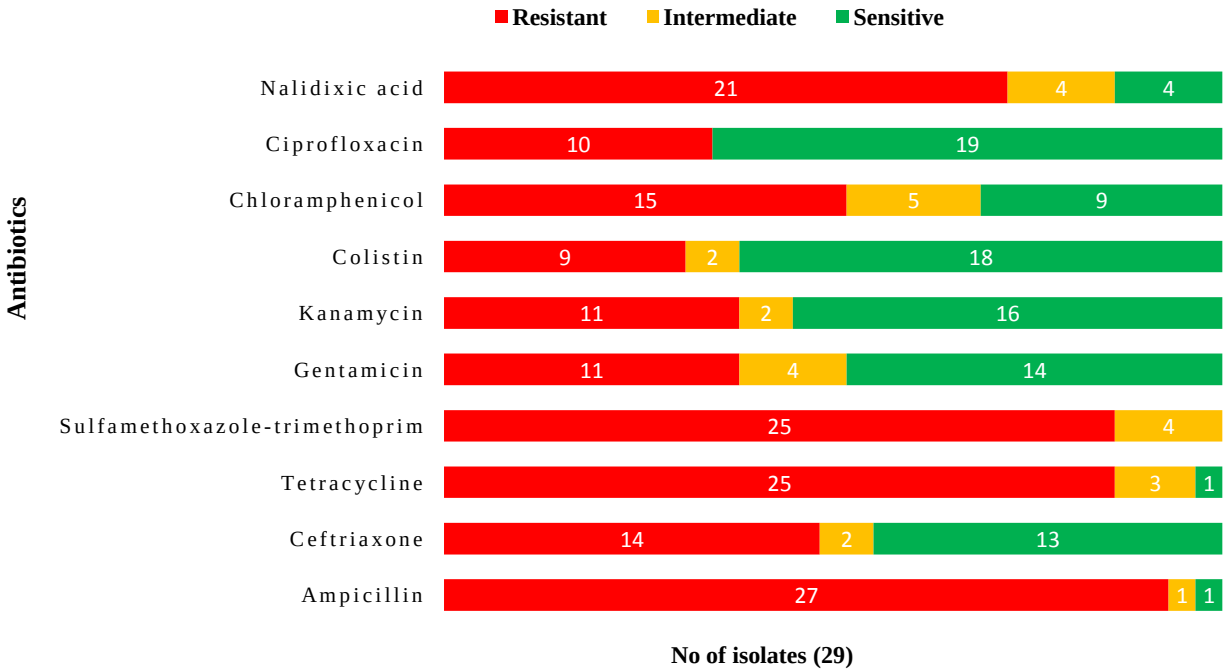

Figure 1. Antibiogram profile of *Salmonella* isolates recovered from cloacal swab samples of pigeons in a live bird market in Chattogram, Bangladesh

DISCUSSION

In this study, the prevalence of *Salmonella* in pigeons was lower than the previous reports in Bangladesh (Hosain et al., 2012; Saifullah et al., 2016), which reported the prevalence rates of 40.28% and 37.5% in pigeons, respectively. In Copenhagen, pooled fecal samples of pigeons showed a prevalence rate of 22.8% for *Salmonella* (Pasmans et al., 2004), while in Iran, prevalence of *Salmonella* in cloacal samples isolated from pigeons was 15.6% (Akbarmehr, 2010).

In this study, *Salmonella* isolates were highly resistant to ampicillin, followed by tetracycline and sulfamethoxazole-trimethoprim (86.2%) and nalidixic acid (72.4%). A comparable result was obtained by Saifullah et al. (2016), who stated a high rate of resistance to ampicillin (88.2%), while a high sensitivity rate to nalidixic acid (76.5%) in *Salmonella* spp from apparently healthy pigeons. Hosain et al. (2012) reported that 80% of *Salmonella* isolated from pigeons were resistant to ampicillin, followed by tetracycline (60%) and sulfamethoxazole (20%) from Bangladesh. The highest resistance rate of these antibiotics may be due to the long-term use in veterinary practice. Ampicillin, tetracycline, and sulfonamide are regularly prescribed antibiotics in poultry treatment in Bangladesh (Saifullah et al., 2016). Moreover, cross resistance to similar classes of antibiotics is also responsible for the high resistance rates.

In food animals, ciprofloxacin is one of the broadly used antibiotics that is regularly prescribed for poultry practice in Bangladesh (Azad et al., 2019). The resistance to ciprofloxacin is a worldwide issue since it could complicate clinical therapy both in humans and livestock. Resistance to colistin (31%), one of the significant findings of our study which is worrisome. The last resort drug, colistin is being expansively prescribed in veterinary practice, however because of nephrotoxicity and neurotoxicity, its use is restricted in human practice (Hassan et al., 2015). The findings of the present study disclosed that 65.5%, 55.2%, and 48.3% of *Salmonella* isolates were sensitive to ciprofloxacin, kanamycin, and gentamicin, respectively while Hosain et al., (2012) reported 60% sensitivity rate to kanamycin and gentamicin. The present study indicated that 96.6% of *Salmonella* isolates were MDR. A number of previous researches reported MDR *Salmonella* in Bangladesh (Khan et al., 2005; Rahman et al., 2011). A high incidence of MDR strains may be occurred due to the aimless use of antibiotic agents.

In the present study, the antibiotic resistance genes among *Salmonella* isolates were detected. The presence of *bla*TEM, *tet*A, and *sul*2 genes in *Salmonella* isolates were reported by Adelowo et al. (2014) and Messaili et al. (2019)

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that had similar frequencies with our findings. The frequency of antibiotic resistance genes in *Salmonella* is quite variable in poultry, which may be due to differences in antibiotic use patterns in different regions.

CONCLUSION

The prevalence of MDR *Salmonella* in pigeons in live bird markets is a public health concern, therefore, it is recommended to maintain strict hygienic measures, proper cage, and litter management to diminish the load and spread of MDR *Salmonella* and ensure customers health and safety.

DECLARATIONS

Acknowledgments

The authors would like to thanks the pigeon shop owners for their help during sample collections.

Competing interests

The authors declare that they have no conflict of interest.

Authors' contribution

Zamila Bueaza Bupasha, Abdul Ahad and Md Samun Sarker designed the study plan. Zamila Bueaza Bupasha, Md Bayzid and Md Samun Sarker performed the laboratory experiments. Sharna Karmakar and Rahima Akter analyzed the data. Ruhena Begum and Zamila Bueaza Bupasha drafted the manuscript. Abdul Ahad and Md Samun Sarker revised the manuscript. All authors read and approved the final manuscript for publication.

REFERENCES

Adelowo O, Fagade O and Agerso Y (2014). Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. The Journal of Infection in Developing Countries, 8: 1103-1112. DOI: https://doi.org/10.3855/jidc.4222

Akbarmehr J (2010). Isolation of *Salmonella* spp. from poultry (ostrich, pigeon, and chicken) and detection of their hilA gene by PCR method. African Journal of Microbiology Research, 4(24): 2678-2681. DOI: http://www.academicjournals.org/ajmr

Azad MARA, Rahman MM, Amin R, Begum MIA, Fries R, Husna A, Khairalla AS, Badruzzaman ATM, Zowalaty MEE, Lampang KN et al. (2019). Susceptibility and Multidrug Resistance Patterns of *Escherichia coli* Isolated from Cloacal Swabs of Live Broiler Chickens in Bangladesh. Pathogens, 8(3): 118. DOI: https://dx.doi.org/10.3390%2Fpathogens8030118

Begum R, Sarker MS, Ngamsanga P, Pulsrikarn C, Pichpol D, Meeyam T and Chaisowwong W (2018). Prevalence and antimicrobial resistance of *Salmonella* isolated from meat and eggs in Muang district in Chiang Mai province, Thailand. The 5th Food Safety and Zoonoses Symposium for Asia Pacific, Chiang Mai, Thailand, 73-79.

Belaaouaj A, Lapoumeroulie C, Caniça MM, Vedel G, Névot P, Krishnamoorthy R and Paul G (1994). Nucleotide sequences of the genes coding for the TEM-like β-lactamases IRT-1 and IRT-2 (formerly called TRI-1 and TRI-2). FEMS Microbiology Letters, 120(1-2): 75-80. DOI: https://doi.org/10.1111/j.1574-6968.1994.tb07010.x

CLSI (2012). Performance standards for antimicrobial disk susceptibility tests; approved standard, 11th Edn. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute.

Halatsi K, Oikonomou I, Lambiri M, Mandilara G, Vatopoulos A and Kyriacou A (2006). PCR detection of *Salmonella* spp. using primers targeting the quorum sensing gene *sdiA*. FEMS Microbiology Letters, 259(2): 201-207. DOI: https://doi.org/10.1111/j.1574-6968.2006.00266.x

Hassan M, Ahaduzzaman M, Alam M, Bari MS, Amin KB and Faruq AA (2015). Antimicrobial resistance pattern against *E. coli* and *Salmonella* spp. in environmental effluents. International Journal of Natural Sciences, 5: 52–58. DOI: https://doi.org/10.3329/ijns.v5i2.28612

Hosain MS, Islam MA, Khatun MM and Dey RK (2012). Prevalence and antibiogram profiles of *Salmonella* isolated from pigeons in Mymensingh, Bangladesh. Microbes and Health, 1(2): 54-57. https://www.banglajol.info/index.php/MH/article/view/14090

Karczmarczyk M, Martins M, Quinn T, Leonard N and Fanning S (2011). Mechanisms of fluoroquinolone resistance in *Escherichia coli* isolates from food-producing animals. Applied and Environmental Microbiology, 77(20): 7113-7120. DOI: https://doi.org/10.1128/aem.00600-11

Khan M, Rahman M, Khan M and Nazir Khmnh RM (2005). Antibiogram and plasmid profile analysis of isolated poultry *Salmonella* of Bangladesh.Pakistan Journal of Biological Sciences, 8(11): 1614-1619. DOI: http://dx.doi.org/10.3923/pjbs.2005.1614.1619

Lanz R, Kuhnert P and Boerlin P (2003). Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Veterinary Microbiology, 91(1): 73-84. DOI: https://doi.org/10.1016/s0378-1135(02)00263-8

Lillehaug A, Jonassen CM, Bergsjø B, Hofshagen M, Tharaldsen J, Nesse L and Handeland K (2005). Screening of feral pigeon (*Colomba livia*), mallard (*Anas platyrhynchos*) and graylag goose (*Anser anser*) populations for *Campylobacter* spp., *Salmonella* spp., avian influenza virus and avian paramyxovirus. Acta Veterinaria Scandinavica, 46(4): 193-202. DOI: https://doi.org/10.1186/1751-0147-46-193

Messaili C, Messai Y and Bakour R (2019). Virulence gene profiles, antimicrobial resistance and phylogenic groups of fecal *Escherichia coli* strains isolated from broiler chickens in Algeria. Veterinaria Italiana, 55(1): 35-46. DOI: https://doi.org/10.12834/vetit.799.3865.2

Molbak K, Gerner-Smidt P and Wegener HC (2002). Increasing quinolone resistance in *Salmonella enterica* serotype Enteritidis. Emerging Infectious Diseases, 8(5): 514-515. DOI: https://dx.doi.org/10.3201%2Feid0805.010288

Pasmans F, Van Immerseel F, Hermans K, Heyndrickx M, Collard JM, Ducatelle R and Haesebrouck F (2004). Assessment of virulence of pigeon isolates of *Salmonella enterica* subsp. enterica serovar Typhimurium variant Copenhagen for humans. Journal of Clinical Microbiology, 42(5): 2000-2002. DOI: https://doi.org/10.1128/jcm.42.5.2000-2002.2004

Rahman M, Hossain M, Akhter M and Hasan S (2011). Characterization and antibiogram study of *Salmonella* serovars isolated from duck, quail and pigeon in Dinajpur district of Bangladesh. International Journal of Sustainable Agricultural Technology, 7(2): 23-29.

Saifullah MK, Mamun MM, Rubayet RM, Nazir KNH, Zesmin K and Rahman MT (2016). Molecular detection of isolated from apparently healthy pigeon in Mymensingh, Bangladesh and their antibiotic *Salmonella* spp. resistance pattern. Journal of Advanced Veterinary and Animal Research, 3(1): 51-55. DOI: http://doi.org/10.5455/javar.2016.c131

Sánchez S, Martínez R, García A, Benítez J, Blanco J, Blanco JE, Blanco M, Dahbi G, López C and Mora A (2010). Variation in the prevalence of non-O157 Shiga toxin-producing *Escherichia coli* in four sheep flocks during a 12-month longitudinal study. Small Ruminant Research, 93(2-3): 144-148. DOI: https://doi.org/10.1016/j.smallrumres.2010.05.014

Sarker MS, Ahad A, Ghosh SK, Mannan MS, Sen A, Islam S, Bayzid M and Bupasha ZB (2019b). Antibiotic-resistant *Escherichia coli* in deer and nearby water sources at Safari parks in Bangladesh. Veterinary World, 12(10): 1578–1583. DOI: https://dx.doi.org/10.14202%2Fvetworld.2019.1578-1583

Sarker MS, Mannan MS, Ali MY, Bayzid M, Ahad A and Bupasha ZB (2019a). Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh. Journal of Advanced Veterinary and Animal Research, 6(3): 272-277. DOI: https://doi.org/10.5455/javar.2019.f344

Sergeant, ESG (2019). Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au

Sunde M (2005). Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. Journal of Antimicrobial Chemotherapy, 56(6): 1019-1024. DOI: https://doi.org/10.1093/jac/dki377

Szmolka A and Nagy B (2013). Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Frontiers in Microbiology, 4: 258. DOI: https://dx.doi.org/10.3389%2Ffmicb.2013.00258

Tanaka C, Miyazawa T, Watarai M and Ishiguro N (2005). Bacteriological survey of feces from feral pigeons in Japan. Journal of Veterinary Medical Science, 67(9): 951-953. DOI: https://doi.org/10.1292/jvms.67.951

Tenover FC (2006). Mechanisms of antimicrobial resistance in bacteria. The American Journal of Medicine, 119(6): S3-S10. DOI: https://doi.org/10.1016/j.amjmed.2006.03.011

Weber W (1979). Pigeon associated people diseases. Paper presented at the Bird Control Seminars Proceedings. Available at: https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1020&context=icwdmbirdcontrol

To cite this paper: Bupasha ZB, Begum R, Karmakar S, Akter R, Bayzid M, Ahad A and Sarker MS (2021). Multidrug-Resistant *Salmonella* spp. Isolated from Apparently Healthy Pigeons in a Live Bird Market in Chattogram, Bangladesh. *World Vet. J.*, 10 (4): 508-513. DOI: https://dx.doi.org/10.54203/scil.2020.wvj61

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 514-524, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj62

In Vitro Investigation of the Antibacterial Effect of Silver Nanoparticles on ESBL-producing *E. coli* and *Klebsiella* spp. Isolated from Pet Animals

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ORGINAL ARTICLE
pii: S232245682000062-10
Received: 16 Oct 2020
Accepted: 29 Nov 2020

ABSTRACT

Despite the presence of modern antibacterial drugs, bacterial infections are still a major threatening problem due to the enormous increase in multi-drug-resistant bacteria. Nanoparticles have been extensively used as an applicable and safe alternative to antibiotics. The present study aimed to explore the inhibitory effect of silver nanoparticles on Extended Spectrum Beta lactamase (ESBL) producing *E. coli* and *Klebsiella* spp. in vitro as well as their effect on the expression of antibiotic resistance genes. Different samples (i.e., wound swabs, Fecal swabs, and urine samples) were collected from dogs and cats. Phenotypic and molecular identification, antibiotic susceptibility testing, and double-disk synergy test were carried out for the identification of ESBL producing *E. coli* and *Klebsiella* spp. Silver nanoparticles were tested for their in vitro antibacterial potential and there were reports of their minimum inhibitory concentration and minimum bactericidal concentration. Moreover, the effect of silver nanoparticles on the expression of antibiotic resistance genes (i.e., *blaTEM, blaSHV,* and *blaCTX*) was assessed as well as their effect on the structural integrity of the bacterial cells using Scanning Electron Microscope (SEM). Results revealed that 23 isolates (19.16%) (*E. coli*=17, *Klebsiella* spp.=6) were confirmed as ESBL producing. Silver nanoparticles indicated a promising antibacterial effect where the minimum inhibitory concentration of AgNPs for ESBL producing *E. coli* was measured as 0.31 mg/ml, and 0.62 mg/ml for ESBL-producing *Klebsiella* spp., while the minimum bactericidal concentration of ESBL-producing *E. coli* and *Klebsiella* spp. was reported as 0.15 mg/ml and 0.3 mg/ml, respectively. Consequently, the expression of antibiotic resistance genes was downregulated in both bacteria species and there was a noticeable toxic effect of AgNPs on *E. coli* and *Klebsiella* spp. cells which was investigated using SEM. It can be concluded that silver nanoparticles have a promising antibacterial activity and could be considered an applicable alternative for the control of ESBL producing bacteria.

Keywords: *E. coli*, ESBLs, *Klebsiella* spp., Pets, Silver nanoparticles

INTRODUCTION

In the current decade, the search for antibiotic alternatives has become one of the most quintessential issues due to the massive expansion of antibiotic resistance. The usage of silver nanoparticles has been widely recognized due to their known bactericidal as well as bacteriostatic actions against different types of bacterial and fungal pathogens (Saeb et al., 2014). Silver nanoparticles (AgNPs) have a broad-spectrum antimicrobial effect due to their large surface area providing the chance for better contact with microbes (Li et al., 2010). Furthermore, silver nanoparticles have a lower propensity to induce microbial resistance than other antimicrobial agents (Ansari et al., 2014). It is also worth mentioning that these nanoparticles have a non-toxic effect on the human at low concentrations (Bindu et al., 2015). They are characterized by their powerful antioxidant and antibacterial effect because of bioactive molecules on the exterior surface of silver nanoparticles (Keshari, 2020).

Extended Spectrum β-Lactamase producing bacteria (ESBL) are types of bacteria that show resistance to several types of antibiotics through the hydrolysis of the b-lactam ring of antibiotics (Kizilca et al., 2012) and they can transfer resistance to penicillins, third-generation cephalosporins, and monobactams (Ejaz et al., 2011). Moreover, most of them are not inhibited by other non-b-lactam antibiotics since the resistance encoding genes of other antibiotic classes can also be carried by the plasmids containing the ESBL-encoding genes (Alyamani et al., 2017; Fan et al., 2014). *Escherichia coli* and *Klebsiella* spp. remain the major ESBL-producing microorganisms isolated worldwide. They are considered the most emerging Extended-spectrum β-lactamase (ESBL) with a serious effect on the community (Devrim et al., 2011). The CTX-M-type enzymes are the largest ESBL groups spreading globally, followed by TEM and SHV groups (Sukmawinata et al, 2020). Researchers from different countries have successfully isolated and identified ESBL-producing bacteria from different types of samples, including isolates from human in Bahrain (Shahid et al., 2014), different samples (e.g., fecal swabs, animal feeds, water, and excreta) from different animals (e.g, dogs, cats, sheep,

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goat, chickens, turkey, ducks, and human, (Okapara et al., 2018), isolates of urine samples from dogs and cats in USA and Switzerland (Thungrat et al., 2015; Zogg et al., 2018), also isolates of fecal swabs from dogs and cats in Newzeland (Karkaba et al., 2019). Nanoparticles are known mainly by their direct action on the bacterial cell wall which is different from other antibiotic resistance mechanisms and needs no penetration of bacterial cells (Wang et al., 2019). Subsequently, they are less predisposed to develop resistance, compare to antibiotics. AgNps have proved a good antibacterial and antioxidant activity against *E. coli* and *Klebsiella pneumoniae* isolates (Khan et al., 2020). Moreover, it was demonstrated that AgNPs have a powerful toxic action on ampicillin resistant *Klebsiella Pneumoniae* genes and bacterial proteins as well as bacterial membrane damage and oxidative stress (Hamida et al., 2020). Therefore, this study aimed to explore the inhibitory effect of silver nanoparticles on ESBL-producing *E. coli* and *Klebsiella* spp. in vitro as well as their effect on the expression of resistance genes. In doing so, the phenotypic and molecular identification of ESBL producing *E. coli* and *Klebsiella* spp. was carried on different samples collected from dogs and cats living in various places in Egypt. The bactericidal activity of Ag nanoparticles was assessed via different microbiological and molecular techniques.

MATERIALS AND METHODS

Ethical approval

The study was conducted according to ethical guidelines approved by the Faculty of Veterinary Medicine, Cairo University. There were no experiments applied to human participants.

Samples collection and preparation

A total of 120 samples were collected from diseased dogs (n=55) and cats (n=65). All samples were collected according to the guidelines of the Institutional Animal Care and Use Committee at Cairo University and approved by Vet-CU-IACUC (Vet CU 16072020198), Cairo, Egypt. Written consent was obtained from the animal owners after they were informed on the use of their animal samples in the study. Samples included fecal swabs (n=61), wound swabs (n=17), and urine samples (n=42). Samples were collected from Al-Shaab Veterinary Hospital, Surgery and Medicine Departments at Faculty of Veterinary medicine, Cairo University as well as animal laboratories in Cairo and Giza from March to December 2019. Samples were collected from animals suffering from gastrointestinal tract disturbances, and acute or chronic cystitis. In addition, the wounded animals had certain medical conditions and could provide no appropriate response to the prescribed antibiotics (Huber et al.,2013). All investigated samples were collected under aseptic conditions and safety precautions. Samples were directly inoculated into 9 ml of sterile physiological saline (Okapara et al., 2018)**.** Urine samples were collected from each case via catheter and urine was collected from the distal part under aseptic conditions by the collection of the midstream urine sample (Cystocentesis) as reported by Huber et al. (2013). Samples were appropriately labelled and transported without delay to the laboratory and processed immediately.

Phenotypic characterization and Antibiogram testing for *E. coli* and *Klebsiella* spp. isolates

Wound and fecal swabs were inoculated onto MacConkey agar (Oxoid) supplemented with ampicillin (100 mg/L; Mac-AMP100, Oxoid) according to Okapara et al. (2018). Urine samples were centrifuged, and the sediment was inoculated directly on MacConkey agar (Oxoid). All inoculated plates were incubated at 37˚c for 18-24 hours and examined for bacterial growth. Both lactose fermenter colonies and late lactose fermenter colonies were selected for further examinations. The purified isolates were finally confirmed biochemically with citrate, oxidase, indole, catalase, Voges Proskauer, methyl red, urease, and triple sugar iron (TSI) tests according to Cruickshank et al. (1975). All isolates were tested for their susceptibility to different antimicrobial drugs and antibiotics (Table 1). The antimicrobial susceptibility test was performed using the disc diffusion method (Kibry-Bauer method) on Muller-Hinton agar plates (Oxoid) and the interpretation was performed based on CLSI (2018).

Doubled-disc synergy test

ESBL production was identified using Double Disk Synergy Test (DDST) according to Iqbal et al. (2017). Three antibiotics were used for DDST ceftriaxone (30µg), Amoxicillin-clavulanic acid (20/10µg), and ceftazidime (30µg, Oxoid). Discs were placed at a distance of 1.5cm. ESBL positive organism are showing development of the inhibition zone towards the clavulanate disc at 37°C after 24-hours-incubation.

Molecular characterization of ESBL-genes in *E. coli* and *Klebsiella* spp. isolates

Extraction of DNA was performed using QIAmp DNA Mini Kit instructions (QIAGEN, Germany). Specific primers were used for the amplification of *blaTEM*, *blaSHV*, and *blaCTX* genes (Table 2). The preparation of the PCR Master Mix was performed according to Emerald Amp GT PCR Master Mix (Takara). The reaction mixture consisted of 12.5μl Emerald Amp GT PCR Master Mix (2x premix), 4.5 μl PCR grade water, 1μl of each primer in the concentration

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of (20 pmol), 6μl of Template DNA, and leading to a total of 25μl. The cycling condition was like that used in (Hasman et al., 2005). The ladder was mixed gently by pipetting up and down, and 6 µl of the required ladder was directly loaded. The PCR products were resolved by electrophoresis on an agarose gel according to Sambrook et al. (1989) with some modification.

Table 1. Antibiotics used in antimicrobial susceptibility test and their resistance pattern against *E. coli* and *Klebsiella* spp. isolates

Antibiotic	Disc content	Resistance pattern of *E. Coli*			Resistance pattern of *Klebsiella* spp.		
		Sensitive (%)	Intermediate (%)	Resistant (%)	Sensitive (%)	Intermediate (%)	Resistant (%)
Aminoglycosides							
Amikacin	30 µg	89	5	6	86	6	8
Gentamycin	10 µg	53	18	29	72	0	28
Streptomycin	10 µg	47	0	53	28	20	52
Kanamycin	10 µg	31	13	56	60	12	28
Cephalosporins (1st generation)							
Cephalexin	30 µg	8	0	92	18	6	76
Cephalosporins (3rd generation)							
Cefotaxime	30 µg	9	4	87	52	0	48
Ceftazidime	30 µg	19	0	81	36	4	60
Ceftriaxone	30 µg	23	6	71	38	22	40
Miscellaneous antibiotics							
Chloramphenicol	10 µg	49	27	34	45	31	24
Nitrofurinations	300 µg	69	13	18	58	24	28
Other *β lactam*							
Aztreonam	30 µg	41	6	53	66	10	24
Penicillins							
Ampicillin	10 µg	0	0	100	0	0	100
Amoxycillin	(20/10) µg	37	0	63	32	8	60
Clavulanic acid							
Quinolones							
Ciprofloxacin	5 µg	71	11	18	88	0	12
Nalidixic acid	30 µg	68	3	29	92	0	8
Tetracyclines							
Tetracycline	30 µg	34	6	60	42	22	36

Table 2. Oligonucleotide primers and probes used in PCR and SYBR Green real-time PCR

Gene	Primer sequence (5'-3')	Amplification size	Reference
bla_{TEM}	ATCAGCAATAAACCAGC	516 bp	Colom et al. (2003)
	CCCCGAAGAACGTTTTC		
bla_{SHV}	AGGATTGACTGCCTTTTTG	392 bp	
	ATTTGCTGATTTCGCTCG		
blaCTX	ATG TGC AGY ACC AGT AAR GTK ATG GC	593 bp	Archambault et al. (2006)
	TGG GTR AAR TAR GTS ACC AGA AYC AGC GG		
gyrA (Klebsiella spp.*)*	CGC GTA CTA TAC GCC ATG AAC GTA	-	Brisse and Verhoef (2001)
	ACC GTT GAT CAC TTC GGT CAG G		
16S rRNA (E. coli)	GCTGACGAGTGGCGGACGGG	-	Tivendale et al. (2004)
	TAGGAGTCTGGACCGTGTCT		

In vitro assessment of the antibacterial effect of Ag NPs suspension

Silver nanoparticles powder was purchased from the National Research Center, Egypt. The dispersion process was done using an ultrasonic processor (Cole-Parmer instruments, Illinois U.S.A.). For nanofluid preparation, 10 mg of Ag NPs of average size 58 nm were dispersed in 1ml of sterile Muller Hinton broth and sonicated for 5 minutes at 20000 HZ frequency from 3 to 5 times to avoid aggregation (Tayel et al., 2010). Pure colonies from both *E. coli* and *Klebsiella* spp.

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isolates were picked up and suspended in Muller Hinton broth. Suspensions were adjusted to match McFarland standard 0.5 (1.5×10^8 CFU /ml) to be ready for antimicrobial testing. The tests were adapted according to CLSI (2018). The minimum inhibitory concentration (MIC) of AgNPs was determined in a sterile 96-well microtiter plate. The 100 µL of sterile muller Hinton broth was pipetted into the well No.1 through No.12 of the column. In the next step, 100 µL of the Ag NPs suspension (10mg/ml) were added into well No.1 of column (A), to reach a total volume of 200 µL with a concentration of (5 mg/ml). Two-fold serial dilution was applied starting with the previously mentioned concentration. Later, 100 µL of the *E. coli* suspension (McFarland 0.5) was added to the wells of the column, and then the initial concentration changed to 2.5 mg/ml. Well No.11 served as a negative growth (sterility control) containing Ag NPs suspension plus sterile broth only while well No.12 was served as a positive bacterial control containing broth plus the bacterial inoculum only. These steps were repeated with *Klebsiella* spp. in another microtiter plate. The microtiter plates were incubated at 37 °C for 24 hours in a shaker incubator. The lowest concentration of Ag NPs in the series inhibiting the growth of the bacteria in vitro was taken as the MIC. For MBC determination, 50 µL from each well was spread on MacConkey agar plates and incubated at 37°C for a further 48 hours. Growth-free plates validated that the used concentration inhibited bacterial growth. These tests were performed in triplicate.

Assessment of the inhibitory effect of AgNPs on the expression of resistance genes in *E. coli* and *Klebsiella* spp. using SYBR Green RT- PCR

The effect of sub-MIC dose (1/2) of AgNPs on the expression of *blaTEM, blaSHV,* and *blaCTX* genes was studied in the presence of 16s rRNA for *E. coli* and gyrA for *Klebsiella* spp. as housekeeping genes (Brisse and Verhoef, 2001;Tivendal et al., 2004). The RT-PCR procedure was performed in Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Egypt. Extraction of RNA was performed according to RNeasy Mini Kit instructions (QIAGEN, Germany, GmbH). Oligonucleotide primers and probes used in SYBR Green real-time PCR are shown in Table 2. PCR Master Mix QuantiTect SYBR Green PCR Kit was used. The reaction mixture consisted of 12.5μl 2x QuantiTect SYBR Green PCR Master Mix, 0.25μl Revert Aid Reverse Transcriptase (ThermoFisher, 200 U/µL), 0.5μl of each primer (20 pmol), 8.25μl RNase Free Water, 3μl Template RNA, leading to a total of 25μl. The cycling conditions were performed according to previous studies (Brisse and Verhoef, 2001; Colom et al., 2003; Tivendale et al., 2004; Archambault et al., 2006)**.** Amplification curves and CT values were determined by the strata gene MX3005P software. To estimate the variation of gene expression of the different samples, the CT of each sample was compared with that of the control group according to the " ΔΔCt method CT" stated by Yuan et al. (2006) and samples were tested in triplicates. The dissociation curves of different samples were compared to exclude false-positive results.

Evaluation of morphological changes in *E. coli* and *Klebsiella* spp. upon their interaction with silver nanoparticles

Samples included untreated samples (control) and AgNPs treated *E. coli* and *Klebsiella* spp. colonies. The samples were fixed by glutaraldehyde 2.5% and dehydrated by the serial dilution of ethanol with agitation using an automatic tissue processor (Leica EM TP, Leica Microsystems: Austria). In the next step, they were dried using CO_2 critical point drier (Model: Audosamdri-815, Tousimis; Rockville, Maryland, USA). The samples were coated by a gold sputter coater (SPI-Module, USA). They were examined by Scanning electron microscopy (Model: JSM- 5500 LV; JEOL Ltd –Japan) using a high vacuum mode at the Regional Center of Mycology and Biotechnology, Cairo, Egypt.

Statistical analysis

Statistical analysis was performed using R-programme. One-way ANOVA was run to evaluate the statistical significance between the control and treated samples. *P-value* less than 0.05 was considered statistically significant.

RESULTS

Phenotypic identification of *E. coli* and *Klebsiella* spp. isolates

Out of 120 samples, *E. coli* and *Klebsiella* spp. were detected in 62 (51.6%) and 25 (20.8%) cases, respectively. On MacConkey agar, *E. coli* appeared as medium-sized, smooth, round, lactose-fermenting colonies, pink to red with bile salt precipitate surrounding the colonies. On the other hand, *Klebsiella* spp. was observed as medium-sized, pink, lactose- fermenting, round, shiny, and mucoid colonies. Microscopic examination of Gram-stained pure colonies of *E. coli* and *klebsiella* spp. isolates were gram-negative rod-shaped bacteria. Isolates were confirmed biochemically. *E. coli* was negative in urease, oxidase, Voges Proskauer (vp), citrate tests while positive for catalase, methyl red (MR), Indole tests and A/A with gas production and negative H_2S production for TSI test. *Klebsiella* spp. was positive in urease, oxidase, Voges Proskauer, catalase and citrate test and A/A with gas production and negative indole, Methyl red, and H_2S production for TSI test.

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Antimicrobial susceptibility testing

Using different groups of antibiotics (Table 1), the antibiogram was assessed for *E. coli* and *Klebsiella* spp. isolates. Ampicillin showed the highest percentage for resistance in both *E. coli* and *Klebsiella* spp. as shown in the resistance pattern (Figure 1). The most prominent groups in resistance pattern were Cephalosporins first and third generation represented by Cephalexin (92%, 76%), Cefotaxime (87%, 48%), Ceftazidime (81%, 60%), Ceftriaxone (71%, 40%) for *E. coli* and *Klebsiella* spp., respectively. Moreover, Amoxicillin Clavulanic acid indicated 63% resistance for *E. coli* isolates and 60% for *Klebsiella* spp. The overall result of the antibiogram revealed that about 29 isolates (*E. coli* =20, *Klebsiella* spp.=9) were suspected to be ESBL producing isolates.

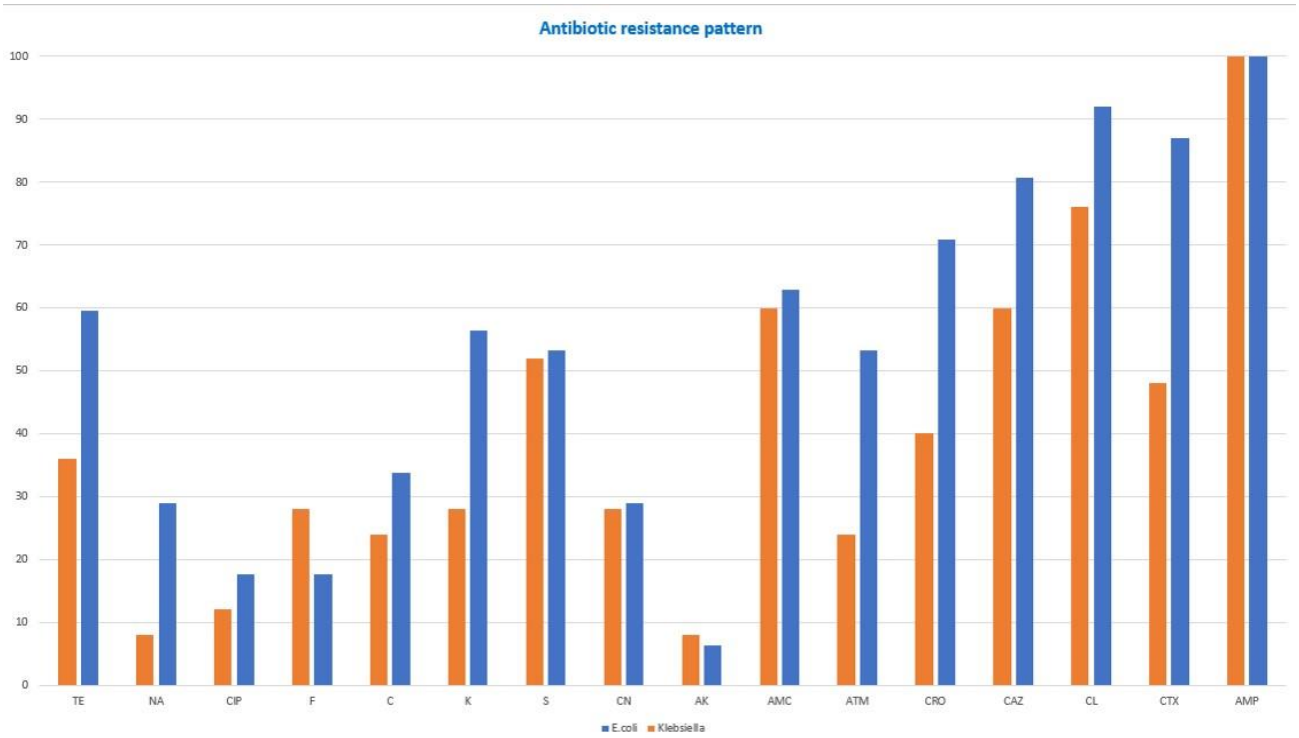

Figure 1. Antibiotic resistance pattern of the used antibiotics against *E. coli* and *Klebsiella* spp. isolates

Double-disc synergy test method

Detection of suspected isolates by DDST revealed that 23 isolates (*E. coli*=17, *Klebsiella*=6) were ESBL producing isolates. The number of isolated ESBL producing *E. coli* and *Klebsiella* spp. for each type of sample is demonstrated in Table 3.

Molecular detection of ESBL -encoding genes

PCR screening of genes encoding ESBL revealed the presence of *blaCTX*, *blaSHV*, and *blaTEM* genes in all the tested isolates except one *Klebsiella* spp. isolate which did not harbor *blaCTX* gene (Figure 2).

In vitro evaluation of the antibacterial effect of Ag NPs

Scanning Electron Microscopy of AgNPs (Figure 3) revealed that Ag NPs were spherical with the average size of the 58 nm. The MIC of AgNPs tested for ESBL-producing *E. coli* was 0.31 mg/ml, and 0.62 mg/ml for ESBL-producing *Klebsiella* spp. The minimum bactericidal concentration (MBC) of ESBL-producing *E. coli* and *Klebsiella* spp. was 0.15 mg/ml and 0.3 mg/ml, respectively.

Effect of sub MIC concentration of AgNPs on the expression of resistance genes.

Expression of *blaTEM*, *blaSHV*, and *blaCTX* genes was downregulated with sub-MIC doses of AgNPs (150 µg/ml for *E. coli* and 310 µg/ml for *Klebsiella* spp.), compared to untreated sample as shown in (Figure 4).

Effect of silver nanoparticles on the integrity of cells

The SEM images of untreated (control) cells showed healthy cells with clear unpenetrated cell membranes while treated cells showed cell membrane damage due to the adherence of AgNPs with the bacterial cell membrane and penetration into the cells causing cell death (Figure 5).

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Table 3. Prevalence of ESBL producers from dogs and cats in Egypt

Origin	No. of samples with ESBL producer (%)		
	EC	KS	Total
Wound swabs (n=17)	-	2 (11.7)	2 (11.7%)
Fecal swabs (n=61)	10 (16.4%)	1 (1.6%)	11 (18%)
Urine samples (n=42)	7 (16.7%)	3 (7.1%)	10 (23.8%)
Total (n=120)	17 (14.16%)	6 (5%)	23 (19.6%)

EC: Escherichia coli; KS: *Klebsiella* spp.

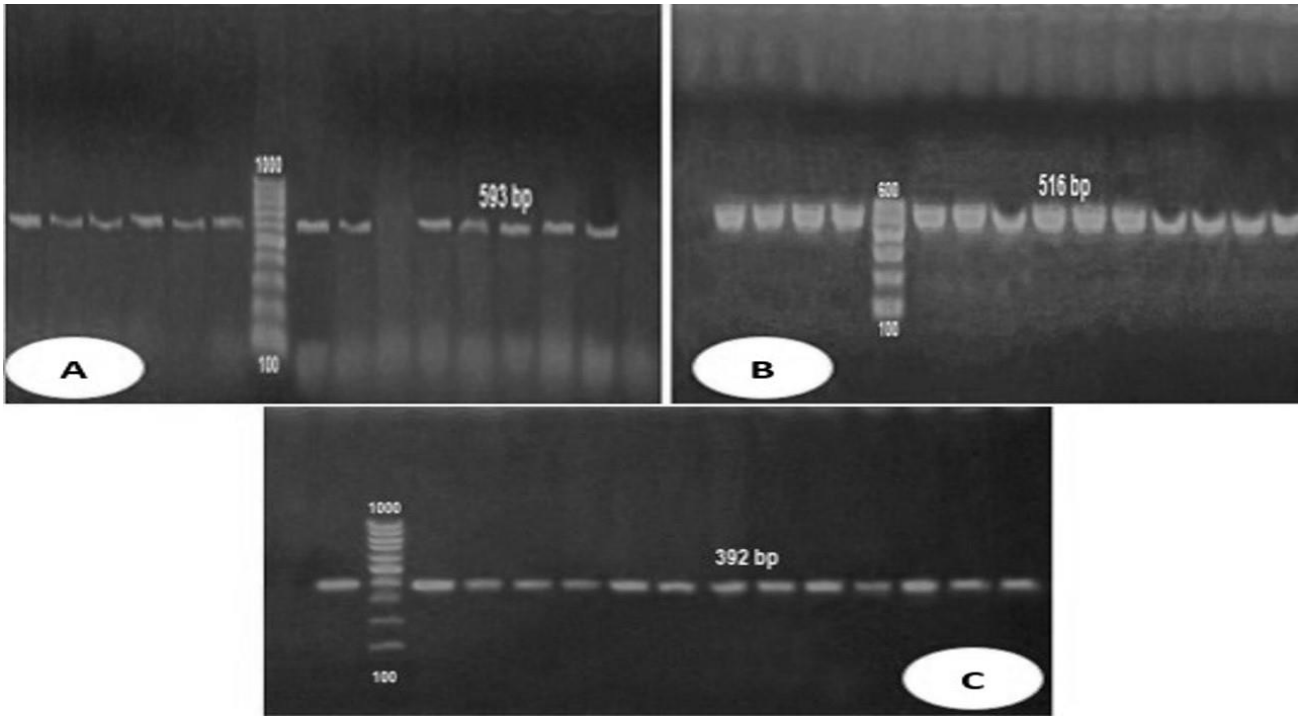

Figure 2. Agarose gel electrophoresis of A- *blaCTX* gene (Amplicon size593 bp), B- *blaTEM* gene (Amplicon size 516 bp), C-*blaSHV* gene (Amplicon size 392 bp) Ladder [Gelpilot100 bp plus ladder (Qiagen, 100-1500 bp)]

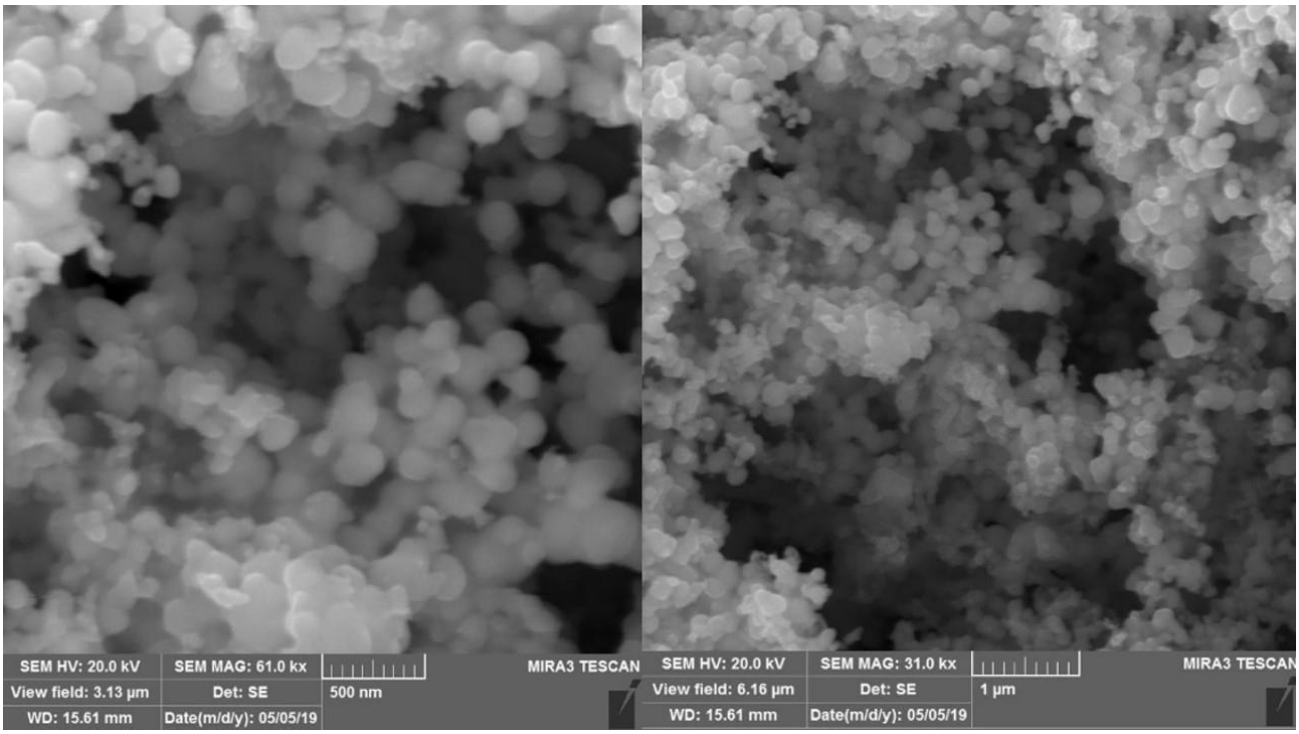

Figure 3. SEM of AgNPs revealed that Ag NPs are spherical in shape and the average size is 58 nm.

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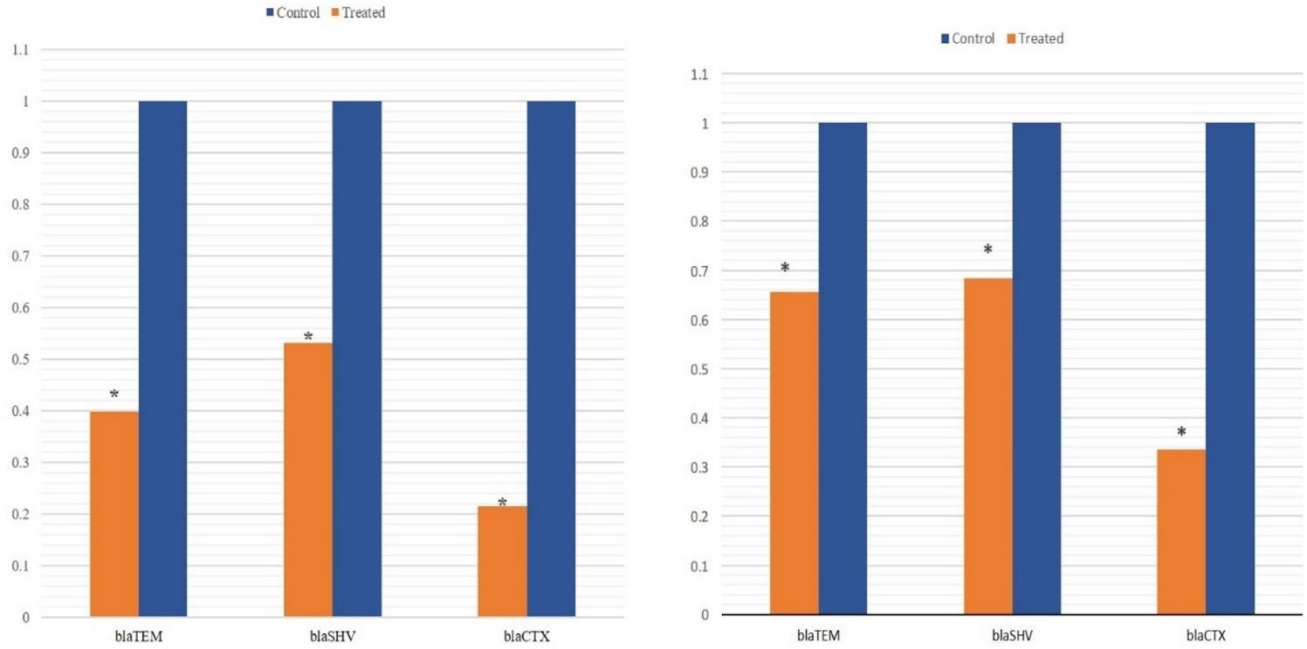

Figure 4. Effect of Silver nanoparticles on the antibiotic resistance genes expression in Left *E. coli*, Right *Klebsiella* spp. Stars indicate significant difference between control and treated samples for each gene.

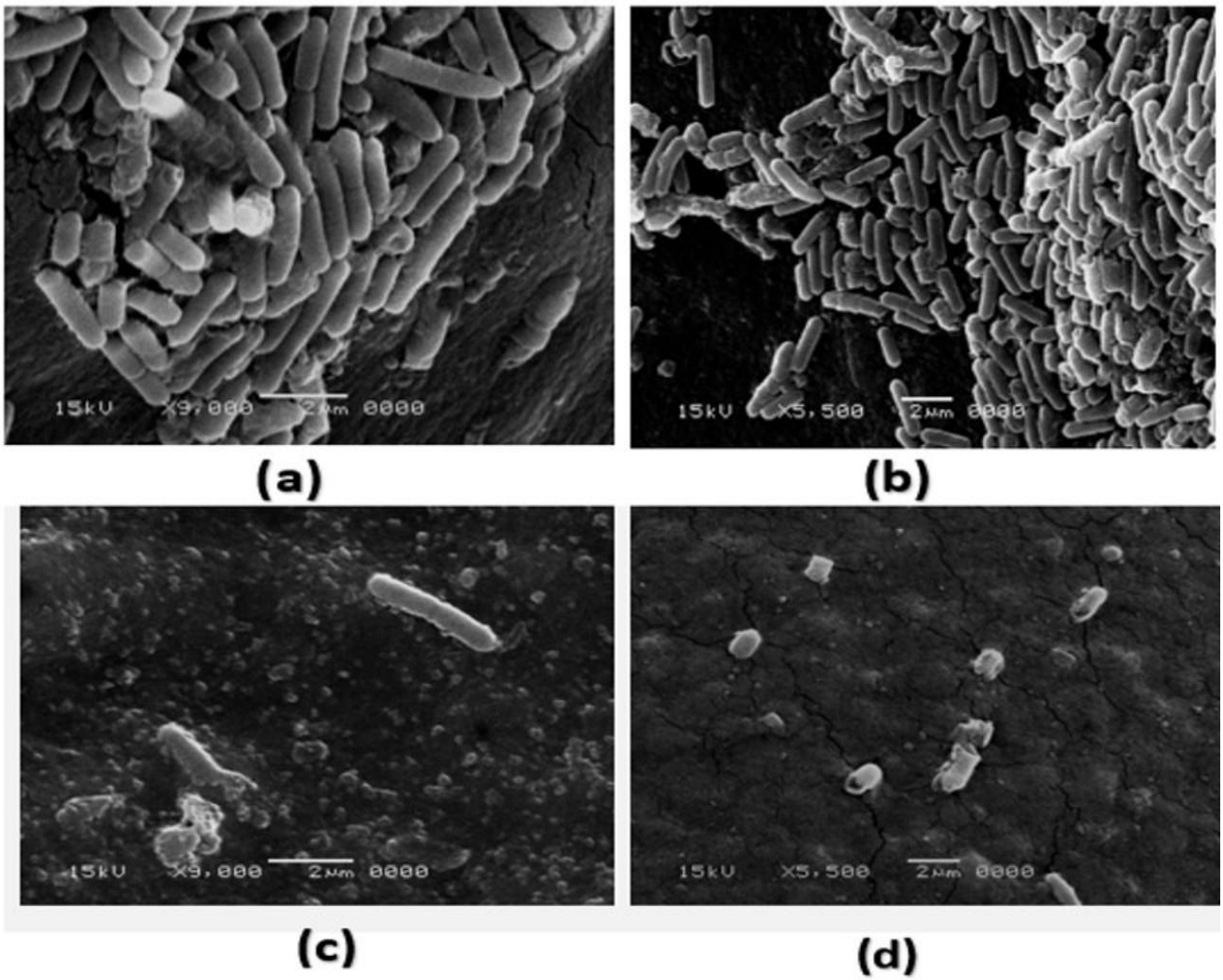

Figure 5. Scanning Election microscopic pictures of AgNPs treated and untreated *E. Coli* and *Klebsiella* spp. The control cells showed normal, clear and unpenetrated cell membrane (**a, b**). AgNps treated cells showed cell membrane penetration and perforation of cells leading to excretion of cell metabolites and cell death (**c, d**)

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DISCUSSION

Despite the presence of various modern antimicrobial agents and antibacterial drugs, bacterial infections are still a major threatening problem due to the enormous increase in multi-drug-resistant bacteria. Mobile genetic elements, such as plasmids, are capable of transferring antimicrobial resistance determinant elements among different bacterial populations and play an important role in the epidemiology of antimicrobial resistance (Carattoli, 2013). The extensive misuse of antibiotics has become a leading cause of the emergence of several hazards to public health, such as superbugs, which resist all the current drugs (Khameneh et al., 2016). The clinical effectiveness of beta-lactams has been diminished owing to the massive increase in resistant bacteria and prolonged patient recovery (Denisuik et al., 2013; Mathers et al., 2015). Extended-spectrum beta-lactamases (ESBLs) in pet animals is a threatening issue which has emerged worldwide. Since their emergence, ESBLs have most often been found in *Escherichia coli* and *Klebsiella pneumonia* (Bonnet 2004; Livermore et al. 2006; Mathers et al. 2015). In the present study, the prevalence of ESBL producing *Escherichia coli* and *Klebsiella* spp. was investigated in different samples obtained from pet animals. MacConkey agar (Oxoid) supplemented by Ampicillin was used for the isolation of ESBL-producing isolates which facilitate screening of the isolates rather than Macconkey supplemented with Cephalosporine as reported by Okapara et al. (2018). ESBL-producing *E. coli* and *Klebsiella* spp. were detected in 23 out of the 120 samples of the current study representing 19.6 % of the total samples. In previous studies, ESBL- producing *K.pneumoniae* were recorded as 41% (Okapara et al., 2018) and 7.5% (Liu et al., 2017). ESBL-producing isolated *E. coli* was detected in 17 (14.16%) samples, 7 from urine, and the other 10 from fecal samples. The obtained results of the current study were indicative of higher percentages, compared to those of ESBL isolated *E. coli* obtained from dogs and cats in Switzerland 8% (Huber et al., 2013) and New Zealand 6.4%, (Karkaba et al., 2019) and lower than those obtained from pet animals in Switzerland 54.7%, (Zogg et al., 2018) and from dairy farms in Germany 75.6%, (Odenthal et al., 2016)**.** Three genes (i.e., *TEM*, *SHV*, and *CTX-M*) are the most predominant in ESBL-producing bacteria (Paterson and Bonomo, 2005). The *blaSHV* and *blaTEM* were detected in all the isolates while *blaCTX-M* was found in 92.3%. Huber et al. (2013) investigated ESBL genes of ESBL producing *E. coli* isolates where *blaCTX-M* was found in 100% of isolates and *blaTEM* in 87.5%. Searching new effective bactericidal alternatives has become an urgent issue for combatting drug resistance. Silver nanoparticles have been established as a promising approach as an alternative for antimicrobial agents in the treatment of several medicinal problems (Beyth et al., 2015; Hassanen and Ragab, 2020**).** They have attracted great concerted attention and have been broadly used in a variety of applications as antibacterial/antifungal agents in a diverse range of products, including air sanitizer sprays, pillows, respirators, wet wipes, detergents, soaps, shampoos, toothpaste, air filters, coatings of refrigerators, vacuum cleaners, washing machines, food storage containers, cellular phones (Sun et al., 2001). Moreover, they do not cause high level of toxicity in human as well as they have broad-spectrum antibacterial actions (Chandran et al., 2006).

In the present study, AgNPs were tested against ESBL-producing *E. coli* and *Klebsiella* spp. isolates in vitro by the detection of MIC and MBC. Manikprabhu and Lingappa (2014) determined the antibacterial effect of AgNPs with size (28-50nm) against ESBL producing *E. coli* where MIC and MBC were found to be (in the range of 0.11 and 0.22 mg/ml**)**. Moreover, biogenic nanosilver of (20-70nm) was used against ESBL-producing *k. pneumoniae* and *E. coli*, where the recorded MIC and MBC were 1.4µg and 2µg, respectively (Subashini et al., 2014). This indicated that AgNPS had a good bacteriostatic effect according to MIC and good bactericidal effect according to MBC on ESBL-producing *E. coli* and *Klebsiella* spp. As reported, the small size of nanoparticles potentiates the antibacterial effect on microorganisms (Smekalova et al., 2016). Also, AgNPs had the same effect against ESBL- and non ESBL- producing bacteria (Ansari et al., 2014).

It should be noted that it is not easy to compare the obtained results of the antibacterial effect of AgNPs in the current research with those of previous studies since different researchers employed different methods to study the antibacterial effect of AgNPs against different types of bacteria. Besides, the effect of AgNPs against microorganisms was influenced by the size, shape, stability, and concentration of AgNPs (Bandyopadhyay et al., 2018). We found that the concentration of AgNPs used to inhibit or kill microorganisms differed from one another as MIC and MBC values of AgNPs against *E. coli* were lower than those of *Klebsiella* spp.

In the current study, SYBR Green RT-PCR was used to investigate the influence of silver nanoparticles against ESBL-producing *E. coli* and *Klebsiella* spp. resistance genes (*blaCTX-M, blaTEM,* and *blaSHV*). The sub-MIC dose (150µg) of silver nanoparticles of average size 58 nm was tested against *E. coli* and showed the downregulation of *blaCTX-M, blaTEM,* and *blaSHV* genes with fold change about 0.21, 0.39, 0.53, respectively, for *E. coli*. Furthermore, the sub-MIC dose (310µg) of silver nanoparticles was tested against *Klebsiella* spp. and indicated the downregulation of genes expression with 0.33, 0.65, 0.68 for *blaCTX-M, blaTEM,* and *blaSHV* resistance genes, respectively. These results indicated that AgNPs could effectively influence the gene expression of *E. coli* (*blaCTX-M, blaTEM,* and *blaSHV*) genes more than that of *Klebsiella* spp, which would subsequently reflect in their resistance pattern.

In the current study, the morphological changes in *E. coli* and *Klebsiella* spp. cells were evaluated before and after the treatment with silver nanoparticles using SEM. The SEM observations in treated cells confirmed cell membrane

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damage due to the adherence of AgNPs with bacterial cell membranes and penetration into the cells causing cell death. On the other hand, untreated cells indicated healthy cells with a clear unpenetrated cell membrane. The bactericidal effect of silver nanoparticles is still of unknown mechanism. Many studies suggest that their binding to the bacterial cell membrane may disrupt cell permeability (Kvítek et al., 2008) while other studies propose that the bactericidal effect did not only caused by contact with cell membrane but also because of penetration into the bacterial cell leading to the inactivation of DNA replication and causing cell death (Morones et al., 2005).

CONCLUSION

In the present study, the antibacterial effect of silver nanoparticles was investigated in vitro against Extended Spectrum Beta lactamase producing *E. coli* and *Klebsiella* spp. The findings revealed that using silver nanoparticles as an alternative to antimicrobial agents had an obvious effect on minimum inhibitory concentration, minimum bactericidal concentration , Bacterial cell wall integrity as well as genetically on the expression of antibiotic resistance genes. This trial is very encouraging for the control of antibiotic-resistant bacteria.

DECLARATIONS

Authors' contributions

Omnia A Khalil, Mona I Enbaawy, Eman Ragab, Hossam Mahmoud, and Taher Salah designed the plan of work, supervised the experiment, and revised the manuscript. Eman Ragab is the corresponding author and responsible for English editing, statistical analysis, and formatting the manuscript.

Competing interests

The authors declare no conflicts of interest.

Consent to publish

Written consent was obtained from the owners of the animals after they were informed on the use of their animal samples in the study

REFERENCES

Alyamani EJ, Khiyami AM, Booq RY, Majrashi MA, Bahwerth FS, and Rechkina E (2017). The occurrence of ESBL-producing *Escherichia coli* carrying aminoglycoside resistance genes in urinary tract infections in Saudi Arabia. Annals of Clinical Microbiology and Antimicrobials, 16(1): 1. DOI: https://www.doi.org/10.1186/s12941-016-0177-6

Ansari MA, Khan HM, Khan AA, Cameotra SS, Saquib Q, and Musarrat J (2014). Interaction of A l2 O 3 nanoparticles with E scherichia coli and their cell envelope biomolecules. Journal of applied microbiology, 116(4): 772-783. DOI: https://www.doi.org/10.1111/jam.12423

Archambault M, Petrov P, Hendriksen RS, Asseva G, Bangtrakulnonth A, Hasman H, and Aarestrup FM (2006). Molecular characterization and occurrence of extended-spectrum beta-lactamase resistance genes among Salmonella enterica serovar Corvallis Thailand, Bulgaria, and Denmark. Microbial Drug Resistance, 12(3): 192-198. DOI: https://www.doi.org/10.1089/mdr.2006.12.192

Bandyopadhyay S, Banerjee J, Bhattacharyya D, Samanta I, Mahanti A, Dutta TK, Ghosh S, Nanda PK, Dandapat P and Bandyopadhyay S (2018). Genomic identity of fluoroquinolone-resistant bla CTX-M-15-Type ESBL and pMAmpC β-lactamase producing *Klebsiella pneumoniae* from buffalo milk, India. Microbial Drug Resistance, 1: 24(9): 1345-1353. DOI: https://doi.org/10.1089/mdr.2017.0368

Beyth N, Houri-Haddad Y, Domb A, Khan W, and Hazan R (2015). Alternative antimicrobial approach: nano-antimicrobial materials. *Evidence-Based Complementary* and *Alternative Medicine*, 2015: 246012. DOI: http://dx.doi.org/10.1155/2015/246012

Bindu D, Mythili S, Inthira KS, palanisamy R, Lingesh KT, and Thandapani S (2015). New Generation of Silver Nanoparticles against Extended Spectrum Beta Lactamase Producing Organisms. International Journal of Drug Development & Research, 7(3): 001-003. Available at: https://www.ijddr.in/drug-development/new-generation-of-silver-nanoparticles-against-extended-spectrum-betalactamase-producing-organisms.pdf

Brisse S, and Verhoef J (2001). Phylogenetic diversity of *Klebsiella pneumonia* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, gyrA and parC gene sequencing and automated ribotyping. *International Journal* of Systematic and Evolutionary *Microbiology*, 51: 915-924.
DOI:: https://www.doi.org/10.1099/00207713-51-3-915

Carattoli A. Plasmids and the spread of resistance(2013) . International Journal of Medical Microbiology 1;303(6-7):298-304. DOI : https://doi.org/10.1016/j.ijmm.2013.02.001

Chandran SP, Chaudhary M, Pasricha R, Ahmad A, and Sastry M (2006). Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnology Progress, 22: 577-583. DOI: https://www.doi.org/10.1021/bp0501423

Clinical and Laboratory Standards Institute (CLSI) (2018). Performance standards for antimicrobial susceptibility testing. 27th Informational Supplement Document M100- S27, CLSI, Wayne, 37(1). Available at :https://clsi.org/media/1469/m100s27_sample.pdf

Colom K, PèrezJ, Alonso R, Fernández-Aranguiz A, Lariño E, and Cisterna R (2003). Simple and reliable multiplex PCR assay for detection of blaTEM,blaSHV and blaOXA-1 genes in Enterobacteriaceae. FEMS Microbiology Letters, 223: 147-151. DOI:https://www.doi.org/10.1016/S0378-1097(03)00306-9

Cruickshank R, Duguid RP, Marmion BP, and Swain RH (1975). Medical Microbiology.New York: Churchill Livingstone, Available at: https://agris.fao.org/agris-search/search.do?recordID=XF2015010062

Denisuik AJ, Lagacé-Wiens PR, Pitout JD, Mulvey MR, Simner PJ, Tailor F, Karlowsky JA, Hoban DJ, Adam HJ, and Zhanel GG (2013), Canadian Antimicrobial Resistance Alliance (CARA). Molecular epidemiology of extended-spectrum β-lactamase-, AmpC β-lactamase-and

carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from Canadian hospitals over a 5 year period: CANWARD 2007–11. Journal of Antimicrobial Chemotherapy, 1: 68(suppl_1): 57-65. DOI: https://doi.org/10.1093/jac/dkt027

Devrim I, Gulfidan G, Gunay I, Agin H, Güven B, Yilmazer MM, and Dizdarer C (2011). Comparison of in vitro activity of ertapenemwith other carbapenems against extended- spectrum beta-lactamase-producing Escherichia coli and Kleibsella species isolated in a tertiary children's hospital. *Expert Opinion* on *Pharmacotherapy*, 12: 845–849. DOI: https://www.doi.org/10.1517/14656566.2011.559460

Ejaz H, Zafa A, Mahmood S, and Javed MM (2011). Urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae*. African Journal of Biotechnology, 10(73): 16661-16666. DOI https://www.doi.org/10.5897/AJB11.2449

Fan NC, Chen HH , Chen CL Ou LS, Lin TY, Tsai MH and Chiu CH (2014). Rise of community-onset urinary tract infection caused by extended-spectrum β-lactamase-producing *Escherichia coli* in children. Journal of microbiology,Immunology and Infection 47(5): 399-405. DOI: https://www.doi.org/10.1016/j.jmii.2013.05.006

Hamida RS, Ali MA, Goda DA, Khalil MI, and Redhwan A (2020). Cytotoxic effect of green silver nanoparticles against ampicillin resistant *Klebsiella pneumoniae*. Royal Socitey of Chemistry Advances, 10: 21136-21146. DOI: https://www.doi.org/10.1039/D0RA03580G

Hasman H, Mevius D, Veldman K, Olesen I and Aarestrup FM (2005). β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. Journal of Antimicrobial Chemotherapy. 1;56(1):115-21. Doi: https://doi.org/10.1093/jac/dki190

Hassanen EI, and Ragab E (2020). In Vivo and In Vitro Assessments of the Antibacterial Potential of Chitosan-Silver Nanocomposite Against Methicillin-Resistant Staphylococcus aureus–Induced Infection in Rats. Biological Trace Element Research, Apr 18: 1-4. DOI: https: www.doi.org/10.1007/s12011-020-02143-6

Huber H, Zweifel C, Wittenbrink MM, and Stephan R (2013). ESBL-producing uropathogenic Escherichia coli isolated from dogs and cats in Switzerland.Veterinary Microbiology, 162: 992-996. DOI: https://www.doi.org/10.1016/j.vetmic.2012.10.029.

Iqbal R, Ikram N, Shoaib M, Asad MJ, Tahir R, Mehmood, Niazi A, Asghar A, Bushra Ishfaq and Faiza Naseer (2017). Phenotypic cofirmatory disc diffusion test (PCDDT), double disc synergy test (DDST), E-test OS diagnostic tool for detection of extended spectrum beta lactamase (ESBL) producing Uropathogens. Journal of Applied Biotechnology and Bioengineering, 3(3): 344–349 DOI: https://www.doi.org/10.15406/jabb.2017.03.00068

Karkaba A, Hillb K, Benschop J, Pleydellc E, and Grinberg A (2019). Carriage and population genetics of extended spectrum β-lactamaseproducing Escherichia coli in cats and dogs in New Zealand.Veterinary Microbiology, 233: 61-67. DOI: https://www.doi.org/10.1016/j.vetmic.2019.04.015.

Keshari AK, Srivastava R, Singh P, Yadav B, and Nath G (2020). Antioxidant and antibacterial activity of silver nanoparticles synthesized by Cestrum nocturnum. Journal of Ayurveda and Integrative Medicine, 11(1): 37-44. DOI: https://www.doi.org/10.1016/j.jaim.2017.11.003

Khameneh B, Diab R, Ghazvini K, and Fazly Bazzaz BS (2016). Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. Microbial Pathogenesis, 95: 32–42. DOI: https://www.doi.org/10.1016/j.micpath.2016.02.009

Khan T, Yasmin A, and Townley HE (2020). An evaluation of the activity of biologically synthesized silver nanoparticles against bacteria, fungi and mammalian cell lines. Colloids and surfaces B: Biointerfaces, 194: 111156. DOI: https://www.doi.org/10.1016/j.colsurfb.2020.111156

Kizilca O, Siraneci R, and Yilmaz A (2012). Risk factors for community-acquired urinary tract infection caused by ESBL-producing bacteria in children. Pediatrics *International*, 54(6): 858-862. DOI: https://www.doi.org/10.1111/j.1442-200X.2012.03709.x

Kvítek L, Panáček A, Soukupova J, Kolář M, Večeřová R, Prucek R, Holecova M, and Zbořil R (2008). Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). The Journal of Physical Chemistry C, 17: 112(15): 5825-5834. DOI: https://www.doi.org/10.1021/jp711616v

Li WR, Xie XB, Shi QS, Zeng HY, OU-Yang YS, and Chen Y (2010). Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. *Applied Microbiology* and *Biotechnology*, 85: 1115-1122. DOI 10.1007/s00253-009-2159-5

Liu Y, Yang Y, Chen Y, and Xia Z (2017). Antimicrobial resistance profiles and genotypes of extended-spectrum b-lactamase- and AmpC b-lactamase-producing *Klebsiella pneumoniae* isolated from dogs in Beijing, China. Journal of Global Antimicrobial Resistance, 10: 219–222. DOI: https://www.doi.org/10.1016/j.jgar.2017.06.006

Livermore DM, Hope R, Fagan EJ, Warner M, Woodford N, and Potz N (2006). Activity of temocillin against prevalent ESBL-and AmpC-producing Enterobacteriaceae from south-east England. Journal of Antimicrobial Chemotherapy. 1:57(5):1012-4. DOI : https://doi.org/10.1093/jac/dkl043

Manikprabhu D, and Lingappa K (2014). Synthesis of silver nanoparticles using the Streptomycces Coelicolor Klmp33 pigment: An antibacterial agent against extended-spectrum beta-lactamase (ESBL) producing Escherichia Coli. Materials Science and Engineering C, 45: 434-437. DOI: https://www.doi.org/10.1016/j.msec.2014.09.034

Mathers AJ, Peirano G, and Pitout JD (2015). The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clinical microbiology reviews. 28(3):565-591. DOI: https://www.doi.org/10.1128/CMR.00116-14

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, and Yacaman MJ (2005). The bactericidal effect of silver nanoparticles. Nanotechnology, Aug 26; 16(10): 2346. DOI: https://www.doi.org/10.1088/0957-4484/16/10/059

Odenthal S, Akineden Ö and Usleber E (2016). Extended-spectrum β-lactamase producing Enterobacteriaceae in bulk tank milk from German dairy farms. International Journal of Food Microbiology, 238: 72–78. DOI: https://www.doi.org/10.1016/j.ijfoodmicro.2016.08.036

Okapara EO, Ojo EO, Awoyomi OJ, Dipeolu MA, Oyekunle MA, and Schwarz S (2018). Antimicrobial usage and presence of extended-spectrum- β-lactamase-producing Enterobactereaceae in animal-rearing households of selected rural and peri-urban communities.Veterinary Microbiology, 218: 31-39. DOI: https://www.doi.org/10.1016/j.vetmic.2018.03.013

Paterson DL and Bonomo RA (2005). Extended_spectrumbeta_lactamases: a clinical update. Clinical Microbiology Review,18: 657-686. DOI: https://www.doi.org/10.1128/CMR.18.4.657-686.2005

Saeb ATM, Alshammari AS, Al-Brahim H, and Al-Rubeaan KA (2014). Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. Scientific World Journal, 1: 704-708. DOI: https://www.doi.org/10.1155/2014/704708

Sambrook J, Fritsch EF, Maniatis T, and Sambrook-Fritsch-Maniatis (1989). Molecular Cloning: A Laboratory Manual ;[prepared for Use in the CSH Courses on the Molecular Cloning of Eukaryotic Genes]. Cold Spring Harbor Laboratory Press, Available at: https://www.cshlpress.com/pdf/sample/2013/MC4/MC4FM.pdf

Shahid M, Al-Mahmeed A, Murtadha MM, Qareeballa A, Eltahir MA, Tabbara KS, Ismaeel AY, Dar FK, Giha HA and Bindayna KM (2014). Characterization of cephalosporin-resistant clinical Enterobacteriaceae for CTX-M ESBLs in Bahrain. Asian Pacific Journal of Tropical Medicine, 7(1): S212-S216. DOI:https://www.doi.org/10.1016/s1995-7645(14)60234-0

Smekalova M, Aragon V, Panacek A, Prucek R, Zboril R and Kvitek L (2016). Enhanced antibacterial effect of antibiotics in combination with silver nanoparticles against animal pathogens. The Veterinary Journal, 209: 174-179. DOI: https://www.doi.org/10.1016/j.tvjl.2015.10.032

To cite this paper: Khalil OA, Enbaawy MI, Salah T, Mahmoud H and Ragab E (2021). In Vitro Investigation of the Antibacterial Effect of Silver Nanoparticles on ESBL-producing *E. coli* and *Klebsiella* spp. Isolated from Pet Animals. *World Vet. J.*, 10 (4): 514-524. DOI: https://dx.doi.org/10.54203/scil.2020.wvj62

Subashini J, Khanna VG, and Kannabiran K (2014). Anti-ESBL activity of silver nanoparticles biosynthesized using soil Streptomyces species *Bioprocess* and *Biosystems Engineering*, 37: 999–1006. DOI: https://www.doi.org/10.1007/s00449-013-1070-8

Sukmawinata E, Uemura R, Sato W, Thu Htun M, and Sueyoshi M (2020) . Multidrug-Resistant ESBL/AmpC-Producing *Klebsiella pneumoniae* Isolated from Healthy Thoroughbred Racehorses in Japan. Animals 10(3):369. DOI: https://doi.org/10.3390/ani10030369

Sun YP, Atorngitjawat P, and Meziani MJ (2001). Preparation of silver nanoparticles via rapid expansion of water in carbon dioxide micro-emulsiuon into reductant solution. Langmuir 17: 5707-5710. DOI: https:// www.doi.org/10.1021/la0103057

Tayel AA, El-Tras WF, Moussa S, El-Baz AF, Mahrous H, Salem MF, and Brimer L (2010). Antibacterial action of zinc oxide nanoparticles against foodborne pathogens. Journal of Food Safety, 31: 211-218. DOI: https://www.doi.org/10.1111/j.1745-4565.2010.00287.x

Thungrat K, price SB, Carpeter DM, and Boothe DM (2015). Antimicrobial susceptibility patterns of clinical Escherichia coli isolates from dogs and cats in the united states:january 2008 through january 2013.Veterinary Microbiology. 179(3-4) 287-295. DOI: https://www.doi.org/10.1016/j.vetmic.2015.06.012.

Tivendale KA, Allen JL,Ginns CA, Crabb BS, and Browning GF (2004). Association of iss and iucA, but not tsh, with plasmid- mediated virulence of avian pathogenic Escherichia coli. Infection and Immunity, 72(11): 6554-6560. DOI: https://www.doi.org/10.1128/IAI.72.11.6554-6560.2004

Wang Y, Hu Y, Cao J, Bi Y, Lv N, Liu F, Liang S, Shi Y,Jiao X, Gao GF, et al. (2019). Antibiotic resistance gene reservoir in live poultry markets. Journal of infection 78(6): 11-53. DOI: https://www.doi.org/10.1016/j.jinf.2019.03.012

Yuan JS, Reed A,Chen F, and Stewart CN (2006). Statistical analysis of real-time PCR data. BMC Bioinformatics, 7-85. Available at: https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-7-

Zogg AL, Zurfluha K, Schmittb S, Nüesch-Inderbinena M, and Stephana R (2018). Antimicrobial resistance, multilocus sequence types and virulence profiles of ESBL producing and non-ESBL producing uropathogenic Escherichia coli isolated from cats and dogs in Switzerland.Veterinary Microbiology, 216: 79-84. DOI: https://www.doi.org/10.1016/j.vetmic.2018.02.011.

To cite this paper: Khalil OA, Enbaawy MI, Salah T, Mahmoud H and Ragab E (2021). In Vitro Investigation of the Antibacterial Effect of Silver Nanoparticles on ESBL-producing *E. coli* and *Klebsiella* spp. Isolated from Pet Animals. *World Vet. J.*, 10 (4): 514-524. DOI: https://dx.doi.org/10.54203/scil.2020.wvj62

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 525-532, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj63

Polymorphism Association of *Pituitary Positive Transcription Factor-1* Gene with Body Weight Traits in BC_1 Hybrid Chicken (*Gallus gallus gallus* Linnaeus, 1758) from Cross Breeding between Female F_1 Broiler and Male Pelung

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ORIGINAL ARTICLE
pii: S232245682000063-10
Received: 08 Sept 2020
Accepted: 06 Nov 2020

ABSTRACT

Pituitary Positive Transcription Factor-1 gene is closely related to chicken growth and productivity. This research was conducted to detect Single Nucleotide Polymorphism in the exon 6 Pituitary Positive Transcription Factor-1 gene and its association with the bodyweight growth in the first backcross hybrid chicken. Procedures of the research included crossbreeding female first filial broiler chicken with male Pelung chicken to obtain first backcross hybrid chicken, Day Old chick hatched were maintained during 49 days, the bodyweight on the Day-Old chick measured every seven days, DNA was isolated by Chelex 5% method, Pituitary Positive Transcription Factor-1 gene was amplified by PCR, DNA band was visualized utilizing electrophoresis, and the PCR product was sequenced using Sanger method. The DNA sequence was aligned using Clustal omega software to gain Single Nucleotide Polymorphism. The Single Nucleotide Polymorphism was analyzed using the Pearson correlation test between chicken body weights of 49-days-old chickens with the polymorphism points. The conclusion indicated that the bodyweight of the first backcross hybrid chicken was higher than the Pelung chicken but lower than the first filial broiler chicken. Single Nucleotide Polymorphism was not found on the exon 6 Pituitary Positive Transcription Factor-1 gene in the first backcross hybrid chicken.

Keywords: Growth, Hybrid chickens, PIT-1 gene, SNP

INTRODUCTION

Indonesian native chickens or known as '*ayam buras'* (non-broiler chickens) are very popular by Indonesians, especially in rural areas. Indonesian native chickens are classified into four functional groups such as meat and egg producer, singing chicken, used in traditional ceremonies, fancy, and fighting cock (Hidayat and Asmarasari, 2015). According to (Zein and Sulandari, 2009) a genetic molecular study, informed that all domesticated chicken populations came from one ancestor (monophyletic), namely red jungle fowl (*Gallus gallus*) originated from Southeast Asia. Indonesian local chickens were developed through a process of domestication and known as native chickens. Native chickens were the result of a cross between jungle fowl *Gallus bankiva* and *Gallus varius* scattered in the territory of Indonesia, especially in Java and Nusa Tenggara. Local chickens or often known as '*ayam kampung'* have superiority in the quality of their meat and egg, but this superiority is not followed by good productivity capability in meat and egg (Zein and Sulandari, 2009).

The productivity of local chickens is relatively low, as an implication of the extensive system of maintenance. Indonesian local chickens must be maintained optimally to support the small-scale poultry industry so that it becomes a solution to fulfill the increasing demand for domestic food consumption (Daryono et al., 2010). The efforts to improve the productivity of local chickens include selection and crossbreeding programs. According to Cheng (2010), selective breeding is aimed to produce a superior chicken breed with adjusted phenotype quality according to human needs. The targeted selection program will provide a high economic mean in the use of local chickens, namely by improving the quality of local chickens through the crossing and selective breeding programs of specific characters. Other basic information such as specific characteristics, origin, performance, and productivity of local chickens are needed to optimize the utilization of local chickens in Indonesia. This information is expected to make Indonesian local chickens better known, developed, and preserved, so that they can be used sustainably (Sulandari et al., 2007). Therefore, we need research that can study genetic diversities and identify genes responsible for the growth of hybrid chickens.

With the progress of molecular genetics, the selection program can be carried out earlier through analysis at the DNA level. Pituitary Positive Transcription Factor-1 (PIT-1) gene is the one gene that is closely related to chicken growth and productivity (Miyai et al., 2005). As stated by Jiang et al. (2004) exon 6 in PIT-1 gene has a significant

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relationship to improving the growth of chicken weight. The purpose of this study was to obtain hybrid chickens that inherited superior characteristics from both broodstocks with good growth characteristics resembling broiler chickens and good phenotypic characters, body resistance, good quality of meat and eggs resembling local chickens, and genetic quality improvement through molecular selection. Therefore, this study also analyzed the relationship between exon 6 polymorphisms of the PIT-1 gene with hybrid chicken body weight.

MATERIALS AND METHODS

Ethical approval

The procedure in this research has been conducted following the guidelines of the ethical committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Chicken cross breeding

In this study, the first backcross (BC_1) hybrid chickens were used as a result of crossing between female F_1 broiler chicken and male Pelung chicken. The Day Old Chick (DOC) was maintained for seven weeks with lighting 24 hours using 10 watts light bulb, air temperature ±30℃ and 40-50% humidity, feed by BR I (protein 21,00-23,00% and energy 3000 kcal/kg) made by PT. Japfa Comfeed Indonesia Public Listed Company (Plc) *ad libitum*. 12 broods were consisting of 7 males and 5 females. Furthermore, DOC was raised intensively for 7 weeks in special cages to minimize outside influences that can interfere with health, facilitating growth monitoring, and facilitating chickens' feeding. The DOC body weight measurement every 7 days was aimed to observe DOC growth during the observation period for 7 weeks. Quantitative character measurements and qualitative character observations were carried out on the last day of observation on the 49th day.

DNA isolation

DNA isolation with Chelex 5% method with the modified concentration of Chelex according to the optimization phase. A total of 10 µl of chicken blood was put into a 1.5 mL tube, added with 1 ml of Tris-EDTA (TE) buffer. Then put into a 1.5 mL microcentrifuge tube, centrifuged at a speed of 13,000rpm for 3min. The supernatant was transferred to the new eppendorf tube, then the pellet was added with 200µl of 5 percent Chelex solution, 18 µl of dithiothreitol (DTT) 0.05 M, 2 µl of proteinase K, then mix various samples rapidly 30s with vortex and incubated at 56℃ for 2 h, and vortex in every 15 min. Then incubated at 100℃ for 8 min, and centrifuged at 13,000 rpm for 3 min. The supernatant was transferred to a 1.5 ml microcentrifuge tube, and stored at -20℃ (Butler, 2009).

DNA amplification

The amplification of Pituitary Positive Transcription Factor-1 (PIT-1) gene was carried out by PCR, with the reaction composition of Bioline PCR kit as much as 12.5 µl, 5'-GGCACTTTGGAGAACAAAGC-3' forward primer as much as 1.25 µl, 5'-CTCGTGGTGCTCCTTGATAA-3' reverse primer as much as 1.25 µl, 5 µl of DNA samples, and 5 µl of ddH2O so that the total volume was 25 µl. The specific primer used was MR5 (for exon 6 with access code AJ236855) from *Gallus gallus* (Nie et al., 2008). The used PCR program was 95℃ initial denaturation for 5 min, followed by 35 denaturation cycles at 95℃ for 15s, annealing at 60℃ for 60 s, and extension at 72℃ for 60 s, extra extension at 72℃ for 10 min (Van As et al., 2000).

Agarose preparation

Agarose was weighed according to agar concentration (genome =1%) (PCR yield 1.8-2%). Next, it was put in a beaker glass and added with Tris-borate-EDTA (TBE) according to the chamber volume. Then it was put in the oven, heat until it dissolves (clear). A mold was set and installed with the comb. The agar was added with 2-3 µl of flourosave, then poured into the mold. The agarose was left to solidify.

Electrophoresis

Electrophorator was prepared. Agar was inserted into the electrophorator Mupid-exU™. Tris-borate-EDTA/TBE (immersion) was added until the agar was sinked. The sample was inserted into the well. Electrophorator was closed, turn on, time was set (20-30 min =100 volts, 1h =50 volts) then visualized under UV light by AnalytikJena™ gel imaging system and documented with GelDoc™ Documentation System.

Sequencing with Sanger method

The PCR product was sequenced by the Sanger sequencing method (Sanger et al., 1977) in first Base Company, Selangor, Malaysia.

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Data analysis

The correlation between chicken weights was analyzed using SPSS 16.0 one-way ANOVA program statistical test and post hoc LSD method to assess the significance between chicken strains. The data of DNA sequencing were assembled using the Gene Studio program, multiple sequences were alignment using Clustal Omega software, and Pearson correlation test between chicken body weight with Single Nucleotide Polymorphism (SNP, Arnedo et al., 2007).

RESULTS AND DISCUSSION

Chicken growth

In this study, crossings between female F_1 broiler chickens and male Pelung chickens were carried out and resulted in the first backcross or BC_1 hybrid chickens. The comparison of the weight of BC_1 hybrid chicken, Pelung, broiler, and F_1 broiler chicken for 7 weeks is presented in figure 1.

The average weight of chickens from the lowest to the highest starting from Pelung chicken, BC_1 hybrid chicken, F_1 broiler chicken, and broiler chicken. The average body weight of BC_1 hybrid chickens (161.44 gr) for the seven weeks showed lower results compared to the average weight of F_1 broiler chickens (648.14 g) (Roosdianto, 2010), and broiler chickens (1409.57 gr) (Suryaman, 2010), but higher than the average weight of Pelung chickens (112.38 gr). This was based on the inherited character of the broodstocks, the BC_1 hybrid chickens carried the character of broiler chickens which was rapid growth, and thus BC_1 hybrid chickens had a higher weight than Pelung chickens. The growth and development of chickens were influenced by certain factors, including intrinsic factors such as genetics and sex, and extrinsic factors such as the process of chicken breeding, environmental factors, and types of feed (Oktafiantari, 2016).

The significance of the chicken types to the chicken weights for 7 weeks is shown in table 1. The BC_1 hybrid chicken has a higher growth rate compared to Pelung chicken but has a lower growth rate than broiler chicken and F_1 chicken. Table 1, a significant difference is obtained because the significance value of 0.00 is less than the standard deviation of 0.05. Thus, it means that the types of chicken affect chicken weight. The BC_1 hybrid chicken growth is between the Pelung and broiler Chicken growth lines because BC_1 hybrid chickens have both bloodlines. Therefore, it is important to further investigate the causes of these differences in chicken growth, by assessing the polymorphism of the exon 6 PIT-1 gene which has been recognized as one of the genetic markers for chicken growth.

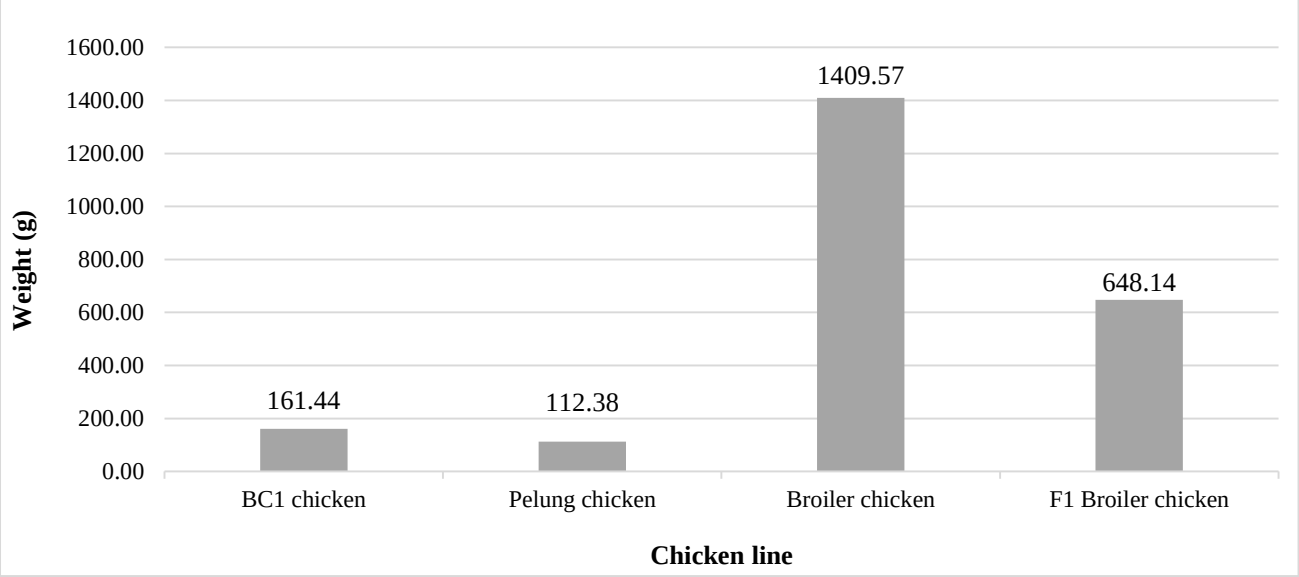

Figure 1. Comparison of mean body weights of BC_1, Pelung, Broiler and F_1 Broiler chickens for seven weeks

Table 1. The differences of chicken weights in BC_1, Pelung, Broiler and F_1 Broiler chickens during seven weeks

Chicken line	Age (week)						
	1	2	3	4	5	6	7
Hybrid BC_1	48.67± 6.18^b	65.33± 9.64^b	95.17± 24.37^b	122± 4306^b	156.4± 43.79^b	230.92± 45.49^b	419.08± 100.6^b
Broiler	194.0± 0.00^d	461.00±0.00^d	842.00±0.00^d	1309±0.00^d	1817± 0.00^d	2347± 0.00^d	2897± 0.00^d
Pelung	32.33±2.52^a	44.33±11.93^a	57.33±17.21^a	84.00±27.22^a	124.3±30.10^a	185.67±42.19^a	258.67±54.09^a
F_1 broiler	94.30±0.00^c	230.00±0.00^c	387.00±0.00^c	583.70±0.00^c	833± 0.00^c	1100.3±0.00^c	1308.70±0.00^c

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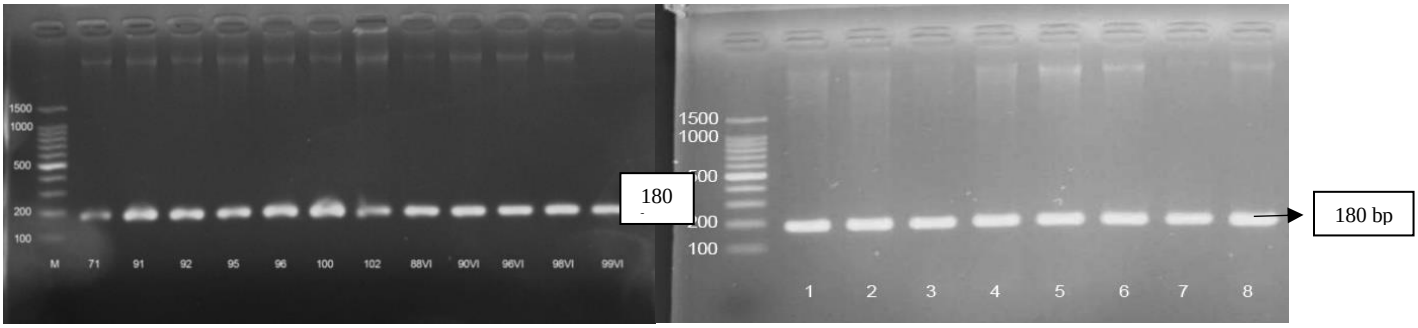
Pituitary positive transcription factor-1 gene polymorphism

The molecular selection was performed as a way to improve genetic quality. This study was aimed to detect the presence of PIT-1 gene polymorphism on the weight growth of hybrid chickens. The Pituitary Positive Transcription Factor-1 (PIT-1, POU1F1, or GHF1) gene in chickens was located on chromosome 1 with a length of 14 kb as a genetic marker that had been used to aid in the early selections based on the relationship between markers and the expected quantitative traits (Yamada et al., 1993). The PIT-1 gene was one of the genes that were closely related to the growth and productivity of chickens because the PIT-1 gene controlled the expressions of the coding genes for growth hormone and prolactin hormone (Miyai et al., 2005). Therefore, it could be express that the PIT-1 gene was a gene candidate that had the prospect of being used as a genetic marker in the local chicken selection program. Electrophoresis was carried out to determine the results of DNA fragments amplification by PCR. The results of electrophoresis can be seen in figure 2 and figure 3.

Based on exon 6 PIT-1 gene visualization, the 12 BC_1 samples had a nucleotide length of 180 bp (Figure 2a). In the PCR result of the exon 6 PIT-1 gene (Figure 2b), the samples 1-4 are broiler chickens that have nucleotide length 180 bp and the samples 5-8 are Pelung chickens also have nucleotide length 180 bp. DNA amplification results showed good fragments that were shown by the appearance of thick and clear DNA bands, then from the amplification results by PCR, a sequencing process was carried out to determine the nucleotide sequences of the genes. The alignment of the exon 6 PIT-1 gene is shown in table 2.



(a) (b)

Figure 2. The results of exon 6 Pituitary Positive Transcription Factor-1 gene (180 bp) amplification by PCR (a) hybrid BC_1 chickens (b) 1, 2, 3, 4: Broiler chickens; 5, 6, 7,8: Pelung chickens

Table 2. The single nucleotide polymorphism of exon 6 pituitary positive transcription factor-1 gene

Sample No.	PIT-1 gene polymorphism		Haplotype	Chicken weight on day 49 (g)
	Exon 6			
	Substitution	Substitution		
AJ236855	A	T	Reference	-
Hybrid BC_1 1	A	T	Reference	680.00
Hybrid BC_1 2	A	T	Reference	490.00
Hybrid BC_1 3	A	T	Reference	493.00
Hybrid BC_1 4	A	T	Reference	471.00
Hybrid BC_1 5	A	T	Reference	369.00
Hybrid BC_1 6	A	T	Reference	414.00
Hybrid BC_1 7	A	T	Reference	352.00
Hybrid BC_1 8	A	T	Reference	348.00
Hybrid BC_1 9	A	T	Reference	368.00
Hybrid $BC_1$10	A	T	Reference	338.00
Hybrid BC_1 11	A	T	Reference	349.00
Hybrid BC_1 12	A	T	Reference	357.00
Broiler 1	G	A	1	-
Broiler 2	G	A	1	-
Broiler 3	G	A	1	-
Broiler 4	G	A	1	-
Pelung 1	G	A	1	321.00
Pelung 2	G	A	1	224.00
Pelung 3	G	A	1	231.00

Notes: *A: Adenine; G: Guanine; T: Thymine

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Based on table 2, the exon 6 PIT-1 gene in BC_1 hybrid chickens has the same nucleotide structure as the reference (AJ236855), so that these nucleotide sequences do not make new haplotypes. But Pelung chicken and broiler chicken have 2 SNP located in the coding region. The SNPs consist of 2 substitution points including Adenine to Guanine and Thymine to Adenine. Pelung chicken and broiler chicken form the same nucleotide sequence so that from the 2 SNP it will form 1 same haplotype. Table 3 is the results of the Pearson correlation test used to determine the correlation between the weight of chickens and the points of polymorphism. The results of the study showed that the two points of polymorphism were A928G substitution and T929 substitution. The substitution A928G consisted of GG genotype (mutant phenotype) in Pelung chicken with a 49th day and average weight of 258.7 g, and the AA genotype (wild type phenotype) in BC_1 hybrid chicken with weight average 419.08 g. For the second polymorphism point, T929A consisted of genotype TT (mutant phenotype) in Pelung chicken with an average weight of 49 days to 258.67 g and AA genotype (wild type phenotype) in hybrid chicken BC_1 has weight chicken average 419.08 g. Genotype frequency at A928G substitution point and T929A substitution point have the same value which is 0.5. The correlation coefficient at both points was –0.588. The A928G substitution point and T929A substitution point had a significance value smaller than 0.05, which was 0.021. Based on table 3 it can be described the mutant phenotype at both points affecting the decrease in chicken weight. So that it can be concluded that the relationship between the point of polymorphism and chicken weight was a significantly negative medium correlation. Whereas in a previous study conducted by Jiang et al. (2004) that on MR5 or exon 6 PIT-1 gene there were SNP associated significantly with the phenotypic characters of chickens' growth. A deletion occurred in C nucleotide which caused a change in the amino acid arrangement after the point of mutation, which was caused by a frameshift mutation. As a result of the frameshift mutation, the protein structure changed which was resulted in an error of protein function, or a decrease in protein formation.

Table 3. The correlation test results of Pituitary Positive Transcription Factor-1 gene polymorphism on the chicken mean weight on the 49th day

Polymorphism	**A928G Substitution**		**T929A Substitution**	
Genotype	GG (mutant)	AA (wild type)	AA (mutant)	TT (wild type)
Genotype frequency	0.5	0.5	0.5	0.5
Mean chicken weight on day 49 (g)	258.67	419.08	258.67	419.08
Correlation coefficient (r)	-0.588		-0.588	
Significant level	0.021 (P < 0.05)		0.021 (P < 0.05)	
Conclusion	Significant with moderate negative correlation		Significant with moderate negative correlation	

CONCLUSION

The conclusion showed that first backcross hybrid chickens resulting from a cross between female F_1 broiler chicken and male Pelung chicken body weight was lower with the average weight at the 7th week was 419,08 g compared to F_1 broiler chickens, but higher than the Pelung chickens. There was not exon 6 Pituitary Positive Transcription Factor-1 gene polymorphism found in first backcross hybrid chickens resulting from a cross between female F_1 broiler chickens and male Pelung chickens.

DECLARATIONS

Author`s contributions

D. Retnosari designed the plan of study, collected data and samples, contributed to analyses, and wrote the manuscript. R.Kilatsih and I.S. Maulidi checked the final form of the manuscript. Trijoko revised the research article and facilitating the experimental work. B.S. Daryono helped in designing the plan of study, facilitating the experimental work, providing the experimental tools, revising the research article.

Acknowledgments

This research was funded by the Ministry of the Higher Education Republic of Indonesia (Kemenristekdikti) through Applied Research (Penelitian Terapan) Funding scheme PT 2020: No. 1997/UN1/DITLIT/DIT-LIT/PT/2020. Authors also would like to express the gratitude to Gama Ayam Research Team, Faculty of Biology Universitas Gadjah Mada, and Pusat Inovasi Agroteknologi (PIAT) UGM for their kind assistance during this research work.

Competing interests

The authors have not declared any conflict of interest.

To cite this paper: Retnosari D, Kilatsih R, Maulidi IS, Trijoko and Daryono BS (2021). Polymorphism Association of *Pituitary Positive Transcription Factor-1* Gene with Body Weight Traits in BC_1 Hybrid Chicken (*Gallus gallus gallus* Linnaeus, 1758) from Cross Breeding between Female F_1 Broiler and Male Pelung. *World Vet. J.*, 10 (4): 525-530. DOI: https://dx.doi.org/10.54203/scil.2020.wvj63

REFERENCES

Arnedo M, Taffé P, Sahli R, Furrer H, Hirschel B, Elzi L, Weber R, Vernazza P, Bernasconi E Darioli R et al. (2007). Contribution of 20 single nucleotide polymorphisms of 13 genes to dyslipidemia associated with antiretroviral therapy. Pharmacogenet Genom,17:755-764. DOI: https://www.doi.org/10.1097/FPC.0b013e32814db8b7.

Butler JM (2009). DNA extraction from forensic samples using Chelex. Cold Spring Harbor Protocols, 4: 6. DOI: https://www.doi.org/10.1101/pdb.prot5229.

Cheng HW (2010). Breeding of tomorrow's chickens to improve wellbeing. Poultry Science, 89: 805-813. DOI: https://www.doi.org/10.3382/ps.2009-00361.

Daryono BS, Roosdianto I and Saragih HTS (2010). Phenotypical characters in hybrids chicken of crossbreeds between pelung and cemani. Jurnal Veteriner, 11 (4): 257-263. Available at: https://ojs.unud.ac.id/index.php/jvet/article/view/3460/2494.

Hidayat C and Asmarasari SA (2015). Native chicken production in Indonesia: a review. Indonesian Journal of Animal Science, 17 (1): 1-11. DOI: https://www.doi.org/10.25077/jpi.17.1.1-11.2015.

Jiang R, Li J, Qu L, Li H and Yang N (2004). A new single nucleotide polymorphism in the chicken pituitary-specific transcription factor (POU1F1) gene associated with growth rate. Animal Genetics, 35: 344-346. DOI: https://www.doi.org/10.1111/j.1365-2052.2004.01164.x.

Miyai S, Yoshimura S, Iwasaki Y, Takekoshi S, Lloyd RV and Osamura R (2005). Induction of GH, PRL, and TSH beta mRNA by transfection of PIT-1 in a human pituitary adenoma-derived cell line. Cell Tissue,322:269-277. DOI: https://www.doi.org/10.1007/s00441-005-0033-z.

Nie Q, Meixia F, Liang X, Min Z, Zhangmin L, Zipin L, Guohuang W, Wensen B, Canjian L, Wei Z et al. (2008). The PIT-1 gene polymorphisms were associated with chicken growth traits. Genetics, 9: 20. DOI: https://www.doi.org/ 10.1186/1471-2156-9-20.

Oktafiantari R (2016). Ghrelin gene polymorphisms encoding growth in chicken [(*Gallus gallus gallus* (Linnaeus, 1758)] second generation of backcross derived crosses between ♀ Pelung with ♂ the first generation of Backcross. Thesis. Faculty of Biology Universitas Gadjah Mada, Indonesia, p. 30.

Roosdianto I (2010). Inheritance of Phenotypic Characters of chickens (F_1) results of crosses of chicken (*Gallus gallus domesticus* Linnaeus, 1758) Pelung with Broiler. Thesis. Faculty of Biology Universitas Gadjah Mada, Indonesia, pp. 24-48.

Sanger F, Nicklen S and Coulson AR (1977). DNA sequencing with chainterminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America, 74 (12): 5463-5467. DOI: https://www.doi.org/10.1073/pnas.74.12.5463.

Sulandari S, Zein MSA, Payanti S, Sartika T, Astuti M, Widyastuti T, Sujana E, Darana S, Setiawan I and Garnida D (2007). Indonesian Local Chicken Biodiversity Resources: Benefits and Potential. Biology Research Center. Bogor, LIPI, Indonesia.

Suryaman (2010). The Comparison of morphometry of native chicken, pelung chicken and first breed chicken (F_1) result from crossbreeding between Pelung and native chickens age 5-12 Weeks. Thesis. Faculty of Animal Science, IPB University, Bogor, Indonesia. Available at: http://repository.ipb.ac.id/handle/123456789/12721.

Van As P, Buys N, Onagbesan OM and Decuypere E (2000). Complementary DNA cloning and ontogenic expression of pituitary-specific transcription factor of chickens (*Gallus domesticus*) from the pituitary gland. General and Comparative Endocrinology, 120: 127–136. DOI: https://www.doi.org/10.1006/gcen.2000.7529.

Yamada S, Hata J and Yamashita S (1993). Molecular cloning of fish Pit-1 cDNA and its functional binding to promoter of gene expressed in the pituitary. Journal of Biological Chemistry, 268(32): 24361-24366. Available at: https://www.jbc.org/content/268/32/24361.long.

Zein MSA and Sulandari S (2009). Pedigree investigation of Indonesian chicken by sequences hypervariable-1 d-loop mitochondrial dna. Jurnal Veteriner, 10(1): 41-49. Available at: http://ojs.unud.ac.id/index.php/jvet/article/view/3345.

To cite this paper: Retnosari D, Kilatsih R, Maulidi IS, Trijoko and Daryono BS (2021). Polymorphism Association of *Pituitary Positive Transcription Factor-1* Gene with Body Weight Traits in BC_1 Hybrid Chicken (*Gallus gallus gallus* Linnaeus, 1758) from Cross Breeding between Female F_1 Broiler and Male Pelung. *World Vet. J.*, 10 (4): 525-530. DOI: https://dx.doi.org/10.54203/scil.2020.wvj63

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 531-539, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj64

Seroprevalence and Associated Risk Factors of Brucellosis in Livestock and Residents of New Valley Governorate, Egypt

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ORIGINAL ARTICLE
pii: S232245682000064-10
Received: 08 Oct 2020
Accepted: 22 Nov 2020

ABSTRACT

Brucellosis is a worldwide zoonotic disease which is now considered endemic in most parts of Egypt. A cross-sectional study was carried out from December 2018 to February 2020 to investigate the seroprevalence of brucellosis in humans and livestock residing in two regions located in New Valley Governorate, Egypt. A total of 1254 animals (673 cattle, 348 sheep, and 233 goats) and 523 human serum samples were examined for brucellosis using Rose Bengal test (RBT) and then randomly selected sera (15 from cattle, 7 from sheep, 3 from goats, and 45 from humans) were further analyzed by complement fixation test, enzyme-linked immunosorbent assay to compare and detect the sensitivity and specificity of RBT. The prevalence of brucellosis was 0% in cattle, sheep, and goats while it was 23.9% in humans using RBT. Concerning humans, there was a higher percentage of infection in EL Kharga (33.6%). The prevalence of this infection was also at a higher level among individuals aged above 40 years (28.57%). Furthermore, men (26.11%) were more inclined to be inflicted, compared to women (22.5%) with no significant difference. Considering the human occupation, abattoir workers were the most predominant group of people at risk (33.3%), followed by farmers (31.25%) and animal keepers (20.6%) while the lowest prevalence was demonstrated in the housewives where the prevalence was 18.8 %. As a result, risk factors of the age range, locality, time of infection, contact with animals, and occupational groups could significantly affect the prevalence of human brucellosis in the New Valley Governorate. In conclusion, brucellosis is an alarming problem among residents of the New Valley Governorate. Thus, reducing the prevalence in humans and animals in the region of study may include restriction of the marketing the raw milk and enhancing public health awareness.

Keywords: Brucellosis, Cattle, Complement fixation test, ELISA, Human, Rose Bengal test, Sheep and goats.

INTRODUCTION

Brucellosis is one of the most common worldwide zoonotic diseases, which requires major economic considerations. The reason is that it can intervene in the normal daily activities of the inflicted infected patients leading to a serious impact on public health. It can also have a detrimental effect on animal production by decreasing the reproductive efficiency, milk yield, as well as the increase of abortion (Corbel, 2006). The etiological agents causing brucellosis belong to the genus *Brucella*, and the classical zoonotic type is *Br. abortus*, *Br. melitensis*, *Br. suis*, and *Br. canis* (Pappas and Memish, 2007; Godfroid, 2017).

Humans can be infected with brucellosis by contacting animals (i.e., secretion, carcasses, or ingestion) or by consuming their products, mainly unpasteurized dairy products (Aparicio, 2013). Human symptoms mainly include undulant fever, malaise, insomnia, arthralgia, sweating, fatigue, weight loss, headache, and joint pain also, some cases may have neurological complications, endocarditis and testicular or bone abscess formation (Acha and Szyfres, 2003; Corbel, 2006). Human brucellosis proved to be a serious occupational health hazard to livestock handlers particularly abattoir workers, butchers, and veterinarians in Egypt (Zakaria et al., 2018)

Brucellosis was first reported in Egypt in 1939, but now it is endemic (Refai, 2002; Eltholth et al., 2015). In 2007, the prevalence rates of brucellosis in livestock were significantly higher in Beni Suef than other regions of Egypt (Samaha, 2008). The disease has been predominantly detected in ruminants with varied prevalence and some regions of Egypt, such as New Valley province, reported no inflicted case of Brucellosis (Wareth et al., 2014; Eltholth et al., 2017).

Isolation of *Brucella* is considered as a gold standard and the most reliable method of diagnosis; however, it is difficult and time-consuming to perform with a great risk of infection for laboratory workers, which necessitates specific biosafety measures (Mathew et al., 2015). In this regard, a variety of serological tests, such as Rose Bengal Test (RBT), Complement Fixation Test (CFT), Enzyme-Linked Immunosorbent Assay (ELISA), tube agglutination test, and buffered acidified plate antigen test can be used for the recognition of *Brucella* specific antibodies (Fatima et al., 2016). The RBT

To cite this paper: Diab MS, Zidan ShAA, Hassan NAA, Elaadli H and Bayoumi AM (2020). Seroprevalence and Associated Risk Factors of Brucellosis in Livestock and Residents of New Valley Governorate, Egypt. *World Vet. J.*, 10 (4): 531-539. DOI: https://dx.doi.org/10.54203/scil.2020.wv64

is simple, good, rapid, and easy to perform and can be used as a herd screening test at remote places (Teng et al., 2017; Diab et al., 2018). Moreover, CFT and ELISA can be utilized as a confirmatory test for *Brucella* diagnosis (Ashraf et al., 2014). The combination of RBT and CFT can be suggested as the best method for the diagnosis of brucellosis (Chisi et al., 2017).

The current study aimed to provide the first report of the prevalence rate of brucellosis in cattle, sheep, goats, and humans residing in New Valley Governorate, Egypt, using RBT confirmed by CFT and ELISA**.**

MATERIALS AND METHODS

Study area and period

The study was carried out in New Valley Governorate from December 2018 to February 2020. The study population consisted of cattle, sheep, goats, and humans from two regions located in the New Valley Governorate to study the seroprevalence of brucellosis.

Samples

Animal samples

A total of 1254 serum samples of farm animals (i.e., 673 cattle, 348 sheep, and 233 goats) were collected from December 2018 to December 2019, and the full history of each animal, including sex, age, season, and locality, was recorded.

Human samples

A total of 523 human serum samples were collected from patients (males or females) with the age range of 10-70 years from New Valley Fever hospital and various clinic laboratories in New Valley Governorate from December 2018 to February 2020. Demographic information of participants (i.e., gender, age, locality, and time of infliction) was also documented in the current study.

Sample collection and processing

The samples in the present study included 5-7 ml of blood from the jugular vein of the investigated animals and the cephalic vein of human cases using sterile disposable syringes. Immediately after collection of the blood sample in a sterile glass tube, the blood was left to stand still for about 30 minutes and then centrifuged at 3000 rpm for 10 minutes to obtain non-haemolyzed clear serum using sterile Pasteur pipettes followed by keeping the samples in Eppendorf tubes and labeling the tubes. The separated serum was stored in a labeled tube at -20 °C until serological examination. The number of serum samples examined from cattle, sheep, goats, and humans in two areas of New Valley Governorate are shown in table 1.

Table 1. Number of serum samples examined from cattle, sheep, goats, and humans in two areas of New Valley Governorate, Egypt

Samples	El Kharga	EL Dakhla	Total
Cattle	484	189	673
Sheep	252	96	348
Goat	186	47	233
Total	922	332	1254
Human	327	196	523

Serological test

Rose Bengal Test

All tested serum samples (i.e., 673 cattle, 348 sheep, 233 goats, and 523 humans) were examined using antigen stained with Rose Bengal and buffered to a low pH (3.65 + 0.05), the reagent was obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt. The test was performed simply by adding 25 µl of both tested serum and the reagent were placed next to the plate and then mixed thoroughly using glass rode or toothpick, shaking the plate with an electric rocker for four minutes. Then the degree of agglutination was recorded. The results were considered positive if agglutination was detected and negative if no agglutination was found.

Complement fixation test

Randomly selected samples were retested for anti-*Brucella* antibodies with CFT. Components were obtained from VSVRI, Abbassia, Cairo, Egypt, and the CFT was performed at *Brucella* Unit in Central Laboratory Evaluation for Veterinary Biologics, Abbasia, Cairo, Egypt. The test was performed according to Alton et al. (1988).

Enzyme linked immunosorbant assay

The selected samples were randomly retested for anti-*Brucella* antibodies using ELISA. The cELISA was performed by using the ID Screen® Brucellosis Serum Indirect Multi-species (ID-Vet, France) and the Human *Brucella*

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IgM ELISA Test Kit (Diagnostic Automation /Cortez Diagnostics, USA.) for animal and human sera. The results were interpreted according to the instructions of the manufactures.

Statistical analysis

Data analysis was run using the Chi-square test. The P-value is the probability of the event occurring by chance if the null hypothesis is true. P-value less than 0.05 was considered statistically significant.

Sensitivity = True Positives (TP) / [True Positive (TP) + False Negative (FN)] ×100.

Specificity = True Negatives (TN) / [True Negative (TN) + False Positives (FP)] ×100.

Ethical approval

All procedures in the current study, including human and animal sera collection, were in accordance with the Egyptian ethical standards of the national research committee. All human subjects gave their consent for the collection of the serum samples, with the agreement that any identifying details of the individuals should not be published.

RESULTS

The obtained results of RBT revealed no seroprevalence of brucellosis in cattle, sheep, and goats (Table 2). However, the seroprevalence of brucellosis by CFT and ELISA was reported 20%, and 0% in cattle, respectively. No seroprevalence of brucellosis was observed in sheep and goats using both CFT and ELISA (Table 3).

As can be seen in table 4, the analysis of seroprevalence of brucellosis in humans using RBT was 23.9%, while this rate was reported as 28.9% utilizing CFT and 31.1% employing ELISA (Table 5). As observed in table 6, the investigation of risk factors of age indicated that there was a significant relationship between human age and infection with *Brucella* ($p < 0.05$). Accordingly, the high percentage of infection was found in the age group > 40 years (28.57%) followed by the age group <40 years (i.e., 17.20 %). Regarding locality, there was a highly significant relationship between locality and infection with brucellosis in humans ($p < 0.05$), the highest infection was recorded in EL Kharga (33.6%) and the lowest was for EL Dakhla (7.7%). Although gender showed no significant relationship with brucellosis in humans, the occurrence of brucellosis was higher in males (26.11%) than females (22.5%). The finding addressing the time of infliction demonstrated that the highest percentage of infection was for days with hot weather (33.63%).

Concerning occupational factors, there was a significant relationship between contact with animals and infection**,** seroprevalence of brucellosis was higher in individuals at close contact with animals (28.4%) than those non-contacts (18.8%). Although there was no significance between the consumption of raw dairy products and infection**,** the raw milk consumers group (25.5%) showed a higher prevalence of infection, compared to non-milk consumer groups (22.4%). The highest seroprevalence was recorded in the abattoir workers (33.3%) followed by farmers (31.25%), and then Animal Keepers (20.6%), while the lowest prevalence was demonstrated in the housewives where the prevalence was 18.8 %. As tabulated in table 7, the sensitivity of RBT and ELISA concerning results of CFT in cattle, sheep, and goats was reported 0%, while the specificity of both tests was 100%. The sensitivity and specificity rates of RBT were respectively 76.92% and 100% for human participants, and the sensitivity and specificity rates of ELISA were estimated at 100% and 96.88%, respectively.

Table 2. Seroprevalence of brucellosis in animals living in New Valley Governorate, Egypt from December 2018 to December 2019 using RBT.

RBT / Farm animals	No. of examined samples	No. of Positive samples	Percentage
Cattle	673	0.0	0.0
Sheep	348	0.0	0.0
Goat	233	0.0	0.0
Total	1254	0.0	0.0

Table 3. Seroprevalence of brucellosis in animals living in New Valley Governorate from December 2018 to December 2019 using CFT and ELISA**.**

Farm animals	No. of examined samples	CFT		ELISA	
		+ve	%	+ve	%
Cattle	15	3	20	0.0	0.0
Sheep	7	0.0	0.0	0.0	0.0
Goat	3	0.0	0.0	0.0	0.0
Total	25	3	12	0.0	0.0

CFT: Complement Fixation Test; ELISA: Enzyme-Linked Immunosorbent Assay

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Table 4. Seroprevalence of brucellosis in humans residing in New Valley Governorate, Egypt from December 2018 to February 2020 using RBT.

RBT	Total (number)	+Ve (number)	Percentage
Humans	523	125	23.9%

RBT: Rose Bengal test

Table 5. Seroprevalence of brucellosis by in humans residing in New Valley Governorate, Egypt from December 2018 to February 2020 using CFT and ELISA.

	No. of examined samples	CFT		ELISA	
		+ve (Number)	Percentage	+ve (number)	Percentage
Human	45	13	28.9	14	31.1

CFT: Complement Fixation Test; ELISA: Enzyme-Linked Immunosorbent Assay

Table 6. Prevalence of brucellosis using RBT in humans regarding different risk factors in New Valley Governorate, Egypt

Species		Human (523)			Chi-square	P-value
Risk factors		Total no.	Positive no.	Percentage		
Age	<40 Y	308	88	28.57	8.987* significant at p < 0.05	0.002719
	>40 Y	215	37	17.20		
Locality	El Kharga	327	110	33.6	45.498* significant at p < 0.05	0.00001
	El Dakhla	196	15	7.7		
Gender	Male	203	53	26.11	0.8892 * Non-significant at p > 0.05	0.3457
	Female	320	72	22.5		
Weather	Hot weather	327	109	33.63	42.6856 * significant at p < 0.05	0.00001
	Cold weather	196	16	8.16		
Contact with animals	Yes	278	79	28.4	6.6563* significant at p < 0.05	0.009
	No	245	46	18.8		
consumption of Raw dairy products	Yes	255	65	25.5	0.6914 NS Non-significant at P >0.05	0.4
	No	268	60	22.4		
Occupation	Farmers	160	50	31.25	9.8809* significant at p < 0.05	0.019
	Abattoir worker	30	10	33.3		
	Animal Keeper	141	29	20.6		
	Housewives	192	36	18.8		

Table 7. Comparison of the results of RBT and ELISA for the diagnosis of brucellosis in the investigated cattle, sheep, goats, and humans as well as detection of sensitivity and specificity of both tests.

Item	RBT		ELISA	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Cattle	0.0	100	0.0	100
Sheep	0.0	100	0.0	100
Goat	0.0	100	0.0	100
Human	76.92	100	100	96.88

RBT: Rose Bengal test, ELISA: Enzyme-Linked Immunosorbent Assay

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DISCUSSION

Brucellosis is one of the most common worldwide zoonotic diseases, which requires major economic considerations, especially in developing countries, including Egypt (Afifi et al., 2005). The diagnosis is mainly based on the serological tests since it is fast, easy to use, and available, compared to other culture techniques, that are not available in laboratories of endemic countries (Young et al., 2005). The combination of serological testing should be adopted to reduce the false-negative number which contributes to the persistence of the herd problem and also to reduce the false positive number to avoid over condemnation by testing and slaughtering policy (Salem et al., 2016). Therefore, in the present study, RBT was used for the determination of brucellosis in livestock (i.e., cattle, sheep, and goats) as well as humans in the southwestern region of Egypt. As indicated in table 2, the overall prevalence rate of brucellosis in cattle, sheep, and goats was 0% using RBT.

Regarding cattle, the obtained results of the current study were in line with those obtained by Cadmus et al. (2006) and Nagi (2003) who respectively reported the seroprevalence rates of 0% and 0.70% among cattle. In contrast, (Musallam et al., 2015) and (Anka et al., 2013) reported higher rates of seroprevalence for cattle (i.e., 18.1% and 21.8%, respectively). Considering sheep, the findings of the current study yield support the results of a study conducted by Samaha (2008) who found that seroprevalence among sheep was 0.00%. In the same vein, Bekele et al. (2011) and Ebid and Salib (2020) respectively reported similar estimates of 1.2% and 0.48%, which were lower than the estimated rates of 20% and 15% respectively reported in the studies conducted by Abdel-Razik et al. (2007) and Hegazy et al. (2009). Similar to the obtained results of the current study concerning goats, (Samaha, 2008) estimated seroprevalence rates as 0.00%, which was almost near to the reported rates of 1.3% and 1.9% mentioned by Tekleye et al. (1989) and Megersa et al. (2011), respectively. However, the calculated percentages were lower than the ones recorded by Ahmed et al. (2010), Kaoud et al. (2010), Montiel et al. (2013), and Musallam et al. (2015) in the related studies (i.e., 31 %, 18.88 %, 34.3%, 38%, respectively). Accordingly, none of the farm animals were infected with brucellosis in the New Valley Governorate, which can be due to very strict measures on animal importation from outside the governorate. Natural and geographical features of the governorate are not considered an appropriate environment for *Brucella* owing to low humidity, very high temperature, and rare showers. Furthermore, most animal keepers in the country avoid locating different types of animals together in the same place.

The RBT is an ideal screening test for human brucellosis since it is a simple, rapid, and highly sensitive test for individual diagnoses (Teng et al., 2017). In the present study, the overall prevalence of human brucellosis by RBT was 23.9% (Table 4), which was similar to the obtained results of studies conducted by Yohannes et al. (2012), El-Diasty et al. (2016), and Diab et al. (2018), in which they estimated the rates as 26.6%, 21%, and 24.3%, respectively. These estimates were higher than those (i.e., 1.25%, 13.1%, 5.6%, 9.44%, 6.3%) recorded by Elmonir et al. (2016), Salem et al. (2016), Awah-Ndukum et al. (2018), Abdelbaset et al. (2018) and Ramadan et al. (2019)**,** respectively. On contrary, the reported ratios were lower than those calculated in studies conducted by Hussien et al. (2007) and Hassanain and Ahmed, (2012), which were 32.3% and 83.3%, respectively. This dissimilarity in the prevalence of human brucellosis in the current work and others may be due to different geographic locations, age range and gender distribution, variation in occupational contact, and the type of implemented tests (Alton et al., 1988). The presented data in table 6 and Figure 1 indicated that the risk factor of age can lead to significant differences. The higher percentage of infection was observed among individuals aged above 40 (28.57%). This finding was also supported by several studies, including those performed by Abdelbaset et al. (2018), Tumwine et al. (2015), and Saddique et al. (2019). In contrast, some other researchers (Nagati and Hassan, 2016; Salem et al., 2016;Tsegay et al., 2017; Saraya, 2017) reported that the highest infection rate of *brucellosis* was observed among individuals with the age range of 20-44 years, compared to the younger or older ones.

With regard to the prevalence of human brucellosis and locality, it was found that there was a significant association between the seroprevalence of brucellosis among humans and locality in New Valley Governorate (table 6 and figure 2). This finding was in line with the obtained results of a study by Nossair and Haggag, (2016), where there was a significant association between the seroprevalence of brucellosis among humans and locality ($p < 0.0001$). Addressing the effect of gender on human brucellosis prevalence, it was observed that the seroprevalence of brucellosis was higher in males (26.11%) than females (22.5%) although gender had no significant effect on the prevalence of brucellosis (table 6 and figure 3). Similarly, (El Mabrouk, 2013) found a non-significant association between the prevalence of brucellosis and gender. Analysis of the effect of weather on brucellosis showed that weather conditions had a significant effect on brucellosis with the highest infection rate during related to the seasons with hot weather (table 6 and figure 4) which was confirmed by Lolika et al. (2017) and Ayoub et al. (2019). However, (Diab et al., 2018) noticed that the highest infection rate occurred during the winter season (43.1%)**.** As can be seen in figure 5, contact with animals have a significant effect on brucellosis with the highest infection rate in individuals at close contact with animals (28.4%) than those with no contact with animals (18.8%) supported by Diab et al. (2018). Figure 6 illustrated that the seroprevalence of brucellosis was higher in the raw milk consumers group (25.5%) than non-milk consumers groups

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(22.4%) with a non-significant effect on dairy products, which was also confirmed by George et al. (2014). According to figure 7, the highest seroprevalence was recorded in the abattoir workers followed by farmers, animal keepers, and housewives indicating a significant association between different occupations and the prevalence of human brucellosis. This result agreed with those obtained by Nossair and Haggag, (2016) and Ramadan et al. (2019), who found that abattoir workers had the highest percentage followed by farmers, householders, and milker's housewives.

The obtained results of table 7 showed that the sensitivity rates of RBT and ELISA in terms of the diagnosis of brucellosis in human were 76.92% and 100 %, respectively, while the specificity of the two tests were 100 % and 96.88%, respectively, as compared with that of the CFT as a gold standard. These results were nearly similar to those reported by Shaaban et al. (2018), who found that the specificity of RBT was 97.77%, and (Rojas and Alonso, 1998), who found that the sensitivity and the specificity rates of 78.1% and 100% for RBT as well as 100% and 100% for ELISA, respectively.

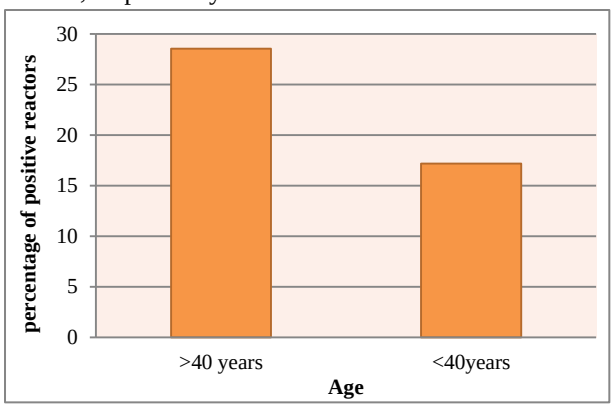

Figure 1. Seroprevalence of brucellosis in human beings using RBT regarding age groups in New Valley Governorate, Egypt

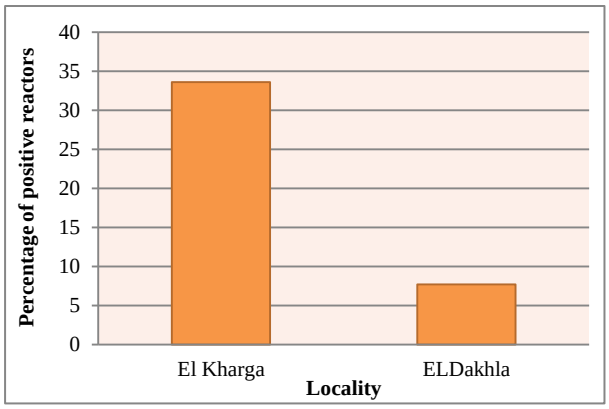

Figure 2. Seroprevalence of brucellosis in human beings using RBT regarding locality in New Valley Governorate, Egypt

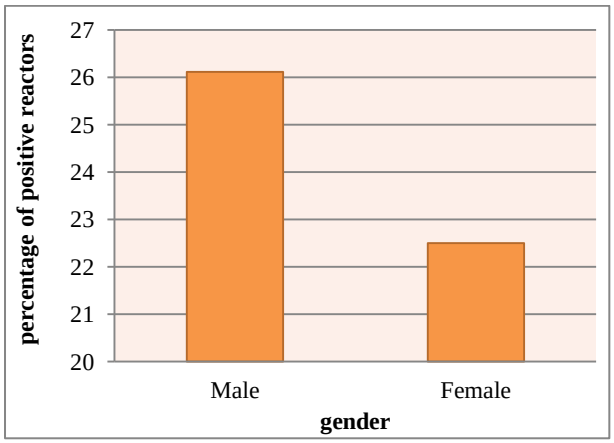

Figure 3. Seroprevalence of brucellosis in human beings using RBT in relation to gender in New Valley Governorate, Egypt

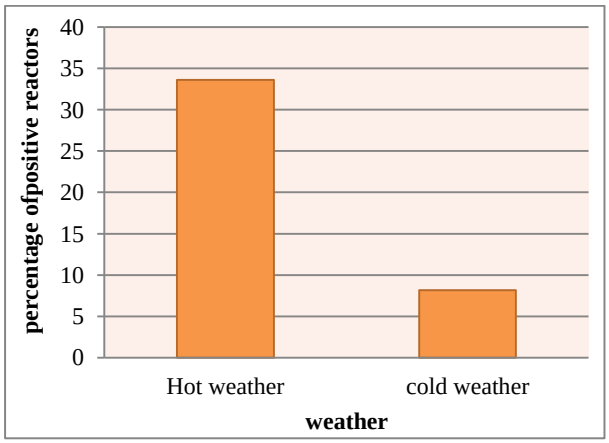

Figure 4. Seroprevalence of brucellosis in human beings using RBT regarding weather in New Valley Governorate, Egypt

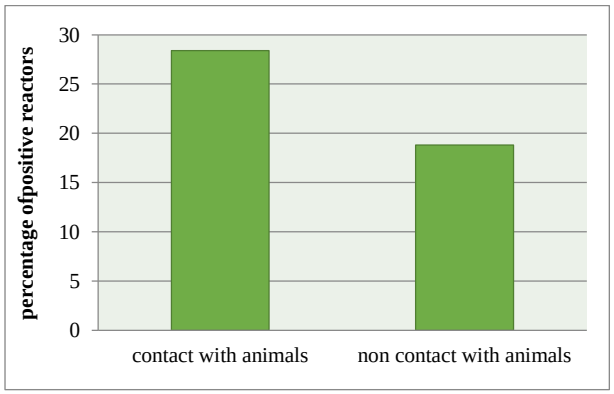

Figure 5. Prevalence of brucellosis in humans regarding contact with different animals in in New Valley Governorate, Egypt

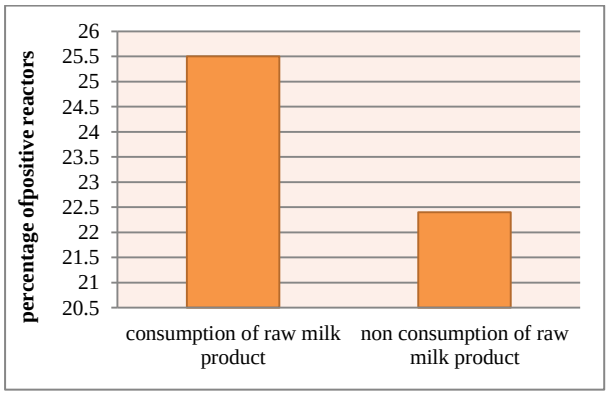

Figure 6. Prevalence of brucellosis in humans regarding consumption of raw milk product in in New Valley Governorate, Egypt

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Figure 7. Prevalence of brucellosis in humans regarding occupation in New Valley Governorate, Egypt

CONCLUSION

According to the setting and the obtained results of the current study (the first report for this area), it can be concluded that there was no reported case of brucellosis among the farm animals of New Valley Governorate. Concerning humans, brucellosis is an alarming problem in New Valley Governorate and threatens the human population through the direct and indirect transmission. There was no relationship between the recorded high prevalence of brucellosis in humans residing in New Valley Governorate and the livestock infection. The highest infection rate was recorded in Abattoir workers and Farmers than in any other occupations. Further investigation is required for other possible sources of infection. A combination of different serological tests is the best method to obtain a well-thought diagnosis of brucellosis.

DECLARATIONS

Acknowledgments

This study was supported by the University of Sadat City and Veterinary Serum and Vaccine Research Institute (VSVRI) in Abbassia, Cairo, Egypt. The authors would also like to thank our colleagues Ghada A. Haddad and Yasser Elnaker.

Competing interests

All authors have no conflict of interest.

Author's contributions

Nermine A. Hassan, Ahmed M Bayoumi, Mohamed S. Diab, and Sherif Abd Allah Zidan conceived and designed the experiments. Nermine A. Hassan, Haitham Eladli and Mohamed S. Diab performed the experiments. Nermine A. Hassan, Haitham Eladli, and Sherif Abd Allah Zidan contributed reagents/ materials/ analysis tools. Nermine A. Hassan and Sherif Abd Allah Zidan wrote the paper

REFERENCES

Abdel-Razik K, Desouky H, and Ahmed W (2007). Investigations on brucellosis in Egyptian baladi does with emphasis on evaluation of diagnostic techniques. Pakistan Journal Biological Sciences, 10: 342-348. DOI: https://doi: 10.3923/pjbs.2007.342.348.

Abdelbaset AE, Abushahba MFN, Hamed MI, and Rawy MS (2018). Serodiagnosis of brucellosis in sheep and humans in Assiut and El-minya governorates, Egypt. International Journal of Veterinary Science and Medicine., 6 (Suppl): S63-S67. DOI: https://doi.org/10.1016/j.ijvsm.2018.01.007.

Acha PN, and Szyfres B (2003). Zoonoses and communicable diseases common to man and animals: Pan American Health Organization, I: 41-51. Available at:https://www.paho.org/hq/dmdocuments/2012/Acha-Zoonoses-Eng.pdf

Afifi S, Earhart K, Azab MA, Youssef FG, El Sakka H, Wasfy M, Mansour H, El Oun S, Rakha M, and Mahoney F (2005). Hospital-based surveillance for acute febrile illness in Egypt: A focus on community-acquired bloodstream infections. The American journal of tropical medicine and hygiene, 73 (2): 392-399. DOI: https://doi.org/10.4269/ajtmh.2005.73.392.

Ahmed M, Elmeshri S, Abuzweda A, Blauo M, Abouzeed Y, Ibrahim A, Salem H, Alzwam F, Abid S, and Elfahem A (2010). Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, december 2006–january 2008. Eurosurveillance, 15 (30): 19625. Available at: https://www.eurosurveillance.org/content/10.2807/ese.15.30.19625-en.

Alton G, Jones L, Angus R, and Verger J (1988). Techniques for the brucellosis laboratory, institut national de la recherche agronomique, Paris. Verger Versailles Cedex: INRA Publications., 192: 195.

Anka MS, Hassan L, Adzhar A, Khairani-Bejo S, Mohamad RB, and Zainal MA (2013). Bovine brucellosis trends in Malaysia between 2000 and 2008. BMC veterinary research, 9 (1): 230. DOI: https://dx.doi.org/10.1186/1746-6148-9-230.

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Aparicio ED (2013). Epidemiology of brucellosis in domestic animals caused by brucella melitensis, brucella suis and brucella abortus. Revue scientifique et technique, 32(1): 53-60. DOI: https://doi.org/10.20506/RST.32.1.2187.

Ashraf A, El Hofy F, Ramadan KM, and Harb FE (2014). Comparative evaluation of standard serological tests for diagnosis of ovine brucellosis. Banha veterinary medical journal, 2: 423-429. Available at: https://www.bvmj.bu.edu.eg/issues/27-2/42.pdf.

Awah-Ndukum J, Mouiche MMM, Kouonmo-Ngnoyum L, Bayang HN, Manchang TK, Poueme RSN, Kouamo J, Ngu-Ngwa V, Assana E, and Feussom KJM (2018). Seroprevalence and risk factors of brucellosis among slaughtered indigenous cattle, abattoir personnel and pregnant women in Ngaoundéré, Cameroon. BMC infectious diseases,18(1): 611. DOI: https://dx.doi.org/10.1186%2Fs12879-018-3522-x.

Ayoub M, Badran A, and Nosair M (2019). Raw milk as a vehicle of brucella infection for human in Behera province. Damanhour Journal of Veterinary Sciences, 1(2): 7-10. Available at: https://djvs.journals.ekb.eg/article_47989_9a2cabeed725c55b104eac9921974406.pdf.

Bekele M, Mohammed H, Tefera M, and Tolosa T (2011). Small ruminant brucellosis and community perception in Jijiga district, Somali regional state, eastern Ethiopia. Tropical animal health and production, 43 (4): 893-898. DOI: https://doi.org/10.1007/s11250-011-9781-9.

Cadmus SIB, Ijagbone IF, Oputa HE, Adesokan HK, and Stack JA (2006). Serological survey of brucellosis in livestock animals and workers in Ibadan, Nigeria. African Journal of Biomedical Research, 9 (3). DOI: https://doi.org/10.4314/ajbr.v9i3.48900.

Chisi SL, Marageni Y, Prebashni Naidoo P, Zulu NG, George W, Akol G, and Van Heerden H (2017). An evaluation of serological tests in the diagnosis of bovine brucellosis in naturally infected cattle in Kwazulu-Natal province in South Africa. Journal of the South African veterinary assoscition, 88: 1381. DOI: https://dx.doi.org/10.4102%2Fjsava.v88i0.1381.

Corbel MJ (2006). Brucellosis in humans and animals: World Health Organization. Available at: https://www.who.int/csr/resources/publications/Brucellosis.pdf

Diab MS, Elnaker YF, Ibrahim NA, Sedeek EK, and Zidan SA (2018). Seroprevalence and associated risk factors of brucellosis in sheep and human in four regions in Matrouh governorate, Egypt. World's Veterinary Journal, 8 (4): 65-72. Available at: https://wvj.science-line.com/vol-8-no-4-dec-2018.html.

Ebid MEM A, and Salib F (2020). Seroprevalence of brucellosis in sheep and goats in the Arabian Gulf region. Veterinary World, 13(8): 1495-1509. DOI: https://www.doi.org/10.14202/vetworld.2020.1495-1509.

El-Diasty MM, Ahmed HA, Sayour AE, El Hofy FI, Tahoun ABMB, and Shafik SM (2016). Seroprevalence of brucella spp. In cattle, molecular characterization in milk, and the analysis of associated risk factors with seroprevalence in humans, Egypt. Vector-Borne and Zoonotic Diseases, 16(12): 758-764. DOI: https://doi.org/10.1089/vbz.2016.1985.

El Mabrouk KS (2013). Serological investigation of some bacterial zoonotic diseases transmitted through ruminants MVSc, Thesis (Zoonoses), Faculty of Veterinary Medicine Alexandria University.

Elmonir W, Hegazy YM, Abdel-Hamid NH, and Elbauomy EM (2016). Brucellosis at the human-animal interface in Kafr-Elsheikh governorate, Egypt. Alexandria Journal for Veterinary Sciences, 50(1): 1-7. DOI: https://doi.org/10.5455/ajvs.229337.

Eltholth MM, Abd El- Wahab EW, Hegazy YM and El-Tras WF (2015). Assessing impacts and costs of brucellosis control programme in an endemic area of the Nile Delta, Egypt. World's Veterinary Journal, 5(4):74-81 Available at: http://wvj.scienceline.com/attachments/article/32/World's%20Vet.%20J.%205(4)%2074-81,%20December%2025,%202015.pdf.

Eltholth MM, Hegazy YM, El-Tras WF, Bruce M, and Rushton J (2017). Temporal analysis and costs of ruminant brucellosis control programme in Egypt between 1999 and 2011. Transboundary and emerging diseases, 64 (4): 1191-1199. DOI: https://doi.org/10.1111/tbed.12491.

Fatima S, Khan I, Nasir A, Younus M, Saqib M, Melzer F, Neubauer H and El-Adawy H (2016). Serological, molecular detection and potential risk factors associated with camel brucellosis in Pakistan. Tropical animal health and production,48(8):1711-1718. DOI: https://doi.org/10.1007/s11250-016-1148-9.

George N, Edward S, John O, Patrice G, Eric E, and Alban B (2014). Brucella sero-prevalence and modifiable risk factors among predisposed cattle keepers and consumers of un-pasteurized milk in Mbarara and Kampala districts, Uganda. African health sciences, 14(4):790-796. DOI: https://doi.org/10.4314/ahs.v14i4.3.

Godfroid J (2017). Brucellosis in livestock and wildlife: Zoonotic diseases without pandemic potential in need of innovative one health approaches. Archives of Public Health, 75(1): 34. DOI: https://doi.org/10.1186/s13690-017-0207-7.

Hassanain N, and Ahmed W (2012). Sero-prevalence of brucellosis in Egypt with emphasis on potential risk factors. World Journal of Medical Sciences,7(2):81-86. Available at: https://www.researchgate.net/publication/287567526.

Hegazy YM, Ridler AL, and Guitian FJ (2009). Assessment and simulation of the implementation of brucellosis control programme in an endemic area of the Middle east. Epidemiology and Infection, 137 (10): 1436-1448. DOI: https://doi.org/10.1017/S0950268809002301.

Hussien A, Oraby N, Ismail A, Elias A, and Abdel-Kader H (2007). The use of Elisa for diagnosis and epidemiology of brucella infection in humans in Assiut governorate. Veterinary Medical Journal-Giza, 55: 867-877. Available at: https://vlibrary.emro.who.int/imemr/the-use-of-elisa-for-diagnosis-and-epidemiology.

Kaoud H, Zaki M, El-Dahshan A, and Nasr A (2010). Epidemiology of brucellosis among farm animals. Nature and Science, 8(5):190-197. Available at: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.465.3723&rep=rep1&type=pdf.

Lolika PO, Mushayabasa S, Bhunu CP, Modnak C, and Wang J (2017). Modeling and analyzing the effects of seasonality on brucellosis infection. Chaos, Solitons and Fractals, 104: 338-349. DOI: https://doi.org/10.1016/j.chaos.2017.08.027.

Mathew C, Stokstad M, Johansen TB, Klevar S, Mdegela RH, Mwamengele G, Michel P, Escobar L, Fretin D, and Godfroid J (2015). First isolation, identification, phenotypic and genotypic characterization of brucella abortus biovar 3 from dairy cattle in Tanzania. BMC veterinary research, 11 (1): 156. DOI: https://doi.org/10.1186/s12917-015-0476-8.

Megersa B, Biffa D, Niguse F, Rufael T, Asmare K, and Skjerve E (2011). Cattle brucellosis in traditional livestock husbandry practice in southern and eastern Ethiopia, and its zoonotic implication. Acta Veterinaria Scandinavica, 53 (1): 24. Available at: https://www.ncbi.nlm.nih.gov/pubmed/21473760.

Montiel DO, Frankena K, Udo H, Baer NMK, and Van der Zijpp A (2013). Prevalence and risk factors for brucellosis in goats in areas of Mexico with and without brucellosis control campaign. Tropical animal health and production, 45 (6): 1383-1389. https://doi.org/10.1007/s11250-013-0375-6.

Musallam I, Abo-Shehada M, Omar M, and Guitian J (2015). Cross-sectional study of brucellosis in Jordan: Prevalence, risk factors and spatial distribution in small ruminants and cattle. Preventive veterinary medicine, 118 (4): 387-396. DOI: https://doi.org/10.1016/j.prevetmed.2014.12.020.

Nagati S and Hassan SK (2016). Diagnosis of brucella infection in sheep and goat and evaluation of the associated practices in animal contacts. American Journal of Infectious Diseases and Microbiology, 4(5): 95-101. DOI: https://doi.org/10.12691/ajidm-4-5-1.

Nagi SDG (2003). Brucellosis in Yemen and Sudan seroprevalence, causative agent and evaluation of different methods of diagnosis Ph. D. thesis, University of Khartoum, Sudan.

To cite this paper: Diab MS, Zidan ShAA, Hassan NAA, Elaadli H and Bayoumi AM (2020). Seroprevalence and Associated Risk Factors of Brucellosis in Livestock and Residents of New Valley Governorate, Egypt. *World Vet. J.*, 10 (4): 531-539. DOI: https://dx.doi.org/10.54203/scil.2020.wv64

Nossair MA, and Haggag YN (2016). Using competitive Elisa for accurate diagnosis of brucellosis in humans in Alexandria province with emphasis on the disease epidemiology. Alexandria Journal for Veterinary Sciences, 51 (2): 296-302. DOI: https://doi.org/10.5455/ajvs.239565.

Pappas G, and Memish Z (2007). Brucellosis in the Middle east: A persistent medical, socioeconomic and political issue. Journal of Chemotherapy,19(3):243-248. DOI: https://doi.org/10.1179/joc.2007.19.3.243.

Ramadan ES, Nassar NR, Ibrahim IG, and Zayed AF (2019). Epidemiological and zoonotic surveillance of brucellosis in Beni-Suef governorate. Alexandria Journal for Veterinary Sciences, 61 (1): 22-31. DOI: https://doi.org/10.5455/ajvs.39738.

Refai M (2002). Incidence and control of brucellosis in the near east region. Veterinary Microbiology, 90: 81-110. DOI: https://doi.org/10.1016/S0378-1135(02)00248-1.

Rojas X, and Alonso O (1998). Elisa for the diagnosis and epidemiology of brucella abortus infection in cattle in Chile. Joint FAO/IAEA Division of in Food and Agriculture, Vienna, pp. 77-81. Available at:https://inis.iaea.org/collection/NCLCollectionStore/_Public/29/067/29067678.pdf

Saddique A, Ali S, Akhter S, Khan I, Neubauer H, Melzer F, Khan AU, Azam A, and El-Adawy H (2019). Acute febrile illness caused by brucella abortus infection in humans in Pakistan. International journal of environmental research and public health, 16(21): 4071. DOI: https://doi.org/10.3390/ijerph16214071.

Salem L, Khalifa NO, and Moustafa SM (2016). Sero-diagnosis of brucellosis in Gharbiya governorate, Egypt. Banha veterinary medical journal,31(1):10-16. DOI: https://doi.org/10.21608/BVMJ.2016.31211.

Samaha HAH AM (2008). Viability of brucella melitensis biovar 3, in milk and some dairy products. Egyptian Journal of Medical Microbiology, 17(2): 1916-1918. DOI: https://doi.org/10.3201/eid1412.071452.

Saraya MA (2017). Acute brucellosis: Presentation and complications in adults. Afro-Egyptian Journal of Infectious and Endemic Diseases, 7(4):186-191. DOI: https://doi.org/10.21608/aeji.2017.17802.

Shaaban SI, Ayoub MA, Ghorbal SH, and Nossair M (2018). Calves as a reservoir of some diarrheagenic agents for human contacts in El-Behira province. Alexandria Journal for Veterinary Sciences, 56 (2): 48-53. DOI: https://doi.org/10.1186/s12879-017-2208-0.

Tekleye BKO, Mugurewa M, Sholtens RG, and Tamirat Y (1989). The prevalence of brucellosis in indigenous cattle in central Ethiopia. Bulletin of Animal Health and Production in Africa., 37 (1): 97-98. Available at: https://hdl.handle.net/10568/29305

Teng YH, Teng JJ, Chao S, Chao H, and Waghela SD (2017). Comparison of the rose bengal plate and the complement fixation tests with the tube agglutination test for diagnosis of human brucellosis. Open Journal of Clinical Diagnostics, 7:73. DOI: https://doi.org/10.20506/rst.31.3.2175.

Tsegay A, Tuli G, Kassa T, and Kebede N (2017). Seroprevalence and risk factors of brucellosis in abattoir workers at Debre Zeit and modjo export abattoir, central Ethiopia. BMC infectious diseases, 17 (1): 101. DOI: https://doi.org/10.1186/s12879-017-2208-0.

Tumwine G, Matovu E, Kabasa JD, Owiny DO, and Majalija S (2015). Human brucellosis: Sero-prevalence and associated risk factors in agro-pastoral communities of Kiboga district, central Uganda. BMC public health, 15 (1): 900. DOI: https://doi.org/10.1186/s12889-015-2242-z.

Wareth GH A, Refai M, Melzer F, Roesler U, and Neubauer H (2014). Animal brucellosis in Egypt. Journal of Infection in Developing Countries, 8 (11): 1365–1137. DOI: https://doi.org/10.3855/jidc.4872.

Yohannes M Gill J P, Ghatak S, Singh D K and Tolosa T (2012). Comparative evaluation of the rose bengal plate test, standard tube agglutination test and complement fixation test for the diagnosis of human brucellosis. Revue scientifique et technique, 31(3): 979-984. DOI: https://doi.org/10.20506/rst.31.3.2175.

Young JS, Gormley E, and Wellington EM (2005). Molecular detection of mycobacterium bovis and mycobacterium bovis bcg (pasteur) in soil. Applied and environmental microbiology, 71 (4): 1946-1952. DOI: https://10.1128/AEM.71.4.1946-1952.2005.

Zakaria AM, Ahmed SF, and Motawae MS (2018). Seropositivity in animals and risk of occupational brucellosis among abattoirs personnel associated with poor work practices and absence of safety policy in Egypt. International journal of occupational and environmental health, 24 (1-2): 55-60. DOI: https://doi.org/10.1080/10773525.2018.1516839.

To cite this paper: Diab MS, Zidan ShAA, Hassan NAA, Elaadli H and Bayoumi AM (2020). Seroprevalence and Associated Risk Factors of Brucellosis in Livestock and Residents of New Valley Governorate, Egypt. *World Vet. J.*, 10 (4): 531-539. DOI: https://dx.doi.org/10.54203/scil.2020.wv64

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 540-550, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj65

Ultrastructural and Molecular Characterization of *Sarcocystis* Species Derived from Macroscopic Sarcocysts of Domestic Sheep and Goats in Soran City, Erbil, Iraq

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ORGINAL ARTICLE
pii: S232245682000065-10
Received: 01 Oct 2020
Accepted: 17 Nov 2020

ABSTRACT

This study aimed to identify *Sarcocystis* species isolated from macroscopic sarcocysts from naturally infected domestic sheep and goats using the molecular method, as well as investigating the morphological and the ultrastructural characteristics of the isolated species. A total of 1000 esophagi were collected from sheep and goats and examined for the presence of sarcocysts. Macroscopic sarcocysts were isolated from the infected esophagi, and *Sarcosystis* species were identified molecularly by 18S rRNA gene sequence analysis. Moreover, the ultrastructure of the sarcocysts was investigated by both scanning and transmission electron microscopy. The macroscopic sarcocysts were detected in 9.1% (91/1000) of the esophagi. The results of electron microscopy indicated the characteristic features of the macroscopic sarcocysts. The cysts contained numerous merozoites and banana-shaped bradyzoites. The bradyzoites were characterized by possessing a double-membrane pellicle and consisted of a conoid in one of the apices, numerous micronemes, two rhoptries, as well as a long, convoluted mitochondrion, subterminal nucleus, and several amylopectin granules. The partial analysis of the 18S rRNA gene presented that all isolates produced bands of expected sizes on gel electrophoresis. The findings from the phylogenetic analysis revealed that the identified *Sarcocystis* species were most closely related to *S. gigantea, S. moulei,* and *S. medusiformis*. To the authors' knowledge, this is the first time *S. medusiformis* has been recorded in goats. Goats and sheep can be proposed as alternative intermediate hosts for *S. gigantea* and *S. moulei,* respectively, cross-infection may also occur between them and the host specificity of these species of *Sarcocystis* is questionable.

Keywords: Goats, Phylogeny, *Sarcocystis*, Sheep, Ultrastructure, 18S rRNA

INTRODUCTION

Sarcocystosis is a zoonotic protozoal disease that may cause mortality in many species of domestic and wild animals. Up to date, more than 196 valid species of *Sarcocystis* have been identified (Dubey et al., 2016). This parasite has an obligatory two-host life cycle, asexual stages develop in the intermediate host, which is frequently a herbivores animal, while sexual stages occur in the definitive host that is a carnivore or omnivore animal (Lindsay and Dubey, 2020). There are two sizes of *Sarcocystis* cysts, the microscopic and the macroscopic sarcocysts (Dubey, 2015). Several species of *Sarcocystis* develop macroscopic sarcocysts in the tissue of domesticated animals. Among them, S. *caprafelis* (*S. moulei*) in goats, *S. gigantea* (*S. ovifelis*), and *S. medusiformis* in sheep (Lindsay and Dubey, 2020). Two kinds of macroscopic sarcocysts are identified and classified as fat and thin in sheep. They represent *S.gigantea* and *S. medusiformis*, respectively (Farhang- Pajuh et al., 2014). In Iraq, a couple of different sizes of macroscopic *Sarcocystis* were recognized in the domestic goats. Fat sarcocysts appear to be large, ovoid, and only found in the esophageal muscle. Besides, the thin sarcocysts are less frequent and appear small and slender, and also are found in the diaphragm and skeletal muscle (Barham et al., 2005).

Traditionally, the ultrastructural characteristics of the *Sarcocystis* are considered as the fundamentals for the identification of various *Sarcocystis* spp. within the same intermediate host (Hu et al., 2017; Huang et al., 2019). Nowadays, genomic sequence analysis is a very essential technique to explain whether morphologically similar sarcocysts from different intermediate hosts are identical species or not (Yang et al., 2001). The most common sequences reliable to discriminate *Sarcocystis* spp. are 18S rRNA, 28S rRNA, COX1, and ITS sequences (Bahari et al., 2014; Bittencourt et al., 2016; Whaeeb and Faraj, 2016; Hu et al., 2017; Elmishmishy et al., 2018; El-Morsey et al., 2019; Gjerde et al., 2020; Metwally et al., 2019). In Iraq, two studies were conducted (Whaeeb and Faraj, 2016; Dakhil et al., 2017) concerning the PCR sequencing and phylogenetic analysis of the 18S rRNA gene among sheep for *S. tenella* and water buffalos for *S. fusiformis* and *S.moulei*. There is no molecular and ultrastructural study on *Sarcocystis* spp. of sheep and goats in the Kurdistan region, Iraq. Therefore, the current study was designed to identify macroscopic *Sarcocystis* spp. in naturally infected domestic sheep and goats using phylogenetic analysis of 18S rRNA sequences and

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to evaluate their genetic variants, as well as to study the morphological and the ultrastructural characteristics of the isolated species in Soran, Erbil, Iraq

MATERIALS AND METHODS

Morphologic characterization of macrosarcocysts

The entire esophagi were collected from 400 sheep and 600 goat carcasses at the slaughterhouse of Soran city, Erbil, Kurdistan, Iraq, from August 2018 to August 2019. The tissue samples were transported to the laboratory in ice-cooled boxes for further analysis. The esophagi were longitudinally sectioned to examine its internal and external walls (Bittencourt et al., 2016). The dimensions of the macrosarcocysts were measured by a ruler, and then several of them were selected and processed for the ultra-histological study, while other macrosarcocysts were kept at -20°C until analyses with molecular methods. The microscopic detection was performed by cutting a small portion of the macrocyst, and then it was compressed between two slides, stained with Giemsa stain, and examined microscopically (Juyal et al., 1989).

For ultrastructure study by scanning electron microscopy (SEM), small portions of the macrosarcocysts were fixed with 2.5% glutaraldehyde in sodium cacodylate buffer for 24 h at 4 °C, then washed in 0.1 M cacodylate buffer. After that, the samples were post-fixed with 1% osmium tetroxide for 2 h at 4 °C. The fixed specimens were dehydrated by sequential incubations in increasing concentrations of acetone (35%, 50%, 75%, 95% for 10 min, and 100% for 15 min. Finally, they were sputter-coated with gold and inspected by SEM (LEICA model CPD030, Germany) (Bittencourt et al., 2016; Metwally et al., 2019).

For transmission electron microscopy (TEM), small portions of macrosarcocysts from infected esophagi were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer for 24 h at 4 °C. Post fixation was done for 2 h in 1% osmium tetroxide. The specimens were dehydrated in a graded acetone series, infiltrated with 1:1 acetone and Epon resin mixture, and embedded in Epon resin. The resin was polymerized in an oven at 60 °C overnight. Sections of 700 Å thick were prepared by LKB ultramicrotome with a diamond knife and stained with uranyl acetate and lead citrate. The stained sections were visualized under TEM (LEICA model CPD030, Germany) (El‑ Morsey et al., 2019).

Molecular identification, sequencing, and phylogenetic analysis

The DNA was extracted from 20 macrosarcocysts of both infected sheep and goats according to the manufacturer's instructions of the DNA extraction kit (FAVORGEN®, Cat. No. FATGK001A, Taiwan). Briefly, 25 mg of the macrosarcocyst sample was isolated, grounded by micro pestle in a microcentrifuge tube, and processed. Finally, the extracted DNA was stored at -20 °C until further analysis. The DNA templates were prepared according to the primers manufacturer's protocol (GeNet Bio, South Korea). Lyophilized forward and reverse primers of *Sarcocystis* spp. 18S rRNA genes were prepared according to the manufacturer's instruction. The PCR was performed using the *Sarcocystis* spp. specific primers amplifying a portion of the 18S rRNA gene as described in Table 1.

Table 1. Primers used for PCR amplification and sequencing of the 18S rRNA gene of *Sarcocystis* spp. isolated from goats and sheep

Primers and orientation	Sequence (5′→3′)	Amplicon size (base pair)	References
Primer 2L Forward	GGATAAACCGTGGTAATTCTATG	850	Choi et al. (2018) Kalantari et al. (2016) Yang et al. (2001) Calero-Bernal et al. (2014)
Primer 3H Reverse	GGCAAATGCTTTCGCAGTAG		
Primer S1 Forward	GAATCCAAACCCCTTTCAGAGT	1050	Choi et al. (2018) Formisano et al. (2013)
Primer 1H Reverse	TATCCCCATCACGATGCATAC		
Primer Forward	CGCAAATACTATATCACTCG	690	Hussein et al. (2017)
Primer Reverse	CTAGAAACCAACAAAATAGA		
Primer Forward	CGAATGGCTCATTAAAACAG	570	Hussein et al. (2017)
Primer Reverse	CCAACTACGAGCTTTTTAAC		

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The PCR reaction was performed by using the super cycler PCR system (Kyratec® Thermo Fisher, Germany) according to the manufacturer's protocol for 30-35 cycles consisted of an initial denaturation step at 94 ºC for 2 min, followed by 35 cycles of denaturation at 94 ºC for 20 s, annealing at 60 ºC for 10 s and extension at 72 ºC for 25 s, with a final extension step at 72 ºC for 5 min. The PCR products were visualized by electrophoresis on 1% agarose gel with a 100 DNA marker (Favorgen, Taiwan), and purified using Favorgen® Gel and PCR Clean-up (Favorgen, Taiwan). The purified amplicons were sent to South Korea for sequencing on both strands, forward and reverse. Positive PCR products were directly sequenced by using oligonucleotide primers for identifying specific DNA regions of *Sarcocystis* species.

Forward and reverse sequencing was performed using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit, version 1.1 (Macrogen Inc., South Korea). Sequenced fragments were purified and resolved by capillary electrophoresis with the 3730xl Genetic Analyzer (Macrogen Inc., South Korea). The resulting sequences were aligned with other sequences of *Sarcocystis* spp. from intermediate hosts deposited in the GenBank using the BLAST tool and subjected to phylogenetic analyses (Rubiola et al., 2019). The neighbor-joining method was used to create a guide tree as pairwise and multiple sequence alignment. The Sanger sequencing technique was used for sequencing purposes. Phylogenetic analyses were performed based on nucleotide sequences of the 18S rRNA gene and compared to *Toxoplasma gondii* as an outgroup parasite by using the MEGA7 program (Kumar et al., 2016). The bootstrap method was used by testing 1000 bootstrap replications.

RESULTS

Morphologic characterization

The macroscopic sarcocysts were detected in 9.1% (91/1000) of the inspected esophagi of both sheep and goats. Two different shapes of macroscopic sarcocysts were detected (Figure 1), which were described as thick and thin sarcocysts. The thick sarcocysts were oval to sac-like shape with bluntly rounded ends and were found in sheep and goats. The other type was thin, slender (fusiform), elongate with pointed ends, and was observed in goats only. The size of the thick sarcocysts ranged from 4 × 2.5 to 8 × 5 mm. While the thin sarcocysts varied from 5 × 2 to 10 × 3 mm in size. Bradyzoites were found in all digested macroscopic sarcocysts samples of both animals (Figure 1D). Based on the gross examination, shape, and size of the macroscopic sarcocysts, the thick macroscopic sarcocysts were tentatively identified to *S gigantea* and *S. moule*, whereas the thin type was assigned to *S. medusiformis*.

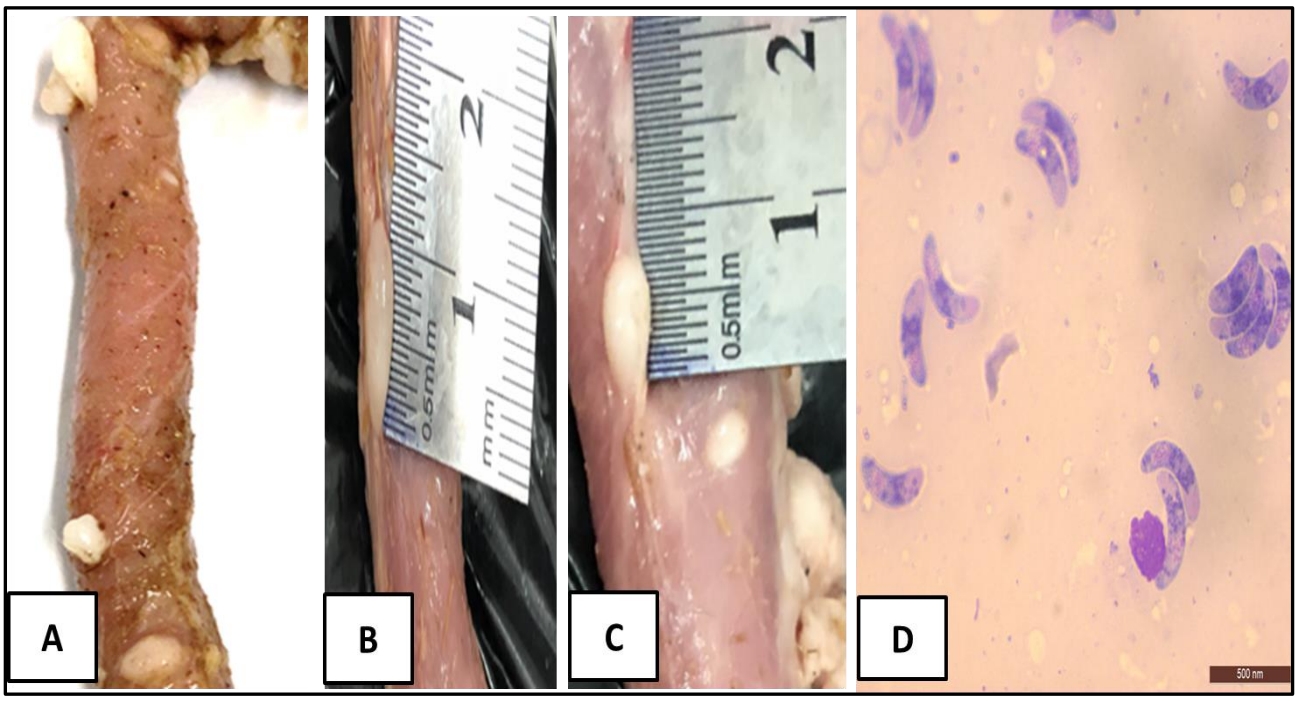

Figure 1. Gross appearance of the macroscopic sarcocysts in the esophageal muscles of infected sheep and goats with *Sarcocystis* spp. **A:** Oval shape sarcocyst in the sheep, **B:** Fusiform slender sarcocyst in the goats, **C:** Oval sac-like sarcocysts in the goats, **D:** Several banana-shaped bradyzoites from macroscopic sarcocyst stained with Giemsa stain (Scale bar = 500 nm).

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The current results of SEM revealed three morphologically distinct sarcocysts types. Based on wall thickness, one of them was thin-walled (<2 µm) without a clear secondary wall (Figure 2A), which preliminary was assigned to *S. medusiformis*. The second sarcocysts were thick-walled (>5.0 µm) with secondary wall (Figure 2B) that was tentatively assigned to *S. moulei*, and the third one was thin-walled (<2 µm) covered with connective tissue secondary wall that was provisionally referred to *S. gigantea* (Figure 3A). Sarcocysts were located within a parasitophorous vacuole in the host cell cytoplasm, consisting of a cyst wall that surrounds the metrocytes or the bradyzoites. Internally, groups of bradyzoites were separated into compartments by septa that arise from the sarcocyst wall as seen in Figure 2A, or they may not be clearly compartmentalized (Figure 2B).

The esophageal tissue appeared in both animals as a sheath covering the cyst with intact adhesion. Regarding the macroscopic sarcocyst which proposed belong to *S. gigantea*, it appeared with a thin wall surrounding by connective tissue secondary capsule as seen in Figure 3B. In one of the examined macroscopic sarcocysts of sheep, numerous recognizable bradyzoites appeared and looked like a banana in bundle shape resembles a rose (Figure 3C). There was no host tissue capsule surrounding the macroscopic sarcocyst of *S. medusiformis* (Figures 2A and 4A), while *S. gigantea* had a host-derived connective tissue (Figure 3A and B). This feature was considered as characteristic for macroscopic sarcocystis of *S. medusiformis*.

The present findings of TEM showed that there are ultrastructure similarities for all macroscopic sarcocysts sampled from sheep and goats. The sarcocysts were filled with a granular substance, merozoites, metrocytes, some intermediate stages between metrocytes and cyst merozoites, and typical cyst bradyzoites. The merozoites had all the organelles observed in the bradyzoites, except the rhoptries. The bradyzoites seemed to be banana-shaped and were arranged separately or tightly in packets separated by septa. The size of the bradyzoite reached 11 × 2.6 µm, and had a double-membrane pellicle, and consisted of a conoid in one of the apices, numerous micronemes, two rhoptries, as well as a long, convoluted mitochondrion, subterminal nucleus, and several amylopectin granules (Figures 5 and 6). The apical complex, numerous micronemes, and up to two rhoptries were located in the anterior region. Inclusion bodies, including amylopectin, lipid, and electron-dense granules were found in the central region of the bradyzoites, and cell organelles, including the nucleus, were located at the posterior region. The micronemes were small in size and appeared ovoid or round in cross-section, and they were located towards the conoid.

Moreover, the metrocyte appeared with a nucleus, ground substance, amylopectin, and without micronemes. Some cysts had muscular tissues adhered to the external layer of the cyst wall. In other cysts, the host muscle tissue was completely absent.

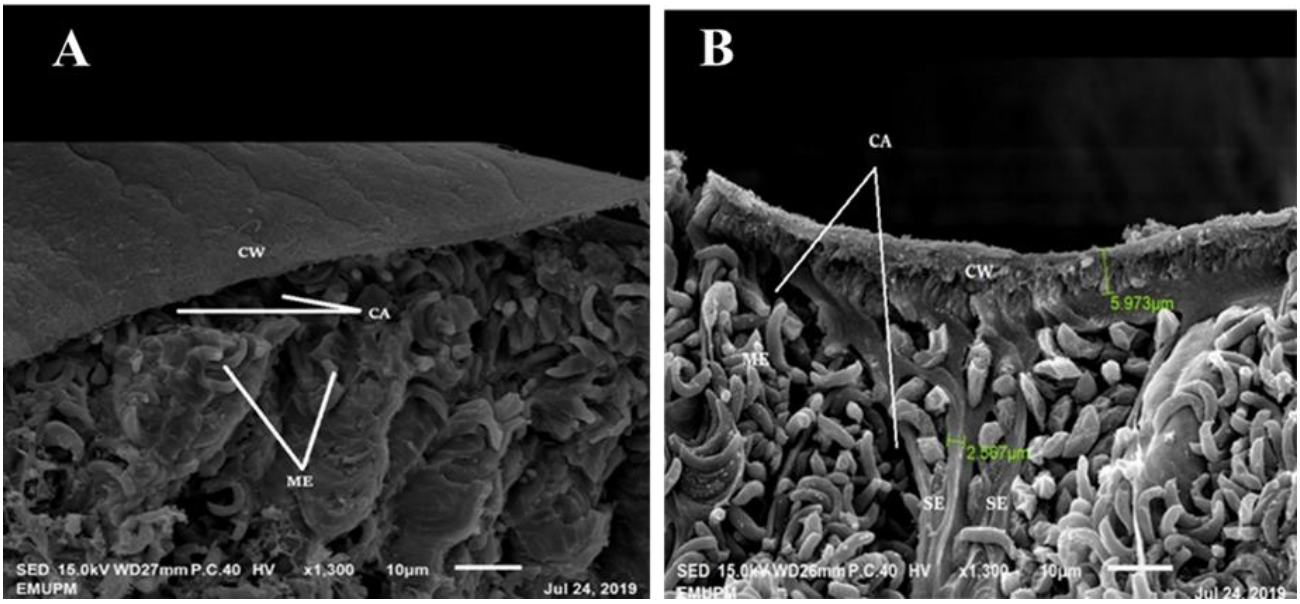

Figure 2. SEM of macroscopic sarcocyst in the esophagi of goat and sheep. **A**: *S. medusiformis* sarcocyst in goat. Note the groups of merozoite (bradyzoites), which appear like a banana shape in bundles or packed in chamber-like compartments separated from each other, and some chamber-like hollows (CA) between them. Scale bar= 10 µm. **B**: *S. moulei* sarcocyst *in* sheep with the thick cell wall (CW), and filled with bradyzoites and metrocytes; the clear septa (Se) that originate from the sarcocyst wall like tree roots. Scale bar=10 µm.

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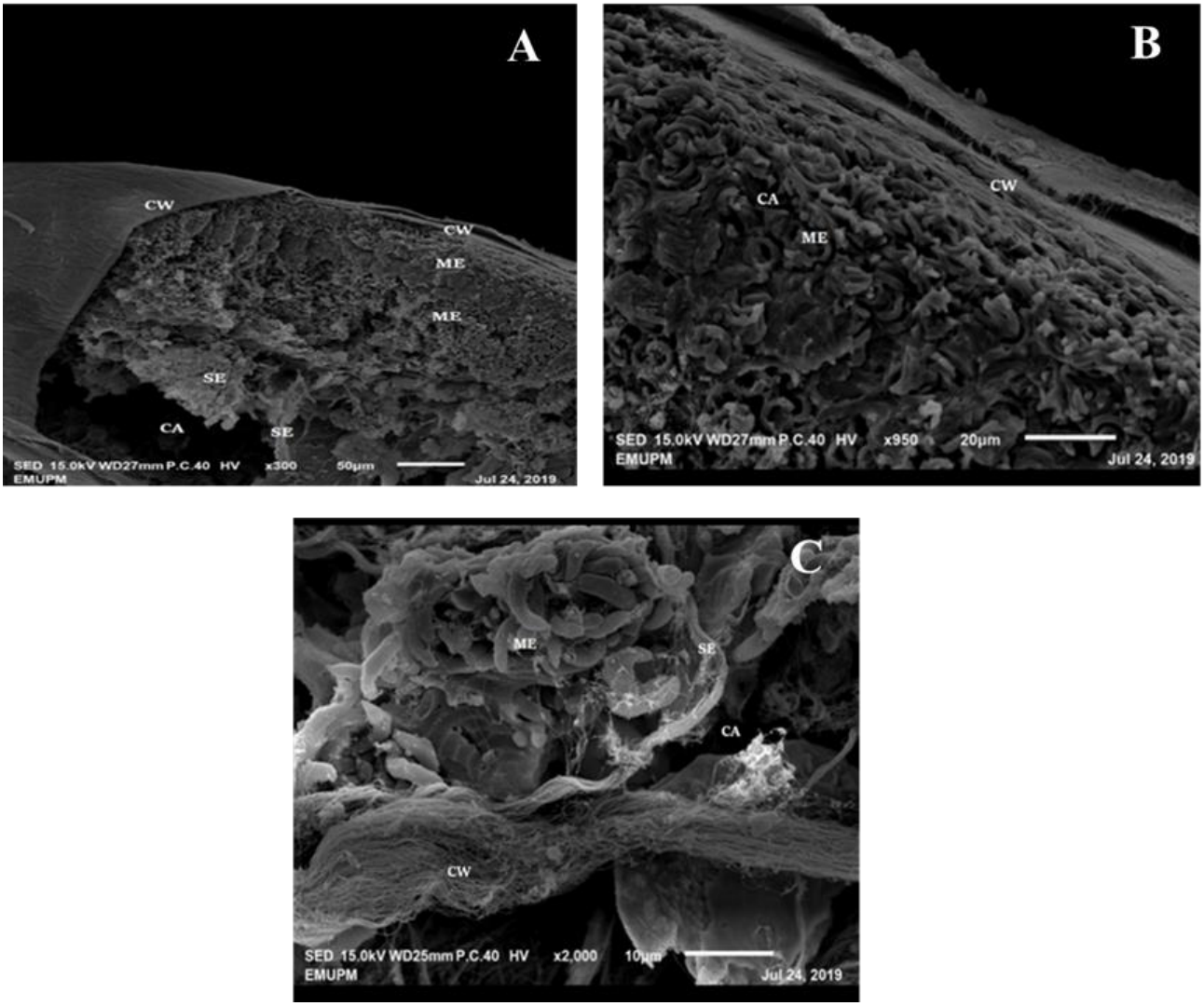

Figure 3. SEM micrograph of a macroscopic sarcocyst within the esophagi of sheep and goat. **A**: *S. gigantea* with thin-walled and highly patched merozoite (ME), the septa (SE) are thin; also, there is an appearance of chamber-like hollows (CA).Scale bar=50 µm; **B**: Peripheral site of macroscopic sarcocyst of *S. gigantea* with a thin wall surrounding connective tissue as a secondary capsule. Scale bar= 20 µm; **C:** Part of sarcocyst showing the cell wall of a cyst with numerous obvious appearances of bradyzoites (Br), which appear like a banana in bundle shape resembles a rose. Scale bar= 10 µm.

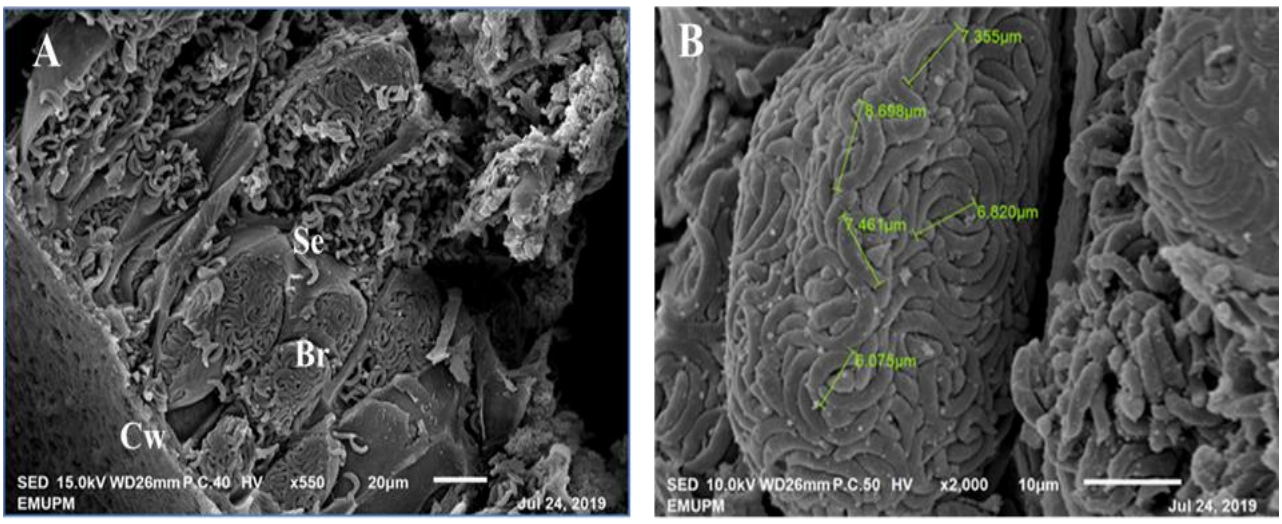

Figure 4. High magnification of macroscopic sarcocyst of *S. medusiformis.* **A**: Note the perforated cell wall (CW) and the numerous bundles of bradyzoites. Scale bar= 20 µm **B**: Huge number of tightly packed bradyzoites of *Sarcocystis* in sheep ranged from 6 to 8.6 µm in length. Scale bar= 10 µm.

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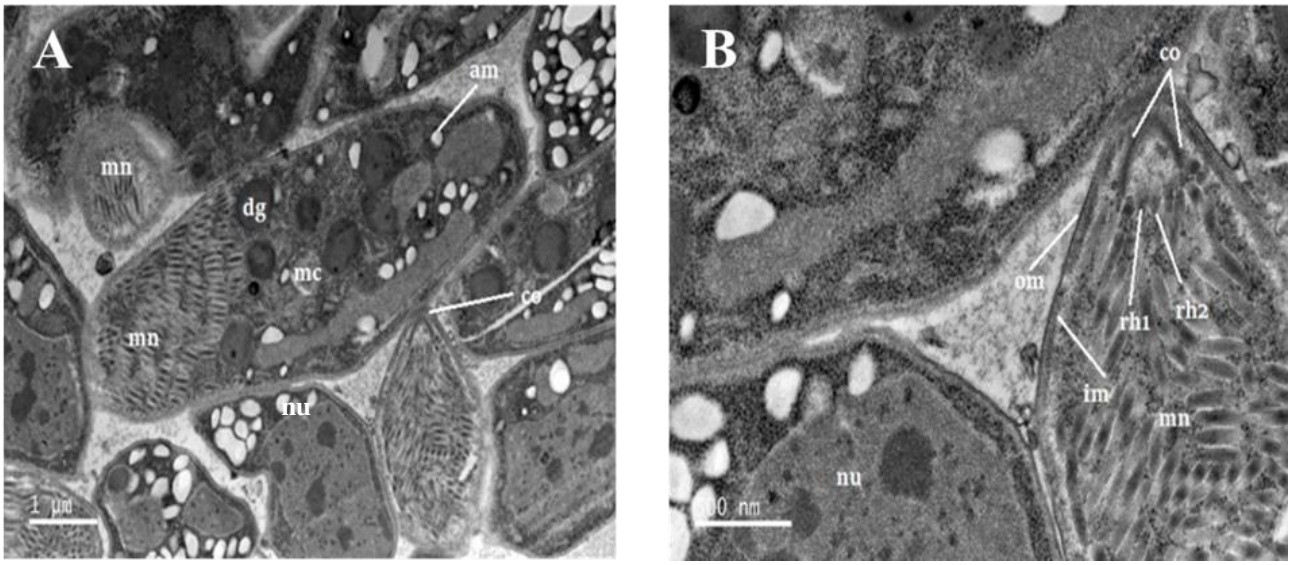

Figure 5. Transmission electron micrographs of a longitudinal section of macroscopic sarcocyst of *Sarcocystis* in goat. **A**: Merozoite of *Sarcocystis*; amylopectin (am), conoid (co), numerous micronemes (mn), nucleus (nu), convoluted mitochondrion (mc), dense granules (dg),Scale bar= 1 µm; **B**: Conoidal (co) end of a bradyzoite of *Sarcocystis* with numerous micronemes (mn), rhoptries (rh), outer plasmalemma membrane (om), inner membrane (im), and tubular mitochondrion, Scale bar = 500nm.

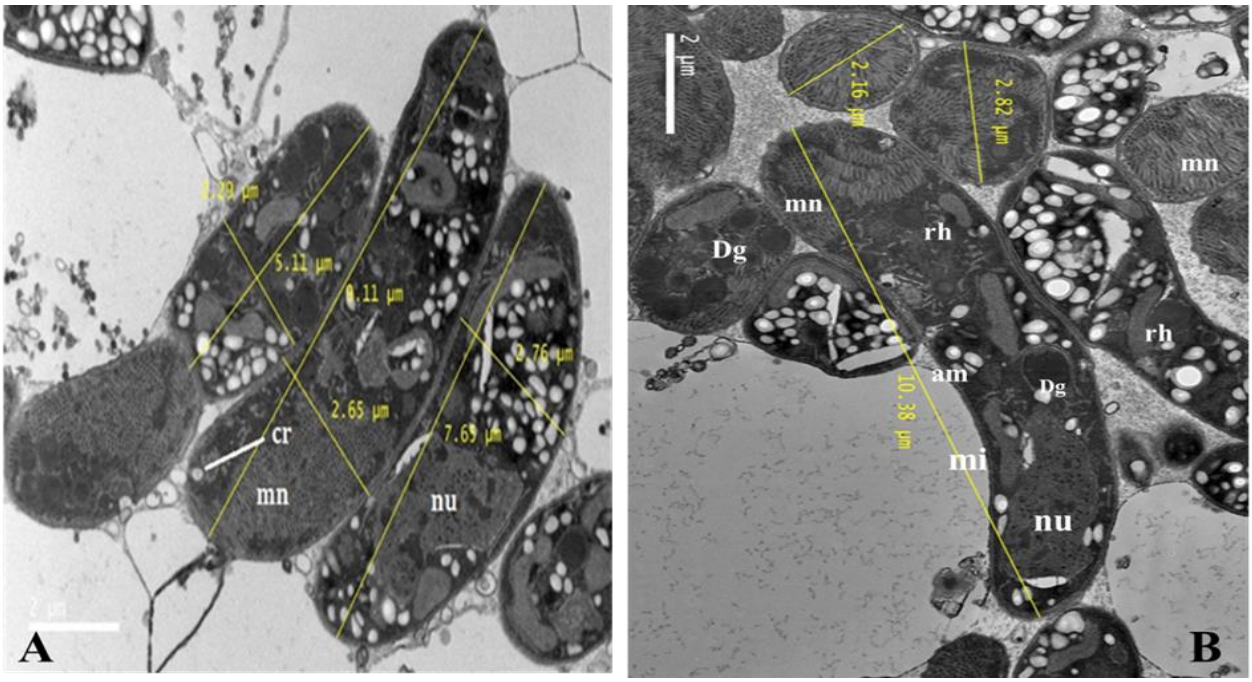

Figure 6. Ultrastructure of bradyzoites of sheep macroscopic sacocysyt of *Sarcocystis*. **A**: Note conoidal ring (Cr), amylopectin (am), numerous micronemes (mn), nucleus (nu). Scale bar=2 µm. B: conoidal ring (cr), numerous micronemes (mn), nucleus (nu), rhoptries (rh), dense granules (dg), amylopectin (am), and mitochondrion (Mi) in sheep esophagus. Scale bar= 2 µm.

Molecular characterization

The PCR amplification revealed that all 20 isolates produced a positive band on gel electrophoresis with different amplicon molecular sizes. The partial 18S rRNA gene was amplified in all tested samples and yielded the expected amplicon PCR size of 1050, 850, 690, and 570 bp in both animals (Figure 7). Sanger sequencing was performed for identifying the *Sarcocystis* spp. by using forward and reverse primers for eight macroscopic sarcocyst samples. The present findings proved that all macroscopic sarcocysts of both sheep and goat belonging to *Sarcocystis* spp and are deposited in the GenBank with accession numbers MN658377 from sheep and MN658381 from a goat. Comparison of one of the obtained sequences with other *Sarcocystis* spp. registered in GenBank was performed. The result of the phylogenetic analysis was compared with *Toxoplasma gondii* as out-group species and compared with *Sarcocystis* 18S rRNA sequences from sequences deposited in the GenBank database. The eight sequences of *Sarcocystis* spp. were placed within the tree, (S1, S3, S4, G1, and G3) and (S2 and G2) were found in both sheep and goats, which related to *S. gigantea* and *S. moulei*, respectively. While G4 was recorded in goats and it was assigned to *S. medusiformis* (Figure 8).

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The sequencing of the amplified products resulted in 16 readable sequences, among those, S4 showed 99.43% identity with previously deposited sequence *S. gigantean in* GenBank with accession number MT026577 from Iran and 98.86% with *S. moulei* with the accession number of KC508513 from Iran. Also, S2 showed 99.46% identity with *S. moulei* under the accession number L76473 and 98.50% with *S. gigantea* with the accession number MK420020 from Spain. In addition, G3 showed 94.35% identity with *S. gigantea* of accession number KP053894 from Iran, Moreover, S1 showed 92.34 % of homology with *S. gigantea* of the accession number MF769582, and G2 showed 94.66% identity with the isolate of accession number L76473. While G4 display 92.61% homology with *S. medusiformis* accession number MK42002.

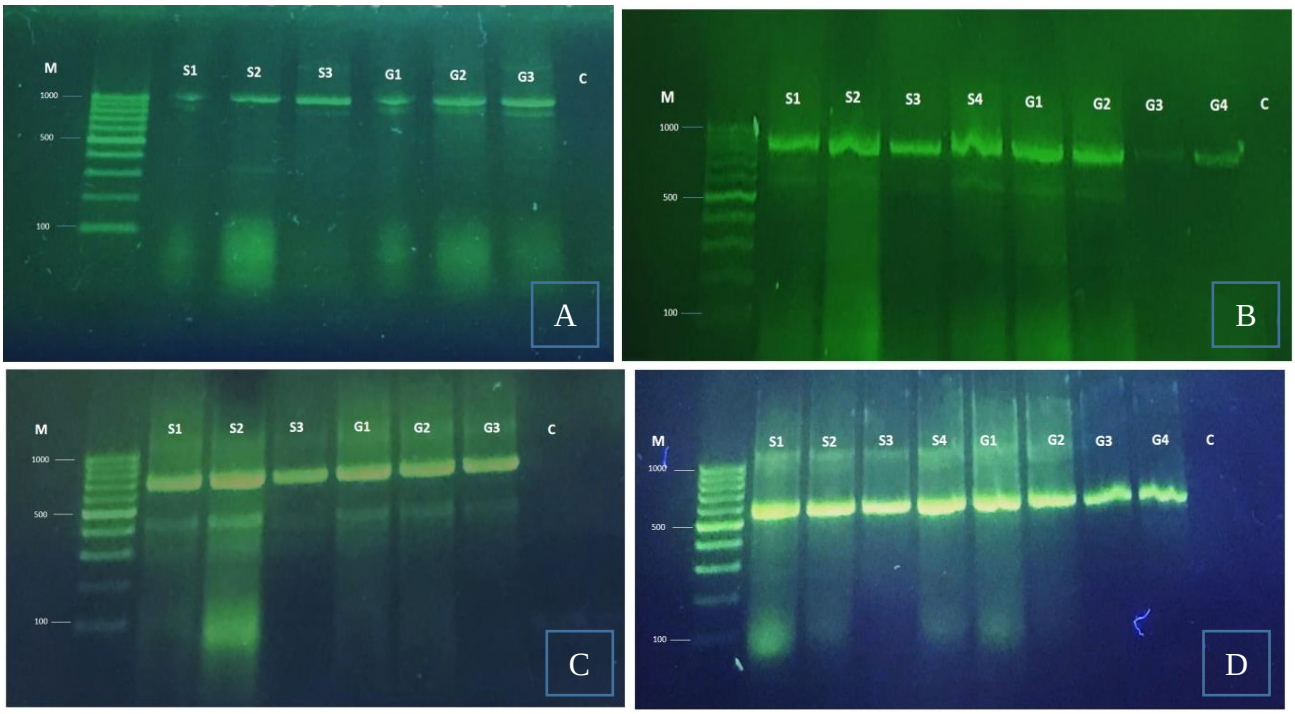

Figure 7. PCR products of partial 18S rRNA gene of *Sarcocystis* spp., presented bands at 1050 bp (A), 850 bp (B), 690 bp (C), and 570 bp (D) on 1% agarose gel. M: 100 bp size marker. Lanes S1-S4: *Sarcocystis* spp. isolated from sheep. Lanes G1- G4: *Sarcocystis* spp. isolated from goats. Lane C: negative control.

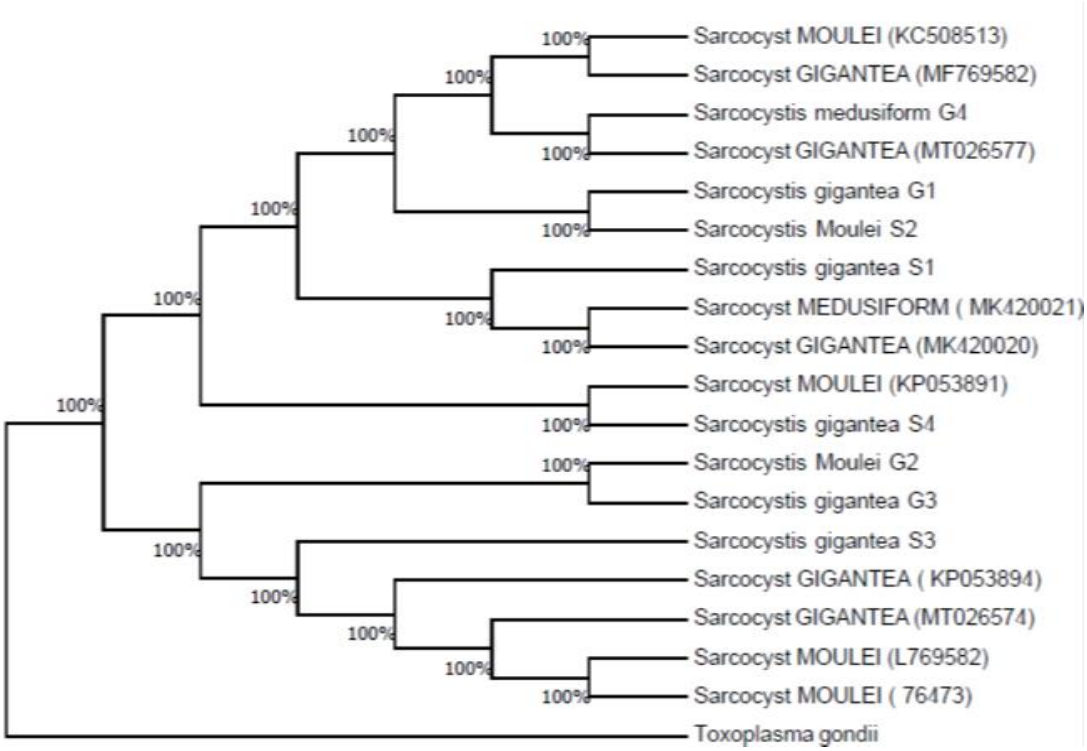

Figure 8. Phylogenetic tree of selected *Sarcocystis* spp by the program Mega v.7 using maximum likelihood-based on 18S r RNA gene sequences. The isolates of the present study represent as S1, S2, S3, S4, G1, G2, G3, and G4. *Toxoplasma gondii* was used as outgroup species.

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DISCUSSION

Sarcocystosis is prevalent in several ruminant livestock animals like cattle, sheep, and goats worldwide. The current study revealed that macroscopic *Sarcocystis* infection is common among sheep and goats. A large variation in the size and the shape of the sarcocysts was observed in the inspected esophagi of both animals, and this may be attributed to the age of the cyst as well as to the species of *Sarcocystis.*

Ultrastructural and molecular analysis of macroscopic sarcocysts showed three distinctive species of *Sarcocystis.* Regarding the sarcocysts from sheep origin, the results indicated that three of them represented *S. gigantea*, whereas the fourth one related to *S.moulei.* There are only two validated macroscopic species of *Sarcocystis* described in sheep; *S. gigantea* and *S. medusiformis. S. gigantea* is distributed worldwide, including Iraq (Al-Hyali et al., 2011), whereas *S. medusiformis* has only been reported from Italy, Iran, New Zealand, Spain, Jordan, and Australia (Collins et al., 1979; Dubey et al., 2016). The occurrence of *S. moulei* in sheep esophagi demonstrated that sheep can be a suitable host for *S.moulei* that is previously documented to parasitize goats. Elmishmishy et al. (2018) stated that there are very close phylogenetic interactions between *S.gigantea* and *S.moulei*, a goat specific species which rarely recorded in sheep and they suggested the cross-transmission between these two hosts. Moreover, a similar result was recorded in Iran and indicated that sheep can be a convenient and alternative host for *S.moulei* (Kalantari et al., 2016). Also, other researchers concluded that the *Sarcocystis* spp. of sheep and goats are highly related and should be together grouped (Metwally et al., 2019).

Concerning host specificity, investigation in Saudi Arabia identified *S. moulei* from sheep and goats (Al-Hoot et al., 2005), as well as a previous study, recorded the infection of goats with *S. ovifelis* (*S. gigantea*) and with *S. moulei* at the same time, besides, they concluded that the goats are the host for at least 3 *Sarcocystis* species that are hidden beneath the first description of *S. moulei* (Ghaffar et al., 1989). Also, a study from Iraq proved, for the first time, the infection of water buffalo with *S.moulei* in the world (Dakhil et al., 2017). The current work showed that the goats harbored three species of Sarcocystis, S. *moulei*, *S. gigantea*, and *S. medusiformis*. It is known that *S. moulei* is the macrosarcocysts of goats (Lindsay and Dubey, 2020).

The current study reported the first infection of goats with *S. medusiformis* in Iraq. This species was identified by morphological, ultrastructural, and molecular characterization, and in comparison with the formerly sequences that already existed in the GenBank of *Sarcocystis spp.* (Obendorf and Munday, 1987; Oryan et al., 1996; Gjerde et al., 2020). The morphologic characteristic of the sarcocysts of *S. medusiformis* as a thin fusiform shape and its size was consistent with the previous description of this species from sheep (Obendorf and Munday, 1987; Farhang-Pajuh et al., 2014). Besides, the muscle tissues infected with *S. medusiformis* were not encapsulated (Figure 3A, Figure 5A), but in the case of *S. gigantea* the muscle cell encapsulated by a host-derived connective tissue, but there was no real secondary wall (Figure 4A and B). This agreed with the findings of several studies (Munday and Obendorf, 1984; Obendorf and Munday, 1987; Ghaffar et al., 1989; Gjerde et al., 2020). The secondary cyst wall was detected in the old macrocyst of *S.moulei;* this agreed with the description of (Ghaffar et al., 1989), whereas in *S. gigantea*, there was no secondary wall, but there was a connective tissue capsule that originated from the host tissue. Furthermore, TEM results showed the typical structure of the bradyzoites, which contain the conoid, inner membrane complex, numerous micronemes, and rhoptries that are presented as electron-dense located at the anterior part. Also, there are dense granules scattered along the bradyzoites, particularly in the middle of it, tubular mitochondrion, amylopectin, and a sub-terminal large nucleus as described previously (Dubey et al., 2014; Dubey et al., 2016; El-Morsey et al., 2019).

In the present study, molecular detection was performed by using partial 18S rRNA genes for genotypic confirmation of the *Sarcocystis* spp. The variable regions of the 18S rRNA gene act as useful targets for the classification and characterization of dissimilar species (Neefs et al., 1991; Yang et al., 2001). Moreover, 18S rDNA is very convenient for phylogenetic investigations due to its high conservation, and examination of its variable regions permits the identification of species within a genus (Maidak et al., 1997; Ng et al., 2015). The PCR amplification of 20 macroscopic sarcocysts revealed that all isolates have a positive band on gel electrophoresis with different amplicons sizes. The partial 18S rRNA gene was amplified in all positive samples and yielded the expected amplicon PCR size of 850, 1050, 690, and 570 bp for both animals.

The current phylogenetic analysis was successfully generated with *Toxoplasma gondii* inferred from near full-length 18S rRNA sequences and compared with previous sequences presented in GenBank. The occurrence of cross-infection may happen as seen in (S1, S3, S4, G1, and G3) belonged to *S. gigantea* in both sheep and goats, (S2 and G2) belonged to *S. moulei*, while *S. medusiformis* was only found in goat. Yang et al. (2001) concluded that morphologically similar species from two different intermediate hosts should be considered as the same species. Therefore, some *Sarcocystis* species seem to have a wider intermediate host range than formerly expected. The findings of the present study agreed with the outcomes of some previous investigations in this respect (Yang et al., 2001; Kalantari et al., 2016). The phylogenetic homogeneity between *S gigantea* and *S. moulei* found in the current study can be reasoned by

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considering them as a sister clade, and this highlights the possibility of the occurrence of cross infection (Kalantari et al., 2016).

The current phylogenetic tree showed that the sequence of G4 from Iraqi goats assigned to *S. medusiformis* was highly similar to *S. gigantea* of accession number Mt026577. Similar findings were reported in a study related to *Sarcocystis* of sheep in Spain and showed that *S. medusiformis* was sister to sequence of *S. gigantean* (Gjerde et al., 2020). Moreover, the macrosarcocysts of *S. medusiformis* were recorded in Iran by two studies, the first one using a gross examination of the morphological features and transmission study, the shape of the isolated macrosarcocysts appeared narrow and elongate (Oryan et al., 1996), and the second by using the PCR-RFLP technique for the 18S rRNA gene, but they did not confirm the species by sequencing of the PCR product (Fahang-Pajuh et al., 2014). Besides, a recent study pointed out that *S.capracanis* of domestic goats is sister species of *S.tenella* of sheep (El-Morsey et al., 2019). The genotypic characteristic result agreed with the results obtained in Iran, Brazil, Argentina, and Spain (Bahari et al., 2014; Bittencourt et al., 2016; Gjerde et al., 2020). Nevertheless, it has documented that the mitochondrial COX1 sequence is considered to be a better marker than 18S rRNA, as COX1 gene could differentiate closely related *Sarcocystis* species in 18S rRNA analysis (Gjerde, 2013).

On the other hand, researchers compared the new sequences of the four genetic markers (18S rRNA, 28S rRNA, mitochondrial *COX1*, and *ITS-1)* for *S. tenella* and *S. arieticanis*, and confirmed that the ITS-1 region could be more useful for distinguishing closely related *Sarcocystis* spp owing to its high divergence (Hu et al., 2017). Also, El-Morsey et al. (2019) stated that the COX1 gene and the *ITS-1* sequences could be more accurate than the 18S rRNA and 28S rRNA genes for differentiating the closely related *Sarcocystis* spp. within the intermediate hosts because of their high divergence.

CONCLUSION

To the authors' knowledge, this is the first molecular and ultrastructural study of ovine and caprine *Sarcocystis* infection in Kurdistan, Iraq. The present findings showed that the isolated *Sarcocystis* spp. were most closely related to *S. gigantea*, *S. moulei*, and *S. medusiformis* and may consider them as sibling strains; the cross-infection may happen among sheep and goats, therefore, the host specificity of several *Sarcocystis* species is questionable. Further investigation with more precise gene markers like the mitochondrial *COX1* gene and the *ITS-1* sequences are recommended to differentiate the closely related species, besides transmission study within the proposed definitive host is needed to provide further clarification of the biology of this enigmatic genus of protozoan parasites.

DECLARATIONS

Authors' contributions

Sara Omar Swar performed the practical work and wrote the draft of the manuscript. Bushra Hussain Shnawa conceived the idea, supervised the study, as well as a manuscript final reading. All the authors approved and agreed to publish the manuscript.

Acknowledgments

The authors gratefully acknowledge Dr. Chanar Najmaddin Abdullah from the Biology Department, Science College, Salahaddin University, for her help in drawing the phylogenetic tree.

Competing interests

The authors have declared that no competing interest exists.

REFERENCES

Al-Hoot AS , Al-Qureishy SA, Al-Rashid K, and Bashtar AR (2005). Microscopic study on *Sarcocystis moulei* from sheep and goats in Saudi Arabia. Journal of the Egyptian Society of Parasitology, 35(1): 295-312. Available at: https://europepmc.org/article/med/15881014

Al-Hyali NS, Kennany ER, and Khalil LY (2011). Fate of macrosarcocyst of *Sarcocystis gigantea* in sheep. Iraqi Journal of Veterinary Sciences, 25(2): 87-91. Available at: http://www.vetmedmosul.org/ijvs

Bahari P, Salehi M, Seydabadi M, and Mohammadi A (2014). Molecular identification of macroscopic and microscopic cysts of *sarcocystis* in sheep in North Khorasan province, Iran. International Journal of Molecular and Cellular Medicine, 3(1): 51-56. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3927390/

Barham M, Stutzer H ,Karanis P , Latif B, and Neiss W (2005). Seasonal variation in *Sarcocystis* species infections in goats in northern Iraq. Parasitology, 130(2): 151-615. DOI: https://doi.org/10.1017/S0031182004006134

Bittencourt MV, Meneses IDS, Ribeiro-Andrade M, de Jesus RF, de Araújo FR, and Gondim LFP (2016). *Sarcocystis* spp. in sheep and goats: frequency of infection and species identification by morphological, ultrastructural, and molecular tests in Bahia, Brazil. Parasitology Research, 115 (4): 1683–1689. DOI: https://www.doi.org/10.1007/s00436-016-4909-5

Calero-Bernal R, Verma SK, Oliveira S, Yang Y, Rosenthal BM, and Dubey JP (2014). In the United States, negligible rates of zoonotic sarcocystosis occur in feral swine that, by contrast, frequently harbour infections with *Sarcocystis miescheriana*, a related parasite contracted from canids. Parasitology, 142(04): 549–556. DOI: https://www.doi.org/10.1017/s0031182014001553

Collins GH, Atkinson E, and Charleston WAG (1979). Studies on *Sarcocystis* species III: the macrocystic species of sheep. New Zealand Veterinary Journal, 27(10): 204–206. DOI: https://doi.org/10.1080/00480169.1979.34651

Dakhil HG, AbdallahB H, and Abdallah FA (2017). Molecular identification of *Sarcocystis fusiformis* and *S. moulei* infecting water buffaloes (*Bubalus bubalis*) in southern Iraq. World Journal of Pharmaceutical Research, 6(3): 215-229. DOI: https://doi.org/10.20959/wjpr20173-8013

Dubey JP, Calero Bernal R, Rosenthal BM, Speer CA, and Fayer R (2016). Sarcocystosis of animals and humans. 2nd Edn. Boca Raton: CRC Press; Taylor & Francis Group, DOI: https://doi.org/10.1201/b19184

Dubey JP (2015). Foodborne and waterborne zoonotic sarcocystosis. Food and Waterborne Parasitology, 1(1): 2–11. DOI: https://doi.org/10.1016/j.fawpar.2015.09.001

Dubey JP, Lane EP, Van Wilpe E, Suleman E, Reininghaus B, Verma SK, Rosenthal BM, and Mtshali MS (2014). *Sarcocystis cafferin.* sp. (Protozoa: Apicomplexa) from the African Buffalo (*Syncerus caffer*). Journal of Parasitology, 100(6): 817–827. DOI: https://doi.org/10.1645/13-467.1

Elmishmishy B, Al-Araby M, Abbas I, and Abu-Elwafa S (2018). Genetic variability within isolates of *Sarcocystis* species infecting sheep from Egypt. Veterinary Parasitology: Regional Studies and Reports, 13: 193–197. DOI: https://doi.org/10.1016/j.vprsr.2018.07.002

El-Morsey A, Abdo W, Sultan K, Elhawary NM, and AbouZaid AA (2019). Ultrastructural and Molecular Identification of the sarcocysts of *Sarcocystis tenella* and *Sarcocystis arieticanis* Infecting Domestic Sheep (Ovis aries) from Egypt. Acta Parasitologica, 64: 501–513. DOI: https://doi.org/ 10.2478/s11686-019-00070-8

Farhang-Pajuh F, Yakhchali M, and Mardani K (2014). Molecular determination of abundance of infection with *Sarcocystis* species in slaughtered sheep of Urmia, Iran. Veterinary Research Forum, 5(3): 181–186. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4279651/

Formisano P, Aldridge B, Alony Y, Beekhuis L, Davies E, Del Pozo J, and Beard PM (2013). Identification of *Sarcocystis capracanis* in cerebrospinal fluid from sheep with neurological disease. Veterinary Parasitology, 193, (1-3): 252–255. DOI: https://doi.org/ 10.1016/j.vetpar.2012.12.016

Ghaffar FA, Heydorn AO, and Mehlhorn H (1989). The fine structure of cysts of *Sarcocystis moulei* from goats. Parasitology Research, 75: 416–418. DOI: https://doi.org/10.1007/BF00931140

Gjerde B (2013) *Sarcocystis* species in red deer revisited: with a re-description of two known species as *Sarcocystis elongatan*. sp. and *Sarcocystis truncata* n. sp. based on mitochondrial sequences. Parasitology, 141(3): 441–452. DOI: https://doi.org/10.1017/s0031182013001819

Gjerde B, de la Fuente C, Alunda JM, and Luzón M (2020). Molecular characterization of five *Sarcocystis* species in domestic sheep (Ovis aries) from Spain. Parasitology Research. 119 (1): 215-231. DOI: https://doi.org/10.1007/s00436-019-06504-6.

Hong EJ, Sim C, Chae JS, Kim HC, Park J, Choi KS, Yu DH, Park CH, Yoo JG, and Park BK (2016). Ultrastructural and molecular identification of *Sarcocystis tenella* (Protozoa, Apicomplexa) in naturally infected Korean native goats. Veterinární Medicína, 61(7): 374–381. DOI: https://doi.org/10.17221/93/2015-vetmed

Hu J-J, Huang S, Wen T, Esch GW, Liang Y, and Li HL (2017). *Sarcocystis* spp. in domestic sheep in Kunming City, China: prevalence, morphology, and molecular characteristics. Parasite, 24: 30. DOI: https://doi.org/10.1051/parasite/2017025

Huang Z, Ye Y, Zhang H, Deng S, Tao J, Hu J, and Yang Y (2019). Morphological and molecular characterizations of *Sarcocystis miescheriana* and *Sarcocystis suihominis* in domestic pigs (Sus scrofa) in China. Parasitology Research, 118: 3491–3496. DOI: https://doi.org/10.1007/s00436-019-06521-5

Hussein DE, Abu-Akkada SS, Bessat MS, Aggour MG, and Otify YZ (2017). Molecular identification of *Sarcocystis* species in imported frozen beef in Egypt. Alexandria Journal of Veterinary Sciences, 53 (2): 72-82. DOI: https://doi.org/10.5455/ajvs.244449

Juyal PD, Ruprah NS, and Chhabra MB (1989). Rapid isolation of intact micro-*Sarcocystis* (Protozoa-Apicomplexa) cysts from muscular tissue. Indian Journal of Animal Health, 28(1): 69–70.

Kalantari N, Khaksar M, Ghaffari S, and Hamidekish SM (2016). Molecular Analysis of *Sarcocystis* Spp. Isolated from Sheep (*Ovis aries*) in Babol area, Mazandaran Province. Northern Iran. Iran Journal of Parasitology, 11(1): 73–80. Available at: https://pubmed.ncbi.nlm.nih.gov/27095971/

Kumar S, Stecher G, and Tamura K (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870–1874. DOI: https://doi.org/10.1093/molbev/msw054

Lindsay DS, and Dubey JP (2020). Neosporosis, Toxoplasmosis, and Sarcocystosis in Ruminants. The Veterinary Clinics of North America. Food Animal Practice, 36(1): 205–222. DOI: https://doi.org/10.1016/j.cvfa.2019.11.004

Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ, and Woese CR (1997). The RDP (Ribosomal Database Project). Nucleic Acids Research, 25(1): 109–111. DOI: https://doi.org/10.1093/nar/25.1.109

Metwally DM, Al-Damigh MA, Al-Turaiki IM and El-Khadragy MF (2019). Molecular characterization of *Sarcocystis* species isolated from sheep and goats in Riyadh, Saudi Arabia. Animals, 9(5): 256. DOI: https://doi.org/10.3390/ani9050256

Munday BL, and Obendorf DL (1984). Morphology of Sarcocystis gigantea in experimentally-infected sheep. Veterinary Parasitology, 16(3-4): 193–199. DOI: https://www.doi.org/10.1016/0304-4017(84)90036-0

Neefs JM, Van de Peer Y, Ruk PDE, Goris A, and Wachter RDE (1991). Compilation of small ribosomal subunit RNA sequences. Nucleic Acids Research, 19: 1987–2015.

DOI: https://www.doi.org/ 10.1093/nar/19.suppl.1987.

Ng YH, Fong MY, Subramaniam V, Shahari S, and Lau YL (2015). Short communication: Genetic variants of *Sarcocystis cruzi* in infected Malaysian cattle based on 18S rDNA. Research in Veterinary Science, 103: 201–204. DOI: https://www.doi.org/10.1016/j.rvsc.2015.10.009

To cite this paper: SO Swar and Shnawa BH (2021). Ultrastructural and Molecular Characterization of Sarcocystis Species Derived from Macroscopic Sarcocysts of Domestic Sheep and Goats in Soran City, Erbil, Iraq. *World Vet. J.*, 10 (4): 540-550. DOI: https://dx.doi.org/10.54203/scil.2020.wvj65

Obendorf DL and Munday BL (1987). Experimental infection with *Sarcocystis medusiformis* in sheep. Veterinary Parasitology, 24(1-2): 59–65. DOI: https://www.doi.org/10.1016/0304-4017(87)90130-0

Oryan A, Moghaddar N, and Gaur SNS (1996). The distribution pattern of *Sarcocystis* species, their transmission and pathogenesis in sheep in Fars Province of Iran. Veterinary Research Communications, 20(3): 243–253. DOI: https://www.doi.org/10.1007/bf00366922

Rubiola S, Chiesa F, Zanet S, and Civera T (2019). Molecular identification of *Sarcocystis* spp. in cattle: partial sequencing of Cytochrome C Oxidase subunit 1 (COI). Italian Journal of Food Safety, 7(4): 7725. DOI: https://doi.org/10.4081/ijfs.2018.7725

Whaeeb ST, and Faraj AA (2016). Molecular identification and phylogeny of microscopic *Sarcocystis* sheep in Baghdad Province. International Journal of Advanced Research in Biological Sciences, 3(12): 50-56. DOI: http://dx.doi.org/10.22192/ijarbs.2016.03.12.006

Yang Z, Zuo Y, Yao Y, Chen X, Yang G, and Zhang Y (2001). Analysis of the 18S rRNA genes of *Sarcocystis* species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species. Molecular and Biochemical Parasitology, 115(2): 283–288. DOI: https://www.doi.org/10.1016/s0166-6851(01)00283-3

To cite this paper: SO Swar and Shnawa BH (2021). Ultrastructural and Molecular Characterization of Sarcocystis Species Derived from Macroscopic Sarcocysts of Domestic Sheep and Goats in Soran City, Erbil, Iraq. *World Vet. J.*, 10 (4): 540-550. DOI: https://dx.doi.org/10.54203/scil.2020.wvj65

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 551-561, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj66

S-Methyl Cysteine Protective Effects in *Oreochromis Niloticus* Fish Contaminated by Thiobencarb Herbicide

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ORIGINAL ARTICLE
pii: S232245682000066-10
Received: 09 Oct 2020
Accepted: 28 Nov 2020

ABSTRACT

Thiobencarb which is a carbamate herbicide is used for managing undesirable weeds during rice cultivation in Egypt. This study was designed to investigate the adverse effects of a field dose of thiobencarb on Nile tilapia and ameliorating the role of the low dose of S-methyl cysteine (SMC). Experimental fishes were divided into four groups; first group was reared without any treatments and served as a control group; the second group was exposed to thiobencarb (36µg/L); the third group was fed on a commercial feed containing 200 mg of SMC/Kg in conjunction with thiobencarb added to aquarium (36µg/L) while, the fourth group was fed on a feed containing 200 mg of SMC/Kg only. Fishes were sacrificed at the end of the experimental course (two months) and sampling was carried out. Catalase, Glutathione S Transferase activities, Glutathione reduced, and Malondialdhyde levels were assayed. Genotoxic effect of thiobencarb and SMC on treated fish was investigated in erythrocytes, gills, and liver tissues using micronucleus and comet assay. Histopathological examination of livers, gills, and brain was also carried out. The results indicated that fish exposed to thiobencarb indicated herbicide dependent oxidative stress and genotoxic effect justified by a significant difference in antioxidant biomarkers as well as nuclear abnormalities and comet parameters compared to control values. Moreover, histopathological findings were in line with other results. SMC ameliorated the adverse effects which were effective in the improvement of DNA and oxidative damage in thiobencarb intoxicated fish.

Keywords: Carbamate, Fish, Genotoxic damage, Histopathology

INTRODUCTION

Potential exposure to the toxic chemicals has been progressively increased in the last decades. Scientists have increased their ecological researches in detecting and controlling the hazard chemical agents responsible for unacceptable damages to the ecosystems and human health (Munger et al., 1997; Gorell et al., 1998; Nwani et al., 2011; Nicolopoulou-Stamati et al., 2016). These agents may have adverse physiological, pathological or biochemical effects to living beings, some of them may possess a mutagenic potential which can express their action later in the next generation of the organisms (Anderson, 2005; Recio et al., 2010). Noxious weeds in common are the most remarkable and prevalent biological problem to crop production in rice fields which are commonly controlled by chemical herbicides (Sapari and Ismail, 2012; Hakim et al., 2015). These herbicides can strike their way to the water bodies through surface runoff (Phong et al., 2006; Papadakis et al., 2015) causing impacts on non-target organisms specially those living in the aquatic environment which differs according to degree of dispersion, concentration and toxicity of the herbicide (Van der Werf, 1996). The residues of these herbicides accumulate in the environment due to its widespread use over the last years which negatively damaged the aquatic organisms and living biota (Silva et al., 2019; Valadas et al., 2019).

Thiobencarb is a dithiocarbamate selective herbicide was developed by Kumiai Chem. Ind. Co. Ltd and introduced in the agricultural field to control the unwanted weeds in the rice fields in 1970 (Ishikawa et al., 1977). It has been used extensively by the farmers in Egypt particularly during the rice cultivation season. Previous studies have been conducted to assess the thiobencarb toxicity to the ecosystem (Bailey, 1993; Fernández-Vega et al., 1999; Saka, 2010).

Genotoxic and mutagenic effects of herbicides have been previously evaluated in several model organisms (Sanderson et al., 2001; Hladik et al., 2008; Benfeito et al., 2014) using several biomarkers for genotoxicity assessment, such as DNA adducts, chromosomal aberrations, DNA strands breaks, micronuclei formation and cell death induction (Stein et al., 1993; Sikka et al., 1990; Bombail et al., 2001). *Oreochromis niloticus* fish have been used in several studies as a model for genotoxicity investigation of various environmental pollutants (Lima et al., 2006; Kandiel et al., 2014; Bacolod et al., 2017). Several studies have been conducted implementing relatively simple methods to verify the mutagenic effects of the herbicides. The comet and micronuclei assays can be applied for evaluation of the genetic material damage in the experimental and field studies (Monteith and Vanstone, 1995). S-Methyl Cysteine (SMC) is an

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organosulfur compound naturally discovered in some plants particularly garlic (*Allium sativum*). A promising protective role of SMC had been prophesied as anti-apoptotic (Nasr et al., 2017), antidiabetic (Senthilkumar et al., 2013) and anticancer (Fukushima et al., 2001). However, a high dose of SMC may induce adverse effects (El-Magd et al., 2017). Present study, aimed to point out the genotoxicity of a field dose of thiobencarb on *Oreochromis niloticus* fish and its modulation by a low dose of SMC.

MATERIALS AND METHODS

Ethical approval

This study was conducted under the ethical approval from the Experimental Animal Care Committee in accordance with guidelines of Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

Chemicals

A technical grade of thiobencarb (95 % purity) from Kafrelzyat Chemicals Company, Egypt was used in presenta experimental study. S-methyl cysteine was purchased from Sigma-Aldrich.

Fish acquisition and maintenance

One hundred and sixty Nile tilapia (*Oreochromis niloticus*) fish were used for this study. The average weight of fishes was 30±3.5 g. The fish were purchased from a private fish farm in Kafrelsheikh governorate and then carefully transferred to the laboratory in water tanks filled with water from the fish collection source to avoid the stress of another water composition and temperature.

Aquariums and fish rearing

Fish were transferred to glass aquariums filled with dechlorinated water, each of them has 200-liter capacity. Fish were acclimatized to the lab conditions for 21 days. Fish were fed on commercial fish diet. Water was replaced every three days during the acclimation period.

Experimental design

Fish were divided into four groups; each group contains 40 fish and then each 40 fish divided into two aquariums (20 fish in each). Fish groups were treated as follows; first group (Control group), fish were exposed merely to dechlorinated tap water; second group, fish were exposed to 36 µg/L thiobencarb (this dose was determined as the average concentration of thiobencarb in 10 water samples collected from water drainage bodies near rice filed in Kafrelsheikh, Egypt using gas chromatography); third group, fish in each aquarium were provided every day with 15 g ration containing SMC (200 mg/Kg). Fourth group: fish were exposed to thiobencarb (36 µg/L) and fed on ration containing SMC (200 mg/kg). The experimental course endured 60 days. The water of all aquariums was replaced once a week during the experimental course. The particular herbicide concentration was supplied for the third and fourth groups whenever the water was changed. The laboratory conditions during the experimental period in common were Light period ~12hrs and temperature 25±2^{o}C.

Sampling

Fishes were sacrificed at the end of experimental course (two months) and sampling was carried out. Eight fish samples were randomly collected from each group (four samples from each aquarium) at the end of experiment. Fishes were sufficiently dried by paper towels. Heparinized blood samples were collected from the caudal vein puncture for micronuclei analysis. Then fish samples were dissected. Liver and gills samples were picked up and divided into three sufficient parts. First one preserved on PBS buffer solution at -80 ^{o}C for carrying out comet assay. Second part processed directly for antioxidants biomarkers assay and the third portion was properly preserved in formalin 10 % for histopathological examination.

Determining antioxidant biomarkers

Mincing and homogenization of Liver and gill samples was carried out in phosphate buffer saline and pH 7.4. The homogenate was centrifuged at 4,000 rpm for 20 minutes and supernatants were isolated for measuring antioxidant biomarkers level which include Catalase (CAT), Glutathione S Transferase (GST) activities, total Glutathione reduced (GSH), and Malondialdehyde (MDA) which were assayed by utilizing specified methods described by Beutler et al. (1963), Habig et al. (1974), Ohkawa et al. (1979) and Aebi (1984).

Micronuclei analysis

Heparinized blood samples were immediately smeared on clean glass slides and then stained with Giemsa stain. Micronuclei was assayed according to the previously described method (Cavaş and Ergene-Gözükara, 2003). The

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number of 1,000 clearly separate cells from the neihboring were scored from each slide by using light microscope (labomed, USA) under 100 × magnifications. Micronuclei were scored, if it was clearly separated from the nucleus (Al-Sabti and Metcalfe, 1995). Other nuclear abnormalities (blebbed nuclei, lobed nuclei, notched nuclei and binuclei) were recorded.

Comet assay

Single cell electrophoresis was carried out according to the published method (Singh et al., 1988). Briefly, 10 uL of fish liver or gills homogenate were thoroughly mixed with 90 uL of low melting point agarose (0.7 % in PBS) at 37º C. This prepared mixture was then added to a fully frosted microscope slide coated with 110 ul of normal melting point agarose (1 % in PBS). A cover slip was directly placed on top of the slide, and the agarose layer was allowed sufficiently to solidify for 10 minutes at 4º C. Afterwards, the cover slip was carefully removed and a second layer of low melting point agarose without cells was added gently, a cover slip was applied, and the slide was held at 4º C for five minutes to allow the agarose layer to solidify. After removal of the cover slip, the slides were placed in lysis buffer (2.5 mol/L NaCl, 100 mmol/L Na2EDTA, 10 mmol/L Tris, (pH 10)) with freshly added 1 % Triton X-100 and 10 % DMSO for at least one hour at 4º C. Later, slides were placed in the electrophoresis chamber and incubated with electrophoresis alkaline buffer (300 mmol/L NaOH, 1 mmol/L Na2EDTA, pH > 13) for 15 minutes at 4º C to allow DNA unwinding and the expression of alkali-labile DNA damage as strand breaks. Electrophoresis was for 30 minutes at 25 V and 300 mA. The slides were washed three times, with 0.4 mol/L Tris with pH 7.5 as neutralization buffer. Finally, slides were stained with 50 uL of ethidium bromide (2 mg/mL), covered with a cover slip and observed under 400 × magnification with a Optika Axioskop fluorescent microscope.

From each sample, 100 randomly selected cells, respectively, were photographed and scanned. The scanned images were analyzed with the comet score analysis system. For each cell, the length of DNA migration (tail length) was measured in PX from the center of the nucleus to the end of the tail. The percentage of DNA in the tail was determined by measuring the total intensity (fluorescence) in the cells. The tail moment, expressed in arbitrary units which was calculated as tail length multiplied by percentage of migrated DNA/100.

Histopathological examination

Samples from the liver, gills and brain were collected from fish of various groups. The samples were fixed in 10 % neutral buffered formalin, dehydrated in alcohol, cleared in xylene, embedded in paraffin and sectioned in 5 µm thickness. Then serial sections were subjected to routine hematoxylin and eosin staining (Bancroft and Layton, 2013).

Statistical analysis

The data was analyzed by ANOVA procedures of the IBM SPSS software program (Version 16.0; IBM Corp., NY, USA). The differences between different means were estimated by the Duncan Multiple Range Test (DMRT). The differences among means with $p < 0.05$ were represented statistically as significant differences.

RESULTS

Antioxidant biomarkers

Catalase, GST activities, GSH and MDA level were demonstrated in homogenate samples from liver and gills of fish of all experimental groups. The results were presented in Figure 1. The CAT activity was significantly ($P<0.05$) decreased in both hepatic (3.38) and gill (3.32) tissues of thiobencarb treated fish compared to control fish (4.49) and (4.37), respectively. The inhibition of CAT activity was ameliorated by SMC concurrently with thiobencarb which was more prominent in gills than liver. GST activity was significantly elevated in thiobencarb intoxicated fish gills (0.44) and liver (0.36) compared to the control values (0.29) and (0.18), respectively. This elevation was diminished with SMC provided fish. GSH and MDA levels were increased significantly ($P<0.05$) in the gills and hepatic tissues of intoxicated fish over the control non treated fish. SMC provided in fish ration decreased the level of GSH and MDA compared to the intoxicated fish. Antioxidant biomarkers that measured in the fourth group were evidence to antioxidant system improvement by SMC.

Micronuclei analysis

The small non refractive parts that present in cytoplasm and are similar to the nucleus in staining is recorded as micronucleus, it is usually one particle fully separated from the nucleus. The determined percentage of micronuclei was significantly increased ($P<0.05$) in the second fish group which treated with thiobencarb, compared to the control group. The increased micronuclei level due to thiobencarb was relieved by SMC in the third group compared to the first and second group. Nuclear abnormalities were also recorded in the examined samples (Table 1). Binucleated cells and abnormal nuclei were significantly increased ($P<0.05$) in fish treated with thiobencarb compared to the control group, fish treated with SMC and thiobencarb revealed improvement in the effect on erythrocyte nuclei. The SMC provided groups revealed non-significant decrease ($P>0.05$) in nuclear abnormalities compared to the control group.

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Table 1. Nuclear abnormalities in erythrocytes of *Oreochromis niloticus* exposed to thiobencarb (36µg/L) and SMC (200 mg/kg ration) compared with control non treated fish

Parameters \ Groups	Control	Thiobencarb	Thiobencarb + SMC	SMC
Micronucleus (%)	0.032±0.0011^{b}	0.040±0.0015^{a}	0.033±0.0012^{b}	0.030±0.0009^{b}
Binucleated	0.020±0.0012^{c}	0.037±0.0005^{a}	0.026±0.0009^{b}	0.018±0.0011^{c}
Abnormal Nuclei	0.012±0.0008^{c}	0.043±0.0012^{a}	0.023±0.0012^{b}	0.012±0.0009^{c}

* Means with different superscripts in each row are significantly differ at P < 0.05. SMC means S-Methyl Cysteine

Comet assay

The DNA damage in liver and gills tissues of *Oreochromis niloticus* exposed to thiobencarb and the potential effect of SMC treatment was investigated by using comet assay. The observed results were presented in Table 2 and Figure 2. Regarding to the comet parameters in control fish group gills, Comet%, Head Diameter, DNA % Head, Tail Length, DNA % Tail and Tail Moment were 13.99 %, 20.29, 82.3 %, 5.8, 19.32 % and 1.34, respectively. Fish group treated with thiobencarb indicated a significant increase (P<0.05) in the values of Comet % (18.45 %), Head Diameter (25.5), Tail Length (7.59), DNA % Tail (23.5 %), Tail Moment (2.47) and significant decrease in DNA % Head value (76.91 %). The fish group treated with SMC in conjunction with thiobencarb indicated significantly ameliorated DNA damage effects exerted on gill tissue compared to thiobencarb treated fish group. the comet criteria in the fourth fish group which treated with SMC alone represented non significantly difference (P>0.05) with control values of Comet % (13.2 %) and DNA %Head (82.17 %), while significant difference (P<0.05) in Head Diameter (18.28), Tail Length (5.26), DNA %Tail (18.02 %) and Tail Moment (1 %) was observed which indicated that the DNA damage in SMC group was rarely than the control group. Observational data of comet parameters evaluating the DNA damage effects in hepatic cells of thiobencarb and SMC treated fishes clarified a significant increase (P<0.05) in Comet % (13.6%), Head Diameter (24.44), Tail Length (7.07), DNA % Tail (22.7%) and Tail Moment (2.22) compared to the control group values including Comet % (12.4 %), Head Diameter (20.87), Tail Length (4.72), DNA % Tail (19.94 %) and Tail Moment (1.35) and significant decrease (P<0.05) in DNA % Head (75.82 %) compared to control (79.63 %) value. A repair in DNA damage was observed in fish treated with Thiobencarb+SMC which were evidenced by improved comet values in this group compared to the thiobencarb treated group. All comet variables for hepatic cells of fish treated with SMC exhibited non significantly differences (P>0.05) with the same parameters in the control group.

Histopathological findings

The histopathological findings were illustrated in Figure 3 and scoring of the lesions was mentioned in Table 3. The liver of the control fish presented normal hepatic and pancreatic tissues. The hepatic tissues consisted of normal hepatocytes with irregular hepatic vacuolation consistent with physiological glycogen storage. The pancreatic tissue was distributed within the hepatic tissues and enclosed venous blood supply. The thiobencarb treated fish presented marked decrease in hepatic vacuolation, marked increase in number of hepatocytes within the examined field, hyperplasia and single cell necrosis within the pancreatic portion. The SMC treated fish had normal hepatic and pancreatic tissues. The Thiobencarb and SMC treated fish demonstrated marked decrease in mitogenic effect which represented with decrease in number of hepatocytes. The gills of control fish revealed normal primary and secondary gill lamellae. Marked loss of secondary gill lamellae associated with necrosis of the lamellae, infiltration of leukocytes and proliferation of goblet cells were noticed within the gills of the intoxicated group. The gills of SMC treated fish were within the normal limits. The intoxicated and SMC treated fish revealed marked decrease in the adhesion of the secondary lamellae and appearance of the secondary gill lamellae. The brain of the normal fish indicated normal nerve cells and nerve fibers. The thiobencarb treated fish revealed multifocal malacia associated with marked gliosis and appearance of gitter-like cells. The brain of SMC treated was normal. The brain of thiobencarb intoxicated fish and SMC treated revealed limited malacic foci with marked decrease in the glia and gitter cells.

Table 2. DNA damage assessed by comet assay in liver and gills of *Oreochromis niloticus* exposed to thiobencarb (36 µg/L) and SMC (200 mg/kg ration) compared with control non treated fish

	Groups	Comet %	Head diameter	DNA % Head	Tail length	DNA %Tail	Tail moment
Gills	Control	13.99±0.155^{c}	20.29±0.631^{c}	82.30±0.994^{a}	5.80±0.119^{c}	19.32±0.196^{c}	1.34±0.054^{b}
	Thiobencarb	18.45±0.370^{a}	25.50±0.350^{a}	76.91±0.601^{c}	7.59±0.143^{a}	23.50±0.407^{a}	2.47±0.134^{a}
	Thiobencarb + SMC	15.90±0.448^{b}	22.64±0.495^{b}	79.96±0.275^{b}	6.40±0.039^{b}	21.28±0.292^{b}	1.47±0.112^{b}
	SMC	13.20±0.465^{c}	18.28±0.362^{d}	82.17±0.365^{a}	5.26±0.067^{d}	18.02±0.185^{d}	1.00±0.034^{c}
Liver	Control	12.04±0.168^{bc}	20.87±0.540^{b}	79.63±1.144^{ab}	4.72±0.335^{bc}	19.94±0.495^{bc}	1.35±0.052^{c}
	Thiobencarb	13.60±0.240^{a}	24.44±0.342^{a}	75.82±1.160^{c}	7.07±0.121^{a}	22.07±0.525^{a}	2.22±0.109^{a}
	Thiobencarb + SMC	12.47±0.428^{b}	20.31±0.781^{b}	78.76±0.622^{b}	5.53±0.455^{b}	20.39±0.398^{b}	1.80±0.062^{b}
	SMC	11.50±0.212^{c}	19.08±0.672^{b}	82.23±0.613^{a}	4.27±0.418^{c}	18.67±0.381^{c}	1.12±0.064^{c}

* Means with different superscripts in each column are significantly differ at P < 0.05. SMC means S-Methyl Cysteine

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Table 3. Semi quantitative scoring of the lesions within the liver, gills and kidney of *Oreochromis niloticus* exposed to thiobencarb (36µg/L) and SMC (200 mg/kg ration) compared with control non treated fish

Organs / Groups	Liver		Gills		Brain	
	Degeneration	Hyperplasia	Adhesion	Inflammation	Malacia	Inflammation
Control	-	-	-	-	-	-
Thiobencarb	++	++++	++++	+++	++++	+++
SMC	-	-	-	-	-	-
SMC+Thiobencarb	+	++	++	+	+	+

+, ++, +++ and ++++ indicate mild, moderate, severe and focal, severe and diffuse lesions respectively. SMC means S-Methyl Cysteine

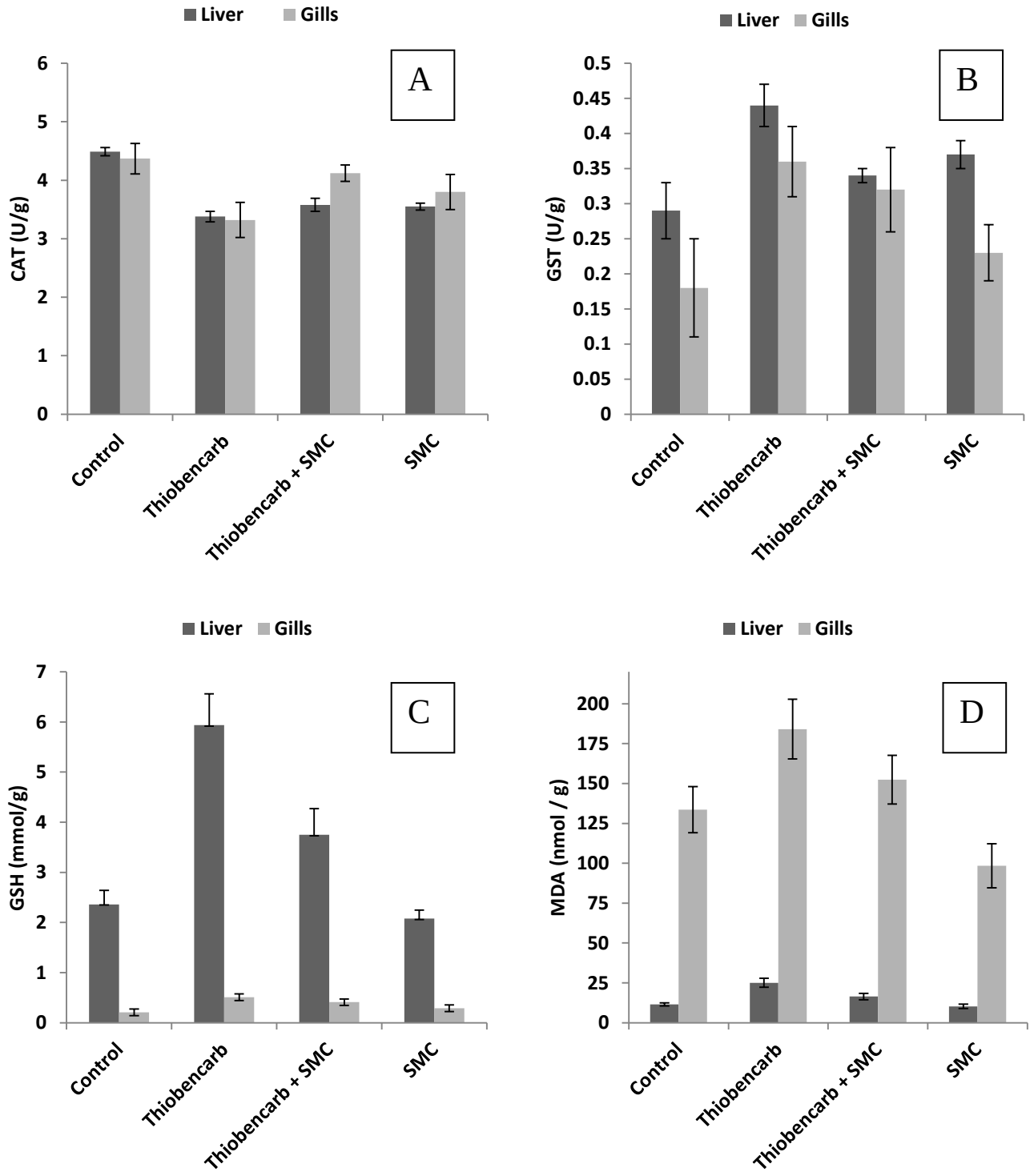

Figure1. Antioxidant biomarkers values in experimental fish (*Oreochromis niloticus*) groups A, B, C and D refers to Catalase, Glutathione S Transferase, Glutathione reduced and Malondialdhyde respectively. SMC refers to S-Methyl Cysteine

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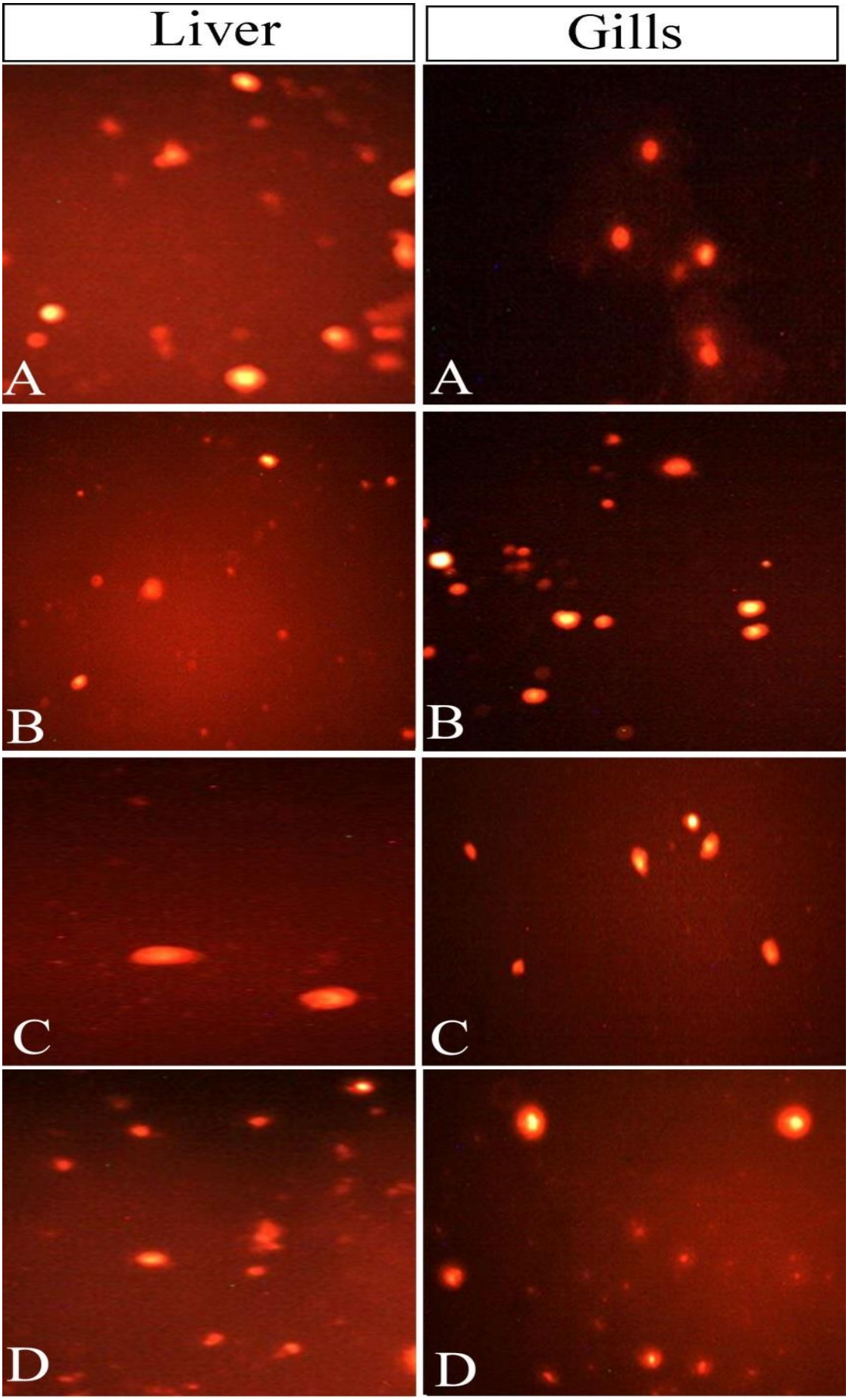

Figure 2. Photomicrograph by using comet assay showing the different degrees of DNA damage in liver and gills of *Oreochromis niloticus* exposed to thiobencarb (36µg/L) and SMC (200 mg/kg ration) compared with control non treated fish. (A), (B), (C), (D) refers to control, Thiobencarb, Thiobencarb+ S-Methyl Cysteine and S-Methyl Cysteine treated fish groups respectively. (Ethidium bromide X40)

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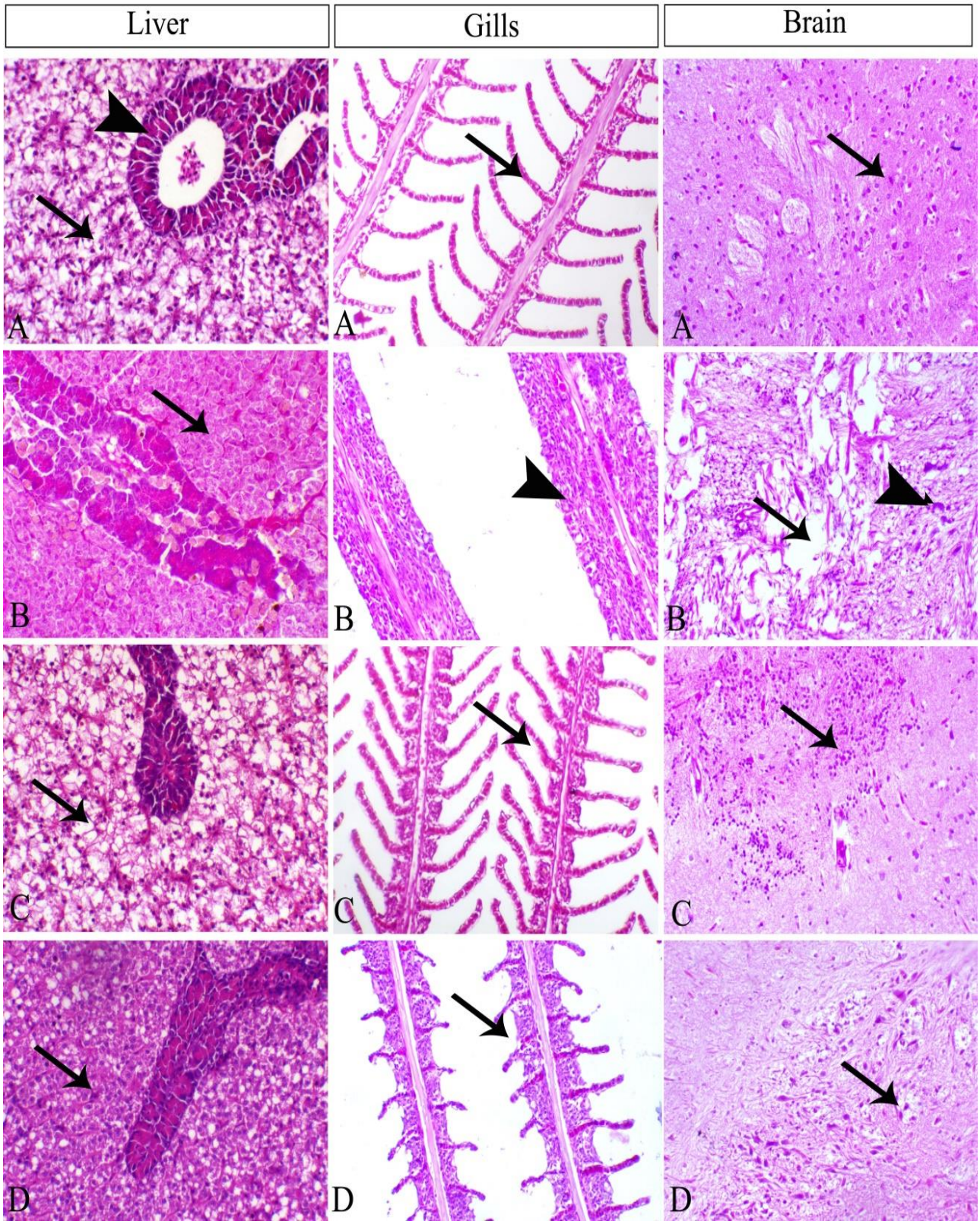

Figure 3. Histopathological changes in liver, gills and brain of *Oreochromis niloticus* exposed to thiobencarb (36µg/L) and SMC (200 mg/kg ration) compared with non-treated fish. The liver of control fish (A) showing normal hepatic (arrow) and pancreatic tissues (arrowhead) (the hepatocytes revealing normal vacuolation consistent with glycogen storage), the liver of thiobencarb-treated fish (B) showing marked increase of hepatocytes indicated with increase the number of nuclei (arrow), the liver of normal fish treated with SMC (C) showing normal hepatic (arrow) and pancreatic tissues, and the liver of thiobencarb and SMC-treated fish (D) showing marked decrease the number of the proliferated hepatocytes (arrow), H&E, X200. The gills of control fish (A) showing secondary gill lamellae (arrow), the gill of thiobencarb-treated fish (B) showing marked loss and adhesion of the secondary lamellae (arrow) with increased number of mucous cells, the gill of normal fish treated with SMC (C) showing lamellae tissues (arrow), and the gills of thiobencarb and SMC-treated fish (B) showing marked decrease the adhesion of the secondary lamellae (arrow), H&E, X200. The brain (telencephalon portion) of control fish (A) showing normal neuronal cells and with normal nerve fibers (arrow), the brain of thiobencarb-treated fish (B) showing malacia (arrow) and gliosis (arrowhead), the brain of normal fish treated with SMC (C) showing neuronal tissues with normal glia cells (arrow), and the brain of thiobencarb and SMC-treated fish (B) showing marked decrease the necrotic changes and neuronal vacuolation (arrow), H&E, X200.

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DISCUSSION

Aquatic environments contamination level with various chemicals has been increased in Egypt during the last years, which may be attributed to the intensive use of chemicals in the agricultural field (Dahshan et al., 2016). Chemical herbicides are a group of widely spread pesticides used for control of exotic weeds, which may be selective or non-selective. Thiobencarb is one of the intensively used selective herbicides in Egypt, which used primarily for the weeds emerging in the rice fields (Tanetani et al., 2013). Furthermore, the uncontrolled use of these chemicals during rice cultivation season poses a crucial source of aquatic environments pollution (Sapari and Ismail, 2012), it finds their way to the near water bodies through the surface runoff (Phong et al., 2006; Papadakis et al., 2015) causing adverse impacts on aquatic livings ranging from mild effects to life threatening ones (Bailey, 1993).

Fish are one of the most important aquatic organisms affected by water chemical pollution (Sommer, 1983). Moreover, it has been considered as a highly sensitive indicator for aquatic environment contamination (Singh et al., 1988). However, previous studies pointed out the adverse effects of water chemical pollutants particularly herbicides in different fish species (Nwani et al., 2011). *Oreochromis niloticus* was selected as an indicator for water pollution (Bacolod et al., 2017). Additionally, fish represent different adverse effects from thiobencarb herbicides (Bailey, 1993; Fernández-Vega et al., 1999; Saka, 2010).

This study pointed out the genotoxic effect of thiobencarb exerted on erythrocytes of *Oreochromis niloticus* which was obvious by significantly increased ($P<0.05$) micronuclei and nuclear abnormalities in thiobencarb treated fish. Moreover, the genotoxic effect was cleared in hepatic and gill tissues by significant increase ($P<0.05$) of comet parameters (Comet %, Head Diameter, Tail Length, DNA %Tail and Tail Moment) and significant decrease ($P<0.05$) of DNA %Head in thiobencarb treated fish compared to control group. Genotoxic effect may be attributed to the oxidative stress of the herbicide (Lima et al., 2006; Bacolod et al., 2017) which was ensured by significantly different antioxidant biomarkers in present results. Furthermore, this adverse toxic effect was reflected on the results of histopathological examination of liver, gills and brain of thiobencarb exposed fish. Hepatic section showed hyperplasia and single cell necrosis, while gills examination revealed marked loss of secondary gill lamellae associated with extensive necrosis of the lamellae, infiltration of leukocytes and proliferation of goblet cells. Also, multifocal malacia associated with marked gliosis and appearance of gitter-like cells was observed in brain tissue of intoxicated fish. The present study results were in line with Elias et al. (2020).

Herbicides induces oxidative stress remains the principal way for production of Reactive Oxygen Species (ROS) which may provoke oxidative damage to DNA, protein and lipids. The ROS detoxification is done by antioxidant system (Fan et al., 2013). Therefore, ROS detoxification failure prompts serious implication on cellular structure, function and life (Bacolod et al., 2017) which was clearly seen in tested parameters of thiobencarb intoxicated fish. The gills are the largest contacting organ of fish with the environment, which play the prime role in fish respiration, osmoregulation, and considered as a crucial part in fish excretion system (Simonato et al., 2008). However, the gills represent the first chief organ critically affected by water contamination, from this stand point the results revealed an elevation in GST activity and GSH level in the gills of intoxicated fish, which means that GST as well as GSH had an important role in thiobencarb detoxification.

SMC is an organosulfur compound naturally found in some plants particularly garlic (*Allium sativum*). A promising protective role of SMC had been prophesied as anti-apoptotic (Nasr et al., 2017), antidiabetic (Senthilkumar et al., 2013) and anticancer (Fukushima et al., 2001). Sulfur is a crucial component of various cellular proteins impacting the cell health particularly by enzymatic and non-enzymatic antioxidant molecules like glutathione and thioredoxin (Atmaca, 2004). In present study, fish exposed to thiobencarb and fed on ration containing SMC presented a curing effect based on the antioxidant biomarkers. The CAT activity was elevated, GST activity was corrected and MDA level was decreased, while sulfur dependent GSH was elevated after treatment with SMC compared with thiobencarb intoxicated fish. SMC ameliorated the oxidative DNA damage based on the correction of erythrocytes micronucleus and abnormal nuclei frequencies and comet parameters. Moreover, histopathological finding in gills, liver and brain of thiobencarb intoxicated fish were disappeared in SMC treated fish.

CONCLUSION

Oreochromis niloticus were sensitive to thiobencarb contamination which was explained by genotoxic and oxidative damage effects. Moreover, histopathological changes related to herbicide impact were recorded. These adverse toxic effects were ameliorated by S-Methyl Cysteine (SMC). Therefore, controlling of thiobencarb which is used as herbicide in rice fields should be done wisely. In addition, careful monitoring of thiobencarb should be carried out to minimize its adverse impacts on the aquatic ecosystems. S-Methyl Cysteine would be helpful for alleviating the toxic effects exerted by thiobencarb on *Oreochromis niloticus*. This study should be supported by more field studies to point out the effects of natural factors of aquatic environment on the use of SMC.

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DECLARATIONS

Acknowledgments

The authors would like to thank Mahmoud S.El-Tarabany, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt for his assistance in statistical analysis of this study.

Competing interests

The authors have not declared any conflict of interest.

Author's contribution

Mostafa Elmadawy, Walied Abdo, Amira Omar, and Nadia Mahfouz designed the study. Mostafa Elmadawy and Amira Omar performed the experiments. Walied Abdo and Nadia Mahfouz supervised the laboratory examinations. Histopathological examination was carried out by Walied Abdo. All authors prepared, read and approved the final manuscript.

Consent to publish

Not applicable

REFERENCES

Aebi H (1984). Catalase in vitro. Methods in Enzymology, 105: 121-126. DOI: https://doi.org/10.1016/S0076-6879(84)05016-3.

Al-Sabti K, and Metcalfe CD (1995). Fish micronuclei for assessing genotoxicity in water. Mutation Research/Genetic Toxicology, 343(2-3): 121-135. DOI: https://doi.org/10.1016/0165-1218(95)90078-0

Anderson D (2005). Male-mediated developmental toxicity. Toxicology and Applied Pharmacology, 207: 506–513. DOI: https://doi.org/10.1016/j.taap.2005.01.022

Atmaca G (2004). Antioxidant effects of sulfur-containing amino acids. Yonsei Medical Journal, 45: 776–788. DOI: https://doi.org/10.3349/ymj.2004.45.5.776

Bacolod ET, Uno S,Villamor SS, and Koyama J (2017). Oxidative stress and genotoxicity biomarker responses in tilapia (*Oreochromis niloticus*) exposed to environmental concentration of 1-nitropyrene. Marine Pollution Bulletin, 124(2): 786-791. DOI: https://doi.org/10.1016/j.marpolbul.2017.01.077

Bailey HC (1993). Acute and Chronic Toxicity of the Rice Herbicides Thiobencarb and Molinate to Opossum Shrimp (*Neomysis mercedis*). Marine Environmental Research, 36: 197-215. DOI: https://doi.org/10.1016/0141-1136(93)90089-I

Bancroft JD, and Layton C (2013). The haemtoxylins and eosin. Bancroft's Theory and practice of histological techniques, Expert consult: Online and print, 7: Bancroft's Theory and practice of histological techniques, p. 173.

Benfeito S, Silva T, Garrido J, Andrade PB, Sottomayor MJ, Borges F, and Garrido EM (2014). Effects of Chlorophenoxy Herbicides and Their Main Transformation Products on DNA Damage and cetylcholinesterase Activity. BioMed Research International, 2014: 709036. DOI: https://doi.org/10.1155/2014/709036

Beutler E, Duron O, and Kelly BM (1963). Improved method for the determination of blood glutathione. The Journal of laboratory and clinical medicine, 61: 882–888. PMID: 13967893.

Bombail V, Dennis AW, Gordon E, and Batty J (2001). Application of the comet and micronucleus assays to butterfish (*Pholis gunnellus*) erythrocytes from the Firth of Forth, Scotland. Chemosphere, 44: 383-392. DOI: https://doi.org/10.1016/S0045-6535(00)00300-3

Cavaş T, and Ergene-Gözükara S (2003). Micronuclei, nuclear lesions and interphase silver-stained nucleolar organizer regions (AgNORs) as cyto-genotoxicity indicators in *Oreochromis niloticus* exposed to textile mill effluent. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 538(1-2): 81-91. DOI: https://doi.org/10.1016/S1383-5718(03)00091-3

Dahshan H, Megahed AM, Abd-Elall AM, Abd-El-Kader MA, Nabawy E, and Elbana MH (2016). Monitoring of pesticides water pollution-The Egyptian River Nile. Journal of Environmental Health Science and Engineering, 14: 15. DOI: https://doi.org/10.1186/s40201-016-0259-6

Elias NS, Abouelghar GE, Sobhy HM, El Miniawy HM, and Elsaiedy EG (2020). Sublethal effects of the herbicide thiobencarb on fecundity, histopathological and biochemical changes in the African catfish (*Clarias gariepinus*). Iranian Journal of Fisheries Sciences, 19(3): 1589-1614. DOI: https://doi.org/10.22092/ijfs.2018.119669

El-Magd MA, Abdo WS, El-Maddaway M, Nasr NM, Gaber RA, El-Shetry ES, Saleh AA, Alzahrani FAA, and Abdelhady DH (2017). High doses of S-methylcysteine cause hypoxia-induced cardiomyocyte apoptosis accompanied by engulfment of mitochondaria by nucleus. Biomedicine & Pharmacotherapy, 94: 589-597. DOI: https://doi.org/10.1016/j.biopha.2017.07.100

Fan JY, Geng JJ, Ren HQ, Wang XR, and Han C (2013). Herbicide Roundup and its main constituents cause oxidative stress and inhibit acetylcholinesterase in liver of Carassius auratus. Journal of Environmental Science and Health B, 48 (10): 844–850. https://doi.org/10.1080/03601234.2013.795841.

Fernández-Vega C, Sancho E, Ferrando MD, and Andreu-Moliner E (1999). Thiobencarb toxicity and plasma AChE inhibition in the European eel. Journal of Environmental Science and Health, Part B Pesticides, Food Contaminants and Agricultural Wastes, 34(1): 61-73. DOI: https://doi.org/10.1080/03601239909373184

Fukushima S, Takada N, Wanibuchi H, Hori T, Min W, and Ogawa M (2001). Suppression of chemical carcinogenesis by water-soluble organosulfur compounds. The Journal of Nutrition, 131(3s): 1049S–1053S. DOI: https://doi.org/10.1093/jn/131.3.1049S

Gorell JM, Jhonson CC, Rybicki BA, Peterson EL, and Ricchardson RJ (1998). The Risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. Neurology, 50(5): 1346-1350. DOI: https://doi.org/10.1212/WNL.50.5.1346

Habig WH, Pabst MJ, and Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. The Journal of Biological Chemistry, 25; 249(22): 7130-7139. Available at: https://www.jbc.org/content/249/22/7130.long

Hakim MA, Juraimi AS, Hanafi MM, Rafii MY, Ismail MR, Karim SM, and Kausar H (2015). Integration of herbicides with manual weeding for controlling the weeds in rice under saline environment. Journal of Environmental Biology, 36(6): 1311-1317. Available at: http://www.jeb.co.in/journal_issues/201511_nov15/paper_10.pdf

Hladik ML, Bouwer EJ, and Roberts AL (2008). Neutral degradates of chloroacetamide herbicides: Occurrence in drinking water and removal during conventional water treatment. Water Research, 42(20): 4905-4914. DOI: https://doi.org/10.1016/j.watres.2008.09.008

Ishikawa K, Nakamura Y, and Kuwatsuka S (1977). Photodegradation of benthiocarb herbicide. Journal of Pesticide Science, 2: 17-25. Available at: https://www.jstage.jst.go.jp/article/jpestics1975/2/1/2_1_17/_pdf

Kandiel MM, El-Asely AM, Radwan HA, and Abbass AA (2014). Modulation of genotoxicity and endocrine disruptive effects of malathion by dietary honeybee pollen and propolis in Nile tilapia (*Oreochromis niloticus*). Journal of Advanced Research, 5(6): 671–684. DOI: https://doi.org/10.1016/j.jare.2013.10.004

Lima PL, Benassi JC, Pedrosa RC, Dal Magro J, Oliveira TB, and Wilhelm Filho D (2006). Time-course variations of DNA damage and biomarkers of oxidative stress in tilapia (*Oreochromis niloticus*) exposed to effluents from a swine industry. Archives of Environmental Contamination and Toxicology, 50(1): 23-30. DOI: https://doi.org/10.1007/s00244-004-0178-x

Monteith DK, and Vanstone J (1995). Comparison of the microgel electrophoresis assay and other assays for genotoxicity in the detection of the DNA damage. Mutation Research, 345(3-4): 97-103. DOI: https://doi.org/10.1016/0165-1218(95)90045-4

Munger R, Isacson P, Hu S, Burns T, Hanson J, Lynch CF, Cherryholmes K, Vandorpe P, and Hausler Jr WJ (1997). Intrauterine growth retardation in Iowa communities with herbicides-contaminated drinking water supplies. Environmental Health Perspectives, 105: 308-314. DOI: https://doi.org/10.1289/ehp.97105308

Nasr EN, Elmadawy MA, Almadaly EA, Abdo W, and Zamel MM (2017). Garlic Powder Attenuates Apoptosis Associated with Lead Acetate-Induced Testicular Damage in Adult Male Rats. Alexandria Journal of Veterinary Sciences, 54(1): 70-78. DOI: https://doi.org/10.5455/ajvs.268149

Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, and Hens L (2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Frontiers in Public Health, 18(4): 148. DOI: https://doi.org/10.3389/fpubh.2016.00148

Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Kumar P, and Lakra WS (2011). Mutagenic and genotoxic assessment of atrazine-based herbicide to freshwater fish *Channa puntatus* (Bloch) using micronucleus test and single cell gel electrophoresis. Environmental Toxicology and Pharmacology, 31: 314-322. DOI: https://doi.org/10.1016/j.etap.2010.12.001

Ohkawa H, Ohishi N, and Yagi K (1979). Assay for lipid peroxides in animal tissues by Thiobarbituric acid reaction. Analytical Biochemistry, 95(2): 351-358. DOI: https://doi.org/10.1016/0003-2697(79)90738-3

Papadakis EN, Vryzas Z, Kotopoulou A, Kintzikoglou K, Makris KC, and Papadopoulou-Mourkidou E (2015). A pesticide monitoring survey in rivers and lakes of northern Greece and its human and ecotoxicological risk assessment. Ecotoxicology and Environmental Safety, 116: 1-9. DOI: https://doi.org/10.1016/j.ecoenv.2015.02.033

Phong TK, Nguyen MH, Komany S, Vu SH, and Watanabe H (2006). Alternative water management for controlling simetryn and thiobencarb runoff from paddy fields. Bulletin of Environmental Contamination and Toxicology, 77(3): 375-382. DOI: https://doi.org/10.1007/s00128-006-1076-x

Recio L, Hobbs C, Caspary W, and Witt KL (2010). Dose-Response Assessment of Four Genotoxic Chemicals in a Combined Mouse and Rat Micronucleus and Comet Assay Protocol. The Journal of Toxicological Sciences, 35(2): 149-162. DOI: https://doi.org/10.2131/jts.35.149

Saka M (2010). Acute toxicity of rice paddy herbicides simetryn, mefenacet, and thiobencarb to *Silurana tropicalis* tadpole. Ecotoxicology and Environmental Safety, 73(6): 1165-1169. DOI: https://doi.org/10.1016/j.ecoenv.2010.05.008

Sanderson JT, Letcher RJ, Heneweer M, Giesy JP, and Van den Berg M (2001). Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. Environmental Health Perspectives, 109(10): 1027–1031. DOI: https://doi.org/10.1289/ehp.01109102712

Sapari P, and Ismail BS (2012). Pollution levels of thiobencarb, propanil, and pretilachlor in rice fields of the muda irrigation scheme, Kedah, Malaysia: Environmental Monitoring and Assessment, 184(10): 6347-6356. DOI: https://doi.org/10.1007/s10661-011-2424-9.

Senthilkumar PS, Thomas S, Sivaraman K, Sankar P, and Bobby Z (2013). Study the effect of s-methyl L-cysteine on lipid metabolism in an experimental model of diet induced obesity. Journal of Clinical and Diagnostic Research, JCDR 7 (11): 2449–2451. DOI: https://doi.org/10.7860/JCDR/2013/7304.3571.

Sikka HC, Rutkowski JP, Kandaswami C, Kumar S, Earley K, and Gupta RC (1990). Formation and persistence of DNA adducts in the liver of brown bullheads exposed to benzo[a]pyrene, Cancer Letters, 49: 81-87. DOI: https://doi.org/10.1016/0304-3835(90)90142-k.

Silva V, Mol HGJ, Zomer P, Tienstra M, Ritsema CJ, and Geissen V (2019). Pesticide residues in European agricultural soils - A hidden reality unfolded. Science of the Total Environment, 25;653: 1532-1545. DOI: https://doi.org/10.1016/j.scitotenv.2018.10.441.

Simonato JD, Guedes CLB, and Martinez CBR (2008). Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. Ecotoxicology and Environmental Safety, 69: 112–120. DOI: https://doi.org/10.1016/j.ecoenv.2007.01.012.

Singh NP, McCoy MT, Tice RR, and Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, 175: 184–191. DOI: https://doi.org/10.1016/0014-4827(88)90265-0.

To cite this paper: Elmadawy MA, Abdo W, Omar AA and Mahfouz NB (2021). S-Methyl Cysteine Protective Effects in *Oreochromis Niloticus* Fish Contaminated by Thiobencarb Herbicide. *World Vet. J.,* 10 (4): 551-561. DOI: https://dx.doi.org/10.54203/scil.2020.wvj66

Sommer TR (1983). Laboratory and field studies on the toxic effects of thiobencarb (bolero) to the crawfish *Procambarus clarkia*. Journal of the World Mariculture Society, 14: 434-440. DOI: https://doi.org/10.1111/j.1749-7345.1983.tb00095.x

Stein JE, Reichert WL, French B, and Varanasi U (1993). 32p-postlabeling analysis of DNA adduct formation and persistence in English sole exposed to benzo[a]pyrene and 7H-dibenzo(c,g)-carbazole. Chemico-Biological Interactions, 88: 55-69. DOI: https://doi.org/10.1016/0009-2797(93)90084-c.

Tanetani Y, Kaku K, Ikeda M, and Shimizu T (2013). Action mechanism of a herbicide, thiobencarb. Journal of Pesticide Science, 38(1): 39–43. DOI: https://doi.org/10.1584/jpestics.D12-047.

Valadas J, Mocelin R, Sachett A, Marcon M, Zanette RA, Dallegrave E, Herrmann AP, and Piato A (2019). Propiconazole induces abnormal behavior and oxidative stress in zebrafish. Environmental Science and Pollution Research, 26(27): 27808-27815. DOI: https://doi.org/10.1007/s11356-019-05977-3.

Van der Werf HMG (1996). Assessing the impact of pesticides on the environment. Agriculture, Ecosystems and Environment, 60: 81-96. DOI: https://doi.org/10.1016/S0167-8809(96)01096-1.

To cite this paper: Elmadawy MA, Abdo W, Omar AA and Mahfouz NB (2021). S-Methyl Cysteine Protective Effects in *Oreochromis Niloticus* Fish Contaminated by Thiobencarb Herbicide. *World Vet. J.,* 10 (4): 551-561. DOI: https://dx.doi.org/10.54203/scil.2020.wvj66

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 564-572, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj67

Microbiological Studies on Naturally Present Bacteria in Camel and Buffalo Milk

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ORIGINAL ARTICLE
pii: S232245682000067-10
Received: 30 Sept 2020
Accepted: 13 Nov 2020

ABSTRACT

The aim of current study was to isolate and identify naturally occurring probiotic *Lactobacillus* species in buffalo milk, camel milk, and camel urine to investigate their susceptibility to antibiotics and their antibacterial activity against pathogenic bacteria. A total number of seven samples which included three milk samples from buffalo, three milk samples from camel, and one urine sample from camel were collected and used in this study. The samples were cultured, and 18 isolated strains were identified by using 16S rRNA multiplex Polymerase Chain Reaction analysis, which was performed following DNA extraction from the isolated bacteria. Buffalo and camel milk were different in their *Lactobacilli* content. All *Lactobacilli* strains that were found in both camel milk and camel urine, were also found in buffalo milk, *Lactobacilli* strains in camel milk and urine were generally more resistant to the antibiotic. *Lactobacilli* isolated from buffalo milk, camel milk, and also camel urine presented variable degrees of antibacterial activity against pathogenic bacteria. Further studies should be conducted with more samples to gain more information in the field of antibacterial activity of probiotic *lactobacilli* and to understand the mechanisms of their activity. Hopefully, they can be used as natural alternatives instead of synthetic antibiotics.

Keywords: Antibacterial, Antibiotics, *Lactobacillus*, Probiotics

INTRODUCTION

Antibiotic resistance is considered as a global health crisis threatening the lives of both humans and animals. Many clinically isolated pathogenic bacteria are becoming increasingly resistant to antibiotics and disinfectants which make infection of these bacteria difficult to treat. During their evolution, bacteria have been developing several sophisticated mechanisms of antibiotic resistance to all types of antibiotics with no exception (Davies and Davies, 2010). The growing threat of antibiotic resistance necessitates the employment of creative approaches towards the discovery of novel alternatives to antibiotics. The use of probiotics is one of the options that is being discussed by the medical community to be used as an alternative to antibiotics (Brunel and Guery, 2017).

Probiotics are living microorganisms which confer health benefits to the host upon their administration in suitable amounts (FAO/WHO, 2011). The beneficial balance of the intestinal microbiota is one of the health-promoting properties that can be presented by probiotic microorganisms. Probiotics have been prescribed for patients with gastrointestinal disease and complaints (Williams et al., 2010). There is a set of cumulative evidence that supports the use of probiotics, both in food products and supplements to provide protection against infectious diseases including respiratory infections (Hao et al., 2011; Ozen et al., 2015). *Lactobacilli*, *Enterococci*, and *Bifidobacteria* are families of Lactic Acid Bacteria (LAB) and they constitute the most frequently used strains of probiotics (Fijan, 2014). The LAB constitute a diverse group of microorganisms that are naturally present in human diet and in both gastrointestinal and urogenital tract of animals (Ruiz Rodriguez et al., 2019) The main objective of the current study was to isolate and identify naturally occurring probiotic *Lactobacilli* in buffalo milk as well as camel milk and urine to investigate their susceptibility to antibiotics as well as their antibacterial activity against representative pathogenic bacterial strains of both Gram-positive and Gram-negative bacteria to assess their potential use as natural alternatives to synthetic antibiotics.

MATERIALS AND METHODS

Ethical approval

Institutional Animal Ethics Committee, local laws and regulations were considered in performing our experiment. All procedures involving the use of the animals were approved by the ethics committee of National Research Centre, Egypt.

Sample collection

A total number of seven samples including three milk samples from three different buffalos, three milk samples from three different camels and one urine sample from a separate camel were collected during the summer of 2016 from

To cite this paper: Abdou AM, Hedia RH, Omara ST, Kandil MM, Bakry MA and Effat MM (2021). Microbiological Studies on Naturally Present Bacteria in Camel and Buffalo Milk. *World Vet. J.*, 10 (4): 562-570. DOI: https://dx.doi.org/10.54203/scil.2020.wvj67

private, individually owned healthy animals in Giza governorate, Egypt. The samples were collected under aseptic conditions in sterile containers and stored on ice. *Lactobacillus* spp. was isolated from the collected samples by using MRS medium as a selective medium. An amount of 1 ml of each of the milk samples as well as 1 ml of the urine sample was dissolved in 100 ml of MRS broth (pH 6.5) and incubated at 37 °C for 24 h in aerobic condition. The initial cultures were subcultured for seven times at 37 °C under low pH (pH 4.5) and anaerobic condition in the presence of 10% CO_2 to eliminate unwanted bacteria. Single colonies were selected and streaked onto MRS agar media at pH 4.8 to obtain pure colonies. Finally, single pure colonies of *Lactobacillus* were selected for further characterization and identification (Shokryazdan et al., 2014).

Characterization of isolated bacteria

The isolated bacteria were evaluated by different biochemical and molecular tests including Gram stain and Catalase test as well as bacterial morphology. The isolate bacteria were identified as *Lactobacilli* based on being Gram-positive, Catalase-negative and having rod-shape under light microscope. The *Lactobacilli* identification of isolated bacteria were further confirmed by using 16S rRNA multiplex polymerase chain reaction (PCR) analysis.

Gram staining

A prepared smear of 24 h cultured bacteria was heat fixed on a slide. Gram staining based on standard technique was then performed and then slides were observed under light microscope (Bergey et al., 1994).

Catalase test

Fresh liquid cultures which contained overnight grown cultures from selected single colonies were used for Catalase test. An amount of 3% hydrogen peroxide solution was dropped onto 1 ml of the culture. The formation of gas bubbles was considered as positive Catalase test and these samples were neglected while the other samples with negative Catalase test were selected since *Lactobacilli* are known to be Catalase-negative.

Molecular identification of probiotic strains

The DNA was extracted from the isolated bacteria and *Lactobacillus* strains were confirmed by using 16S rRNA multiplex PCR analysis (Kwon et al., 2004). The reaction mixture (25 µl) contained 12.5 µl of PCR Master Mix, 5 µl primer mixture comprising 50 pmol of each primer, 4.5 µl of water, and 3 µl of DNA template. PCR amplification was performed in Applied Biosystem 2720 thermal cycler, and DNA fragments were amplified as follows. Initial heating at 94 °C for 2 min, 35 cycles consisting of denaturation at 94 °C for 20 sec, annealing at 51 °C for 40 sec, extension at 68 °C for 30 sec, and final extension step in 7 min at 68 °C. The PCR products were separated on 1.5% agarose gel by electrophoresis and analyzed by RedSafe Nucleic Acid Staining Solution (Intron Biotechnology, Korea).

Antibiotic susceptibility of *Lactobacilli*

A wide panel of 14 antibiotic disks was tested against 7 mixed cultures of probiotic *Lactobacilli* isolated from both buffalo and camel samples (Figures 2-8). Antibiotic susceptibility test was performed by using the disk-diffusion method with some modifications (ISO, 2010). *Lactobacilli* activated cultures were swabbed on MRS agar plates instead of Muller Hinton Agar plates. Fourteen different antibiotic disks were used for the susceptibility test including Trimethoprim/sulfamethoxazole (SXT-25), Ofloxacin (OFX5), Cefuroxime (CXM-30), Amoxicillin with clavulanic acid (AmC-30), Cefotaxime (CTX-30), Cefaclor (CEC-30), Rifampicin (RD-5), Erythromycin (E-15), Vancomycin (Va-30), Amikacin (AK-30), Ampicillin with sulbactam (SAM-20), Cefadroxil (CFR-30), Azithromycin (AZM-15), and Doxycycline (DO-30). All plates were incubated for 24 h at 37^{o}C and inhibition zones were measured.

Antibacterial activity of *Lactobacilli*

The ability of the seven mixed cultured of isolated probiotic *Lactobacilli* to inhibit the growth of pathogenic bacteria was investigated against nine pathogenic standard strains of both Gram-positive and Gram-negative bacteria (Figures 9-15). Gram-positive strains were represented by *Staphylococcus aureus* (ATCC 26923), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pneumoniae* (ATCC 29619), and *Enterococcus faecalis* ATCC (29212). Gram- negative strains were represented by *Pseudomonas aeruginosa* ATCC (27853), *Escherichia coli* ATCC (25922), *Escherichia coli* ATCC (10536), and *Klebsiella pneumoniae* ATCC (700603). Antibacterial activity of probiotic *Lactobacilli* was tested by using Agar-well diffusion method with some modifications (Bauer et al., 1966; Sgouras et al., 2004). Wells of 7 mm diameter were made on Muller-Hinton agar plates. Each plate was swabbed with the respective test pathogen. From each probiotic *Lactobacillus* strain which previously incubated under anaerobic conditions for 24 h at 37^{o}C, 70 µl of MRS liquid culture were placed in the respective wells. After 24 h of incubation at 37 ^{o}C, the inhibition zones were measured and recorded in cm.

Statistical analysis

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The *in vitro* antibacterial activity was conducted in triplicate. All the data were then subjected to SPSS Version 21 (IBM, New York, US). Statistical analysis was performed using two-way ANOVA followed by Duncan's Multiple Range Test to determine significant difference. The given values represented mean ± Standard Deviation (SD). A probability value $P<0.05$ was taken as significant difference (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Lactobacilli isolated from buffalo milk, camel milk, and camel urine were subjected to characterization and identification by using different biochemical and molecular identification methods. A total number of seven samples were collected including three milk samples and one urine sample from camel and three milk samples from buffalo. *Lactobacilli* were isolated by growing the bacterial contents of the samples on MRS medium as selective medium. The bacterial colonies were initially identified as *Lactobacilli* based on being Gram-positive and Catalase-negative as well as being rod-shaped under the microscope. Mixed colonies of each sample in MRS broth medium were used to extract DNA for molecular identification using 16S rRNA multiplex PCR analysis. The mixed colonies of each sample were also used to test antibiotic susceptibility and antibacterial activity of the isolated strains.

Multiplex PCR analysis

The results from 16S rRNA multiplex PCR analysis have been demonstrated in figure 1. A total number of 18 isolated bacteria from buffalo milk, camel milk, and camel urine were identified as *Lactobacilli*. *Lactobacillus* species were identified based on the size of the PCR product (Kwon et al., 2004). The results indicated that buffalo and camel milk were different in their *Lactobacilli* content. There were also differences in *Lactobacilli* content of different milk samples collected from the same species. The results indicated the presence of *L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. plantarum*, *L. gasseri* and *L. delbrueckii* in buffalo milk samples. Meanwhile, both camel milk and camel urine samples expressed the presence of *L. casei*, *L. acidophilus* and *L. plantarum*.

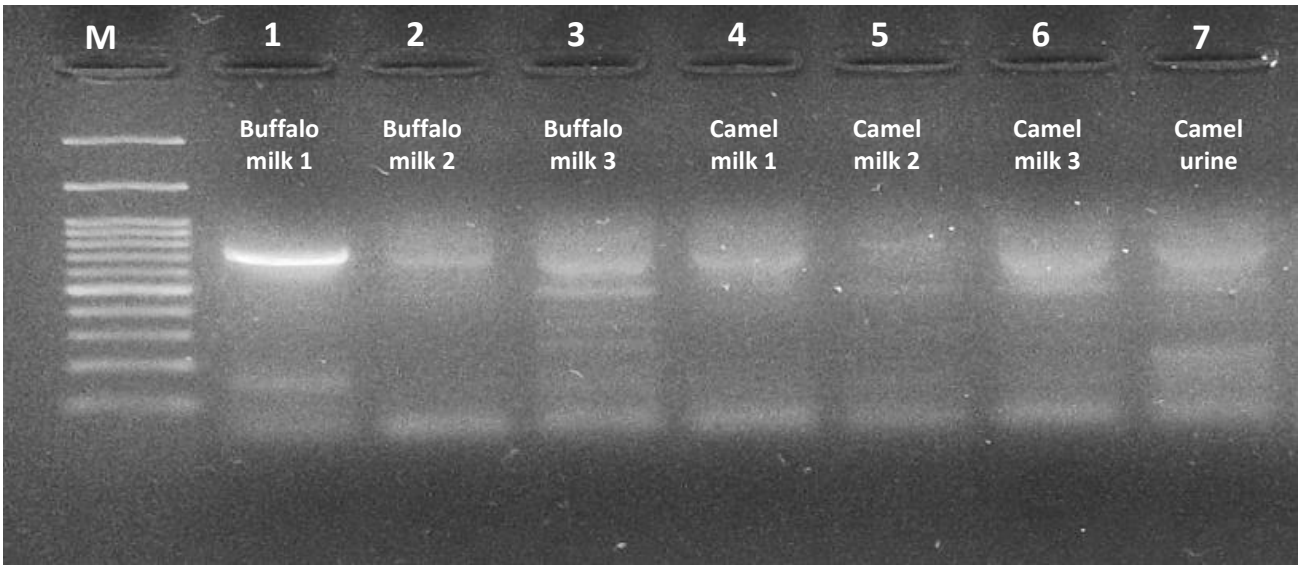

Figure 1. Agarose gel electrophoreses of PCR products from multiplex PCR assays. Multiplex PCR assays were performed with a mixture of seven species-specific or group-specific primers for L. acidophilus, *L. bulgaricus* (same as *L. delbrueckii* subsp. *bulgaricus*), *L. casei*-group *L. gasseri*, *L. plantarum*, *L. reuteri* and *L. rhamnosus* and two bacterial conserved primers. Lanes 1–7 designate the PCR product from each genomic DNA extracted from single or mixed cell suspension isolated from representative host used as PCR template. Lane 1: *L. casei*, *L. delbrueckii*; lane 2: *L. casei*; lane 3: *L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. plantarum* and *L. gasseri*; lane 4: *L. casei*; lane 5: *L. plantarum*; Lane 6: *L. plantarum*; lane 7: *L. plantarum*; lane M: 100 bp-DNA ladder.

Antibiotic susceptibility of *Lactobacilli*

Antibiotic susceptibility of *Lactobacillus* strains was tested by using a panel of 14 antibiotics (Figures 2-8). It was clear that all samples had extremely significant resistant ($p < 0.0001$) to Cefadroxil (CFR-30) with inhibition zones of 0.0 cm. Cefaclor (CEC-30) exclusively did not present any inhibition to the growth of one of buffalo milk samples (buffalo milk 2) and all camel milk and urine samples. While Vancomycin (Va-30) did not cause any inhibition to only one of buffalo milk samples (buffalo milk 1). The rest of antibiotics exclusively presented no inhibition to camel samples which included Cefuroxime (CXM-30), Cefotaxime (CTX-30), Erythromycin (E-15), Ampicillin with sulbactam (SAM-20), and Azithromycin (AZM-15). Furthermore, camel urine sample was the only one to be totally resistant to (AmC-30)

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with 0.0 cm growth inhibition. On the other hand, all samples were sensitive to Trimethoprim/sulfamethoxazole (SXT-25), Ofloxacin (OFX5), Rifampicin (RD-5) and Doxycycline (DO-30) with varying degrees of inhibition.

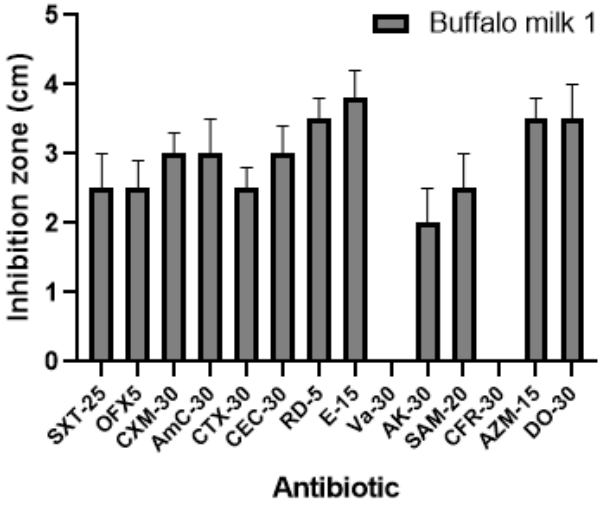
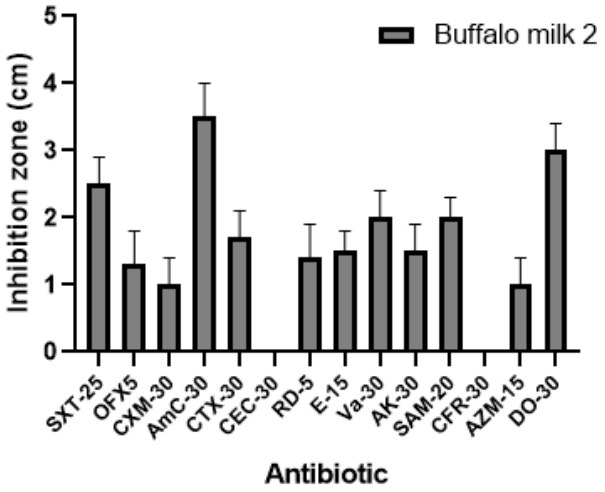


Figure 2



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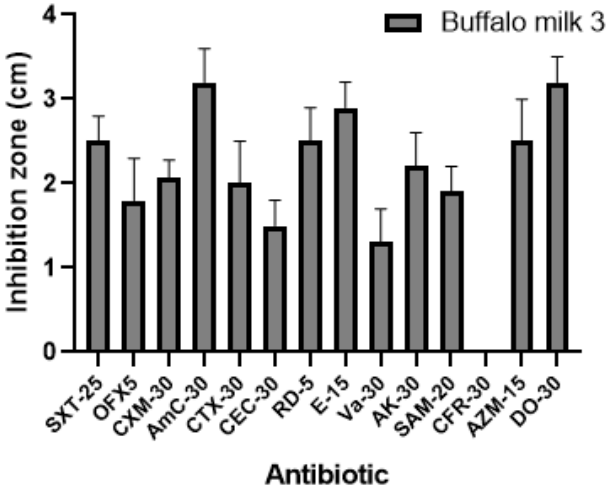

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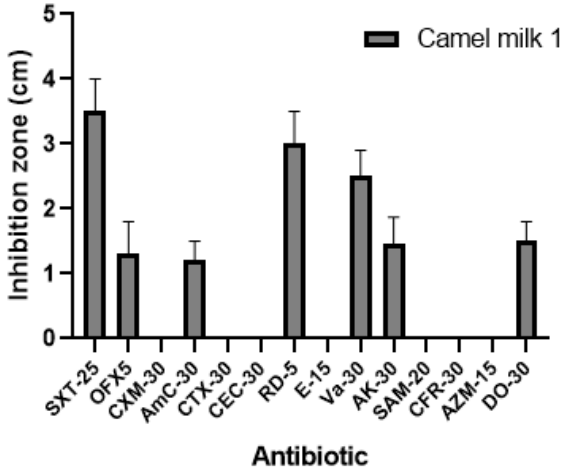

Figure 5

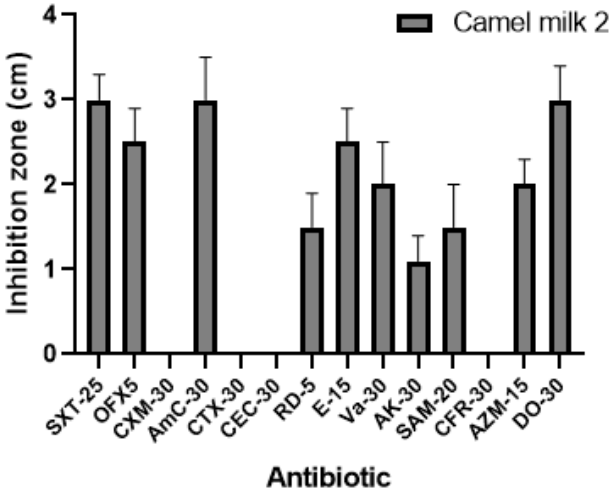

Figure 6

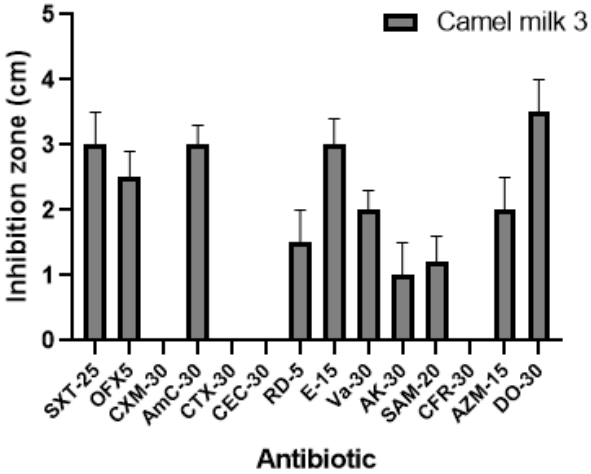

Figure 7

Camel urine

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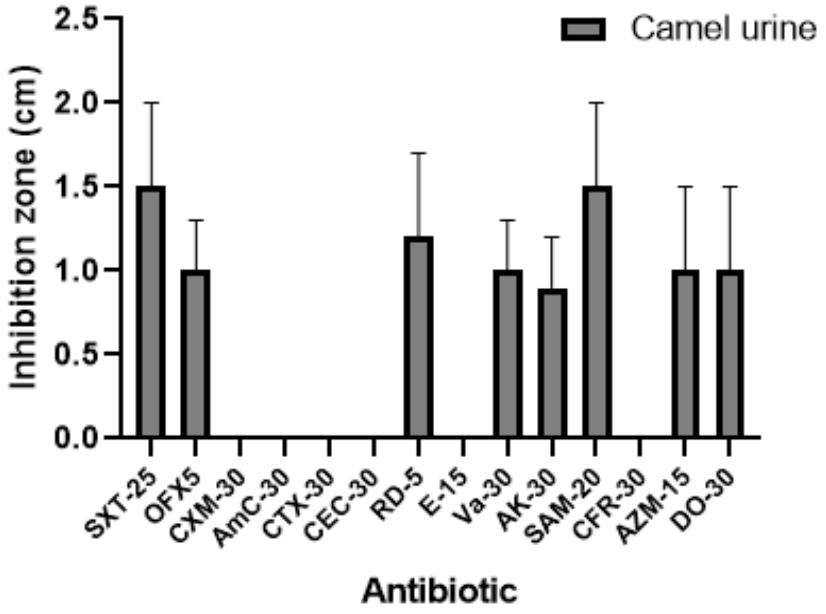

Figure 8

Figures 2-8. Antibiotic susceptibility of the probiotic *Lactobacilli* isolated from buffalo milk, camel milk or camel urine. Fourteen antibiotic disks were used for the susceptibility test including Trimethoprim/sulfamethoxazole (SXT-25), Ofloxacin (OFX5), Cefuroxime (CXM-30), Amoxicillin with clavulanic acid (AmC-30), Cefotaxime (CTX-30), Cefaclor (CEC-30), Rifampicin (RD-5), Erythromycin (E-15), Vancomycin (Va-30), Amikacin (AK-30), Ampicillin with ctam (SAM-20), Cefadroxil (CFR-30), Azithromycin (AZM-15), and Doxycycline (DO-30).

Antibacterial activity of *Lactobacilli*

The antibacterial activity of the isolated probiotic *Lactobacilli* was investigated against nine pathogenic standard strains of both Gram- positive and Gram- negative bacteria (Figures 9-15). The results indicated that the antibacterial activity of *Lactobacilli* which were isolated from camel urine was in general extremely significant lower ($p < 0.0001$) than the antibacterial activity of *Lactobacilli* from both buffalo milk and camel milk against all tested bacterial strains. The antibacterial effect of buffalo milk three was significantly higher ($P = 0.0045$) than all other samples against *E. coli* 25922 while the antibacterial activity of camel milk two was significantly higher against *S. aureus* 29213 ($P = 0.0014$), *S. pneumonia* 29619 ($P = 0.0014$) and *E. faecalis* 29212 ($P = 0.0014$) when compared to its effect against *E. coli* 10536.

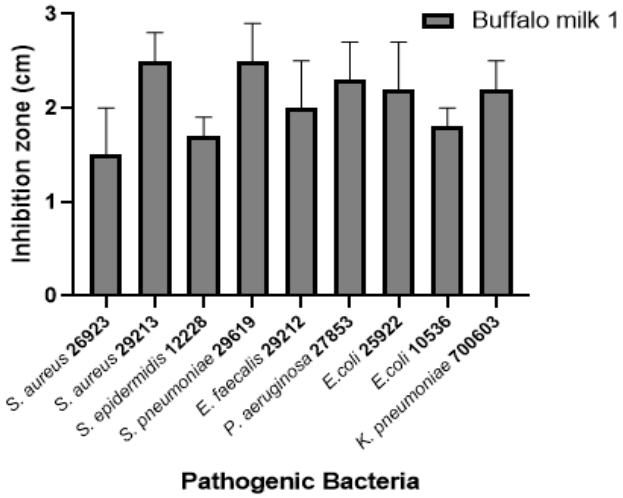

Figure 9

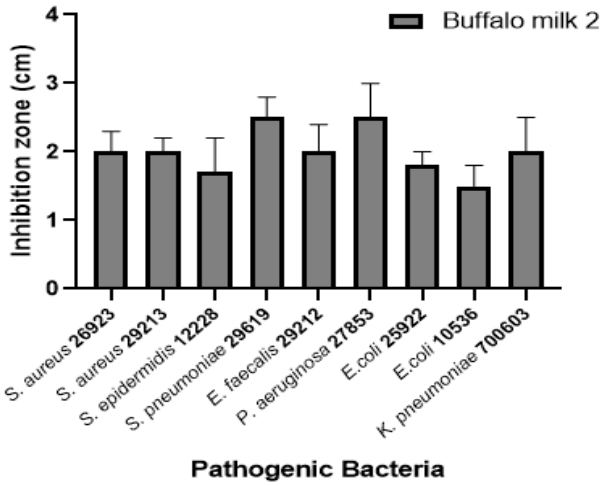

Figure 10

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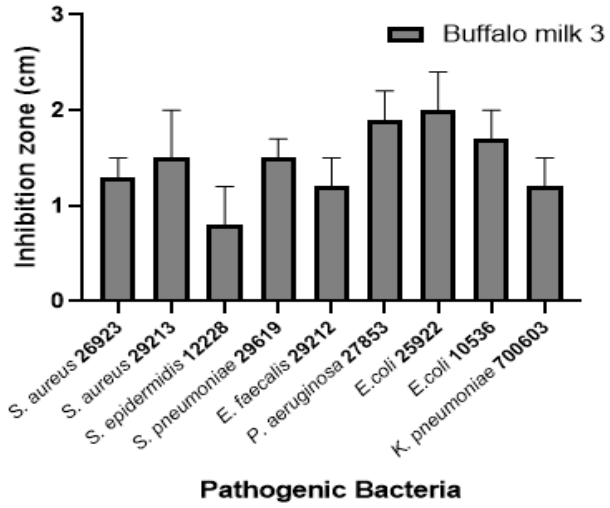

Figure 11

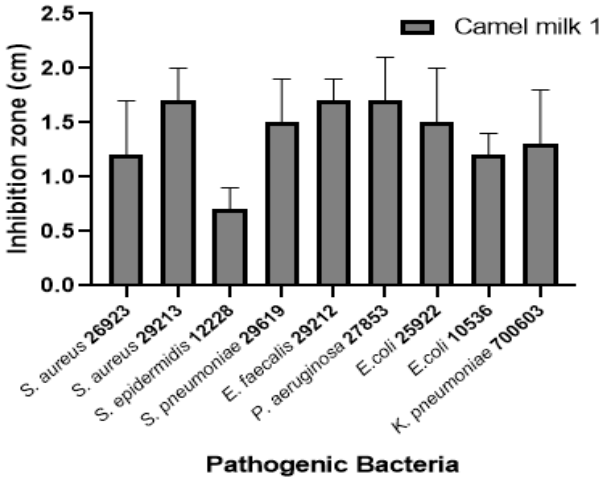

Figure 12

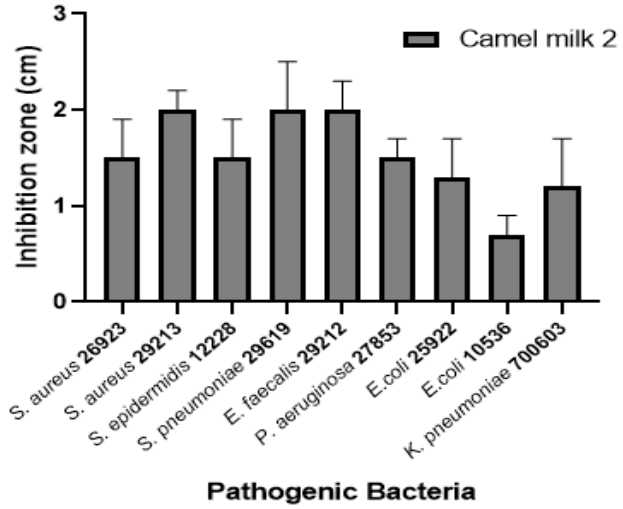

Figure 13

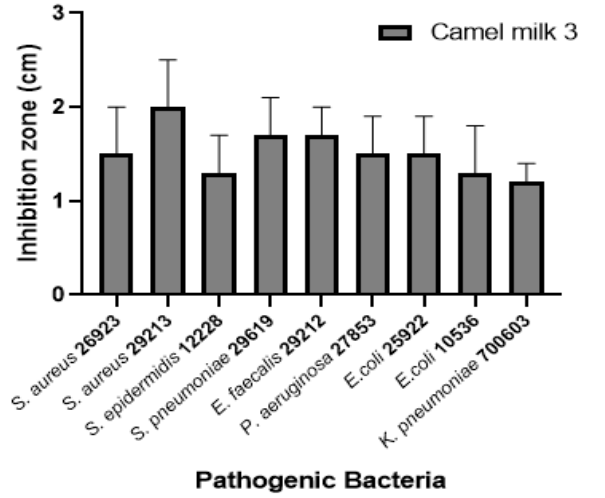

Figure 14

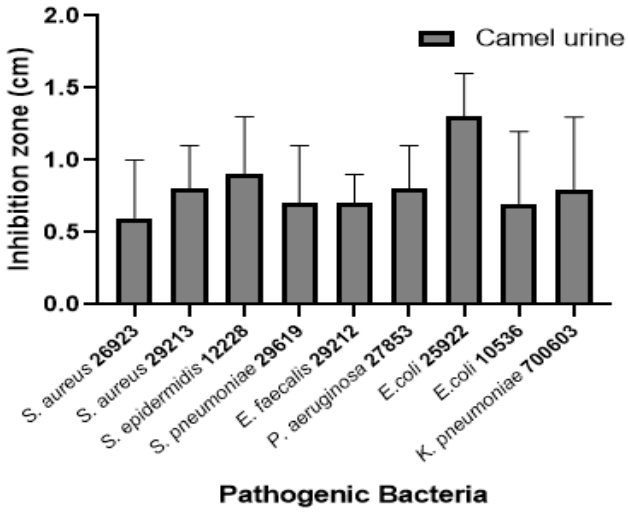

Figure 2

Figures 9-15: The antibacterial activity of the probiotic *Lactobacilli* isolated from buffalo milk, camel milk or camel urine. The antibacterial activity was investigated against both Gram-positive and Gram- negative bacteria using Agar-well diffusion method. Gram-positive strains were represented by *S. aureus* (ATCC 26923), *S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 12228), *S. pneumoniae* (ATCC 29619), and *E. faecalis* ATCC (29212). Gram- negative strains were represented by *P. aeruginosa* ATCC (27853), *E. coli* ATCC (25922), *E. coli* ATCC (10536), and *K. pneumoniae* ATCC (700603).

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DISCUSSION

Probiotic bacteria have been recognized for their beneficial health effects in humans and animals. Their consumption in traditional food was associated with an extended life span and protection against diseases (Kechagia et al., 2013). The mechanisms of their beneficial effects include the protection against infectious disease either by direct competition with pathogenic microorganisms or by the modulation of the immune system and improving the digestion and reduction of metabolic disorders (Azad et al., 2018; Ghosh et al., 2019; Yousefi et al., 2019).

The main source of probiotics is fermented food including fermented milk, cheese and other dairy products. Probiotics are also isolated from both human and animal gastrointestinal tract. Furthermore, probiotic strains have been isolated from non-dairy fermented substrates including meat and fruits. Surprisingly, probiotic strains are also present in both human and animal milk which are originally expected to be sterile (McGuire and McGuire, 2015). These findings are consistent with the findings that breast-fed infants are less affected by gastrointestinal infections and have fewer allergies than formula-fed infants (Fontana et al., 2013). The same is also true about urine which had been thought to be sterile but after the development of sequencing techniques it was found that urine is colonized by normal flora including *Lactobacillus* and *Streptococcus* (Akgul and Karakan, 2018).

The diversity of probiotic *Lactobacilli* which isolated from different animal species has been documented (Abdou et al., 2018;Abdou et al., 2019). This diversity is the result of several factors including nutrition, infections, antibiotics, stress and various disease conditions. The variety of probiotic strains causes different types of benefits for the host.

It was clear from present findings that *Lactobacilli* strains isolated from both camel milk and camel urine were more resistant to the effect of antibiotics than *Lactobacilli* isolated from buffalo milk. This could be useful for restoring the gut microbiota after antibiotic treatment (Gueimonde et al., 2013). Although all *Lactobacilli* strains found in both camel milk and camel urine were also found in buffalo milk, the first two presented more resistance in general to antibiotic. This could be due to the acquisition of plasmids from other bacteria (Gueimonde et al., 2013). Camel milk and urine have been used in traditional medicine for several years to treat many diseases (Hu et al., 2017). In spite of the popularity of buffalo and cow milk and their preference among general public, camel milk is a very important replacement in arid and semi-arid areas where buffalo and cow milk are lacking. The camel milk investigation for bacterial content found it to be rich in LAB (Bin Masalam et al., 2018). In current study buffalo and camel milk were different in their *Lactobacilli* content. This difference might be due to the difference in milk composition (Yoganandi et al., 2014), which may allow the growth of different *lactobacillus* strains. *Lactobacillus plantarum* was isolated from camel milk and it is one of the frequently isolated LAB from raw camel milk (Khedid et al., 2009; Edalati et al., 2019).

Probiotic *Lactobacilli* have the potential to be used as natural alternatives to currently used synthetic antibiotics due to their antagonistic activity against various pathogenic bacteria (Prabhurajeshwar and Chandrakanth, 2017). In current study, it has been indicated that *Lactobacilli* isolated from buffalo milk, camel milk as well as camel urine presented variable degrees of antibacterial activity against pathogenic bacteria. Although present data indicated that isolated *Lactobacilli* from camel urine had the least antibacterial activity when compared to both buffalo and camel milk, the antibacterial, antifungal and antiviral activity of both camel milk and urine were reported previously (Al-Bashan, 2011; Hu et al., 2017). One of the reasons for the least antibacterial activity of camel urine could be using only one sample of it. The antibacterial activities of camel milk and urine in general may be partly due to the presence of different probiotic *Lactobacilli* including *Lactobacillus plantarum* and *Lactobacillus casei* which had been found earlier to represent promising antimicrobial activity (Bin Masalam et al., 2018).

CONCLUSION

The present study indicated the variability in contents of *lactobacillus* strains which isolated from buffalo milk, camel milk, and camel urine. Although some strains were similar among these samples, they presented different susceptibility to antibiotics and had different antibacterial activity against pathogenic bacteria. Further studies should be conducted with more samples to gain more information in the field of antibacterial activity of probiotic *lactobacilli* and to understand the mechanisms of their activity. Hopefully, they will be used as natural alternatives instead of synthetic antibiotics.

DECLARATIONS

Authors' contributions

Amr M. Abdou participated in the molecular identification of probiotic strains, performed the statistical analysis and drafted the manuscript. Riham H. Hedia participated in characterization of isolated bacteria, molecular identification of probiotic strains and antibiotic susceptibility of *Lactobacilli*. Shimaa T. Omara participated in characterization of isolated bacteria, molecular identification of probiotic strains, and antibacterial activity of *Lactobacilli*. Mai M. Kandil

To cite this paper: Abdou AM, Hedia RH, Omara ST, Kandil MM, Bakry MA and Effat MM (2021). Microbiological Studies on Naturally Present Bacteria in Camel and Buffalo Milk. *World Vet. J.*, 10 (4): 562-570. DOI: https://dx.doi.org/10.54203/scil.2020.wvj67

participated in sample collection and participated in the molecular identification of probiotic strains. M. A. Bakry participated in sample collection and characterization of isolated bacteria. Mohammad M. Effat proposed the idea of current study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declared that they had no competing interests.

REFERENCE

Abdou AM, Fouad EA, Alam SS, and Hakim AS (2019). Isolation and Identification of Probiotic Lactobacilli from Non-ruminant Animals. International Journal of Veterinary Science, 8(4): 349-354. Available at: http://www.ijvets.com/.../349-354.pdf

Abdou AM, Hedia RH, Omara ST, Mahmoud MAE, Kandil MM, and Bakry MA (2018). Interspecies comparison of probiotics isolated from different animals. Veterinary World, 11: 227-230. DOI: https://doi.org/10.14202/vetworld.2018.227-230

Akgul T, and Karakan T (2018). The role of probiotics in women with recurrent urinary tract infections. Turkish Journal of Urology, 44: 377-383. DOI: https://doi.org/10.5152/tud.2018.48742

Al-Bashan MM (2011). In vitro Assessment of the Antimicrobial Activity and Biochemical Properties of Camel's Urine Against Some Human Pathogenic Microbes. Middle-East Journal of Scientific Research, **7**: 947-958. Available at: https://bengreenfieldfitness.com/wp-content/uploads/2017/02/In-vitro-Assessment-of-the-Antimicrobial-Activity-and-Biochemical-Properties-of-camels-urine.pdf

Azad MAK, Sarker M, and Wan D (2018). Immunomodulatory Effects of Probiotics on Cytokine Profiles. BioMed Research International, 2018: 8063647. DOI: https://doi.org/10.1155/2018/8063647

Bauer AW, Kirby WM, Sherris JC, and Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4): 493-496. DOI: https://doi.org/10.1093/ajcp/45.4_ts.493

Bergey DHJ, John GH, Noel RK, and Peter HAS (1994). Bergey's Manual of Determinative Bacteriology, 9 th ed. Avaliable at:

https://books.google.com.eg/books?id=jtMLzaa5ONcC&printsec=frontcover&hl=ar&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false

Bin Masalam MS, Bahieldin A, Alharbi MG, Al-Masaudi S, Al-Jaouni SK, Harakeh SM, and Al-Hindi RR (2018). Isolation, Molecular Characterization and Probiotic Potential of Lactic Acid Bacteria in Saudi Raw and Fermented Milk. Evidence-Based Complementary and Alternative Medicine, 2018, 7970463. DOI: https://doi.org/10.1155/2018/7970463

Brunel AS, and Guery B (2017). Multidrug resistant (or antimicrobial-resistant) pathogens - alternatives to new antibiotics? Swiss Medical Weekly, 147: w14553. DOI: https://doi.org/10.4414/smw.2017.14553

Davies J, and Davies D (2010). Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews, 74: 417-433. DOI: https://doi.org/10.1128/MMBR.00016-10

Edalati E, Saneei B, Alizadeh M, Hosseini SS, Zahedi Bialvaei A, and Taheri K (2019). Isolation of probiotic bacteria from raw camel's milk and their antagonistic effects on two bacteria causing food poisoning. New Microbes and New Infections, 27: 64-68. DOI: https://doi.org/10.1016/j.nmni.2018.11.008

FAO/WHO (2011). ICMR-DBT guidelines for evaluation of probiotics in food. Indian Journal of Medical Research, 134(1): 22-25. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3171912/

Fijan S (2014). Microorganisms with claimed probiotic properties: an overview of recent literature. Int International Journal of Environmental Research and Public Health, 11(5): 4745-4767. https://doi.org/10.3390/ijerph110504745

Fontana L, Bermudez-Brito M, Plaza-Diaz J, Munoz-Quezada S, and Gil A (2013). Sources, isolation, characterisation and evaluation of probiotics. British Journal of Nutrition, 109 Suppl 2: S35-50. DOI: https://doi.org/10.1017/S0007114512004011

Ghosh T, Beniwal A, Semwal A, and Navani NK (2019). Mechanistic Insights Into Probiotic Properties of Lactic Acid Bacteria Associated With Ethnic Fermented Dairy Products. Frontiers in Microbiology, 10: 502. DOI: https://doi.org/10.3389/fmicb.2019.00502

Gueimonde M, Sánchez B, G de Los Reyes-Gavilán C, and Margolles A (2013). Antibiotic resistance in probiotic bacteria. Frontiers in microbiology, *4*, 202. https://doi.org/10.3389/fmicb.2013.00202

Hao Q, Lu Z, Dong BR, Huang CQ, and Wu T (2011). Probiotics for preventing acute upper respiratory tract infections. Cochrane Database of Systematic Reviews, CD006895. DOI: https://doi.org/10.1002/14651858.CD006895.pub2

Hu Z, Chang X, Pan Q, Gu K, and Okechukwu PN (2017). Gastroprotective and Ulcer Healing Effects of Camel Milk and Urine in HCl/EtOH, Non-steroidal Anti-inflammatory Drugs (Indomethacin), and Water-Restraint Stress-induced Ulcer in Rats. Pharmacognosy Magazine, 13: 559-565. DOI: https://doi.org/10.4103/pm.pm_135_17

International Organization for Standardization (ISO) (2010). Milk and milk products—determination of the minimal inhibitory concentration (MIC) of antibiotics applicable to bifidobacteria and non-enterococcal lactic acid bacteria (LAB). ISO 10932/IDF 233 standard. Available at: https://www.iso.org/standard/46434.html

Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, and Fakiri EM (2013). Health benefits of probiotics: a review. ISRN Nutrition, Article ID: 481651. DOI: https://doi.org/10.5402/2013/481651

Khedid K, Faid M, Mokhtari A, Soulaymani A, and Zinedine A (2009). Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. Microbiological Research, 164: 81-91. DOI: https://doi.org/10.1016/j.micres.2006.10.008

Kwon HS, Yang EH, Yeon SW, Kang BH, and Kim TY (2004). Rapid identification of probiotic Lactobacillus species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA. FEMS Microbiology Letters, 239: 267-275. DOI: https://doi.org/10.1016/j.femsle.2004.08.049

McGuire MK, and McGuire MA (2015). Human milk: mother nature's prototypical probiotic food? Adv Nutr. Jan 15; 6(1): 112-123. DOI: https://doi.org/10.3945/an.114.007435.

Ozen M, Kocabas Sandal G, and Dinleyici EC (2015). Probiotics for the prevention of pediatric upper respiratory tract infections: a systematic review. Expert Opinion on Biological Therapy, 15: 9-20. DOI: https://doi.org/10.1517/14712598.2015.980233

Prabhurajeshwar C, and Chandrakanth RK (2017). Probiotic potential of Lactobacilli with antagonistic activity against pathogenic strains: An in vitro validation for the production of inhibitory substances. Biomedical journal, 40(5): 270–283. DOI: https://doi.org/10.1016/j.bj.2017.06.008

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Ruiz Rodriguez LG, Mohamed F, Bleckwedel J, Medina R, De Vuyst L, Hebert EM, and Mozzi F (2019). Diversity and Functional Properties of Lactic Acid Bacteria Isolated From Wild Fruits and Flowers Present in Northern Argentina. Frontiers in Microbiology, 10: 1091. DOI: https://doi.org/10.3389/fmicb.2019.01091

Sgouras D, Maragkoudakis P, Petraki K, Martinez-Gonzalez B, Eriotou E, Michopoulos S, Kalantzopoulos G, Tsakalidou E, and Mentis A (2004). In vitro and in vivo inhibition of Helicobacter pylori by Lactobacillus casei strain Shirota. Applied and Environmental Microbiology, 70(1): 518-526. DOI: https://doi.org/10.1128/aem.70.1.518-526.2004

Shokryazdan P, Sieo CC, Kalavathy R, Liang JB, Alitheen NB, Faseleh Jahromi M, and Ho YW (2014). Probiotic potential of Lactobacillus strains with antimicrobial activity against some human pathogenic strains. BioMed Research International, 927268. DOI: https://doi.org/10.1155/2014/927268

Steel RGD, and Torrie JH (1980). Principles and Procedures of Statistics. 2nd ed. McGraw Hill Book Company, New York.

Williams MD, Ha CY, and Ciorba MA (2010). Probiotics as therapy in gastroenterology: a study of physician opinions and recommendations. Journal of Clinical Gastroenterology, 44: 631-636. DOI: https://doi.org/10.1097/MCG.0b013e3181d47f5b

Yoganandi J, Mehta BM, Wadhwani KN, Darji VB, and Aparnathi KD (2014). Evaluation and Comparison of Camel Milk with Cow Milk and Buffalo Milk for Gross Composition. Journal of Camel Practice and Research, 21(2): 259-265. DOI: https://doi.org/10.5958/2277-8934.2014.00046.0

Yousefi B, Eslami M, Ghasemian A, Kokhaei P, Salek Farrokhi A, and Darabi N (2019). Probiotics importance and their immunomodulatory properties. Journal of Cellular Physiology, 234 (6): 8008-8018. DOI: https://doi.org/10.1002/jcp.27559

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2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 571-577, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj68

Canine Parvovirus Infection in Dogs: Prevalence and Associated Risk Factors in Egypt

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ORGINAL ARTICLE
pii: S232245682000068-10
Received: 29 Oct 2020
Accepted: 13 Dec 2020

ABSTRACT

Canine parvovirus (CPV) infection is a global infectious and contagious viral disease of canine, especially in dogs infected by three variants of CPV type. This study aimed to investigate the prevalence and potential risk factors of parvovirus infection in dogs residing in Egypt. A total of 122 dogs suffering from vomiting and diarrhea were screened by antigen rapid CPV/Canine Coronavirus Ag test kit for the diagnosis of CPV infection from March 2012 to February 2013. Age, breed, season, and vaccination of each dog were recorded to study the prevalence of CPV. The overall prevalence of CPV infection in dogs was reported as 59.7%. Dogs between 0 and 3 months of age indicated the highest prevalence of 68% followed by 4-6 months of age which was 53.3%. The lowest prevalence of CPV was reported in dogs above 6 months of age (20%). The maximum prevalence was noticed in non-descript dogs (48.5%) followed by German shepherds (26.7%), Doberman (23.07%), and Griffon (16.6%). Among different risk factors, young, unvaccinated puppies and exotic breeds were more prone to CPV infection. Regarding the season, the higher prevalence was noticed in summer (77.1%) followed by spring (55.5%), autumn (25%), and winter (16.6%). Thus, CPV is an infectious and highly contagious viral disease of dogs. Age and seasonal variations are risk factors in the prevalence of CPV infection. Identification of the potential risk factors associated with the disease may be helpful to construct the ideal preventive measures.

Keywords: Canine parvovirus, Egypt, Epidemiology, Prevalence, Risk factors

INTRODUCTION

Canine parvovirus (CPV) infection is a global infectious and contagious viral disease of canine species especially dogs caused by three variants of canine parvovirus type 2 (Goddard and Leisewitz, 2010; Sykes, 2014; Khare et al., 2019). Canine parvovirus-2 (CPV-2) is the cause of acute enteritis associated with high morbidity and mortality, with very low survival rates in untreated dogs. Although the severity of disease typically occurs in dogs younger than 6 months of age, the immunosuppressed adults may potentially be affected (Decaro et al., 2004; Marcovich et al., 2012; Albaz et al., 2015; Mylonakis et al., 2016; AL-hosary, 2016; Khare et al., 2019). Seasonal prevalence of the disease is affected by geographic variation (Kalli et al., 2010). CPV-2 can survive more than one year in the environment and infect the susceptible dogs through infected feces, or vomitus by fecal-oral route (Decaro et al., 2005a; Sykes, 2014; Albaz et al., 2015). The incubation period following natural or experimental exposure ranges from four to fourteen days, and virus shedding starts a few days before the occurrence of clinical signs, progressively declining 3 to 4 weeks post-exposure (Decaro et al., 2005b; McCaw and Hoskins, 2006).The clinical signs of CPV infection depend on the age and immune status of the dogs, dose of the virus, virulence of the virus, and pre-existing infections (McAdaragh et al., 1982). CPV-2 infection manifests as acute hemorrhagic enteritis and myocarditis. Dogs with enteritis show high fever, depression, loss of appetite, lethargy, vomiting, and severe mucoid or bloody diarrhea (Prittie, 2004; Lamm and Rezabek, 2008; Albaz et al., 2015; Khare et al., 2019). Factors that predispose puppies to parvovirus infection are lack of maternal immunity, gastrointestinal parasites, unsanitary, and stress (Hong et al., 2007; Mylonakis et al., 2016). A definitive diagnosis of CPV-2 in the feces of affected dogs is done by viral isolation, fecal hemagglutination, latex agglutination, immunochromatography, PCR, and electron microscopy (Pollock and Carmichael, 1988; Desario et al., 2005; Wilkes et al., 2015). CPV infection can be treated by symptomatic and supportive therapy (Prittie, 2004; Brown and Otto, 2008; Mylonakis et al., 2016) involving antiemetic, antibiotics, nutritional support, fluid therapy, antiviral treatments, and pain management. Prevention is by vaccination of dogs with either attenuated or modified live vaccines (Martella et al., 2005; Albaz et al., 2015; Mylonakis et al., 2016). Hence, the present study was aimed to know the prevalence and potential risk factors of parvovirus infection in dogs in Egypt.

To cite this paper: Sayed-Ahmed MZ, Elbaz E, Younis E and Khodier M (2021). Canine Parvovirus Infection in Dogs: Prevalence and Associated Risk Factors in Egypt. *World Vet. J.*, 10 (4): 571-577. DOI: https://dx.doi.org/10.54203/scil.2020.wvj68

MATERIALS AND METHODS

Ethical approval

This study was certified, approved and performed according to the ethics of committee of the Faculty of veterinary medicine, Mansoura University, Mansoura, Egypt.

Study area and animals

For one year, an epidemiologic study was carried out at veterinary teaching hospital, faculty of veterinary medicine, Mansoura University, Egypt from March 2012 to February 2013. One hundred twenty-two dogs of different breeds within the age ranging from one day up to 12 months which distributed allover different localities were subjected for clinical examination where the noticed signs were suspected to be parvovirus infection including fever, diarrhea and vomiting, tachycardia, dehydration, and weakness.

Case definition

The diagnosis was made by anamnesis and clinical signs observed. In CPV infection, the main clinical signs were high fever (104-105˚F), vomiting, and bloody diarrhea. If a dog showed signs of high fever, vomiting, bloody diarrhea, anemia, dehydration, it would be suspected as a CPV infection. Anemia was detected by the pale mucous membrane. The degree of dehydration was estimated by a skin fold test (Hasan et al., 2018).

Clinical examination and data collection

The questionnaire was developed according to age, sex, breed, history of vaccination, clinical history, and the data were collected by interviewing the owner. Rectal temperature, heart rate, and respiration rate of the sample dogs were measured. Skinfold test was performed to estimate the degree of dehydration. Then the clinical signs and symptoms were observed. All the clinical signs and symptoms were separately recorded for each clinical case (Kelly, 1990; Hasan et al., 2018).

Samples and sample processing

Fecal samples

Fecal swabs were collected from diseased puppies; each swab was inserted into a screw-capped bottle containing 3 ml of sterilized phosphate buffer saline containing 100 IU of penicillin as well as 100 mg of streptomycin/ml. The swabs were squeezed and removed. All samples were centrifuged at 2000 rpm for 10 minutes. The supernatant solution was transferred to a sterile bijoux bottle and the samples were preserved at -20°C until being examined (Coles, 1986; Wilkes et al., 2015; AL-hosary, 2016).

Blood sample

Two blood samples were collected from diseased puppies via the femoral vein, one with anticoagulant virus isolation and the other without anticoagulant for serological examination.

Viral isolation and identification

Vero cells (African green monkey kidney cells) were used in the virus isolation procedures. Dr. J. House, Plum Island, USA, kindly supplied it. The samples were inoculated at a rate of 100 µL per well in a cell's monolayer in the maximum growth phase (12 hours of culture) with a low cell confluence (60%). After being adsorbed for 1 hour at 37°C, the inoculum was removed, and the minimum essential medium with Eagle salts was added (E-MEM, Sigma-Aldrich®, USA), then the cells were again incubated at 37ºC. Cytopathic effect was monitored daily in the cell culture for 4-5 days. Subsequently, the plate was frozen at -80°C, and submitted to further passages following the same procedure, until the fifth passage or the eventual appearance of cytopathic effect. The cells were examined for the presence of virus by the immunofluorescence according to a 2-day protocol with minor variations according to Virology Manual, 2014, Immunochromatography assay for the qualitative detection of CPV antigen in fecal or rectal swab as described by Schmitz et al. 2009, and Faz et al. 2017 and virus neutralization test according to Rossitter and Jessett (1982), and Hao et al. 2017.

Statistical analysis

All the data including age, breed, sex, bloody diarrhea, vaccination, dehydration, and diagnosis were entered into Microsoft Office Excel-2010.Then the data was cleaned, coded, recoded, and finally analyzed using statistical software STATA Version-11 (STATA Corporation, College Station, Texas). Prevalence was calculated according to different categories of the explanatory variables. A Chi-square ($\chi 2$) test was performed to identify the association between a categorical variable with the occurrence of CPV infection. The association was regarded as significant if the p value was ≤ 0.05, and highly significant when p value was 0.01.

To cite this paper: Sayed-Ahmed MZ, Elbaz E, Younis E and Khodier M (2021). Canine Parvovirus Infection in Dogs: Prevalence and Associated Risk Factors in Egypt. *World Vet. J.*, 10 (4): 571-577. DOI: https://dx.doi.org/10.54203/scil.2020.wvj68

Table 1. Analysis of diagnosed canine parvovirus cases based on age, breed, season and vaccination status, presented to the veterinary teaching hospital, faculty of veterinary medicine, Mansoura University, Egypt from 2012 to 2013

Variable	Category	No of examined animals; n: 67	Positive case	Proportionate prevalence	P value
Age	1-3 month	47	31	65.9	0.068
	4-6 month	15	8	53.3	
	>6 month	5	1	20	
Breed	non-descript dogs	33	16	48.4	0.589
	German shepherd	15	4	26.7	
	Doberman	13	3	23.07	
	Griffon	6	1	16.6	
Season	Summer	35	27	77.1	0.222
	Spring	18	10	55.5	
	Autumn	8	2	25	
	Winter	6	1	16.6	
Vaccination	Vaccinated	26	5	19.2	0.163
	Unvaccinated	41	24	58.5	

RESULTS

Among the 67 clinically sick dogs, 40 were found positive for CPV infection. Prevalence of different risk factors (age, breed, season, localities) associated with CPV disease is summarized in (Table-1). The study showed that the overall prevalence of CPV infection was 59.7%. The prevalence of CPV infection in different age groups differed insignificantly ($P \leq 0.05$), and these were 65.9% for the age range of 1 to 3 months, 53.3% for 4 to 6 months, and 20% for above 6 months of ages. In the table 2, the maximum prevalence was noticed in non-descript dogs (48.5%) followed by German shepherds (26.7%), Doberman (23.07%), and Griffon (16.6%). Among different risk factors young, unvaccinated puppies and exotic breeds were more prone to CPV infection. Regarding the season, the higher prevalence was noticed in summer (77.1%) followed by spring (55.5%), autumn (25%), and winter (16.6%). Among the breeds, the rate of infections was encountered as 48.4% in native dogs, 26.7% in German shepherds, 23.07% in Dobermans, and Griffon (16.6%) were differed insignificantly ($P \leq 0.05$). The study revealed that in CPV infected dogs, 68.6% with diarrhoea, 17.2% with vomiting, 10.7% with tachycardia, and 3.3% with sudden death were recorded (Figure-1). Moreover, 19.2% of vaccinated dogs and 58.9% of non-vaccinated dogs were significantly ($P \leq 0.05$) affected with CPV infection**.**

Table 2. Different clinical signs observed among the canine parvovirus cases presented to the veterinary teaching hospital, faculty of veterinary medicine, Mansoura university, Egypt from 2012 to 2013); n= 122

Variable	Category	Positive case	Percentage of positive cases (%)
Bloody diarrhea	Yes	30	24.5
	No	92	75.5
Mucoid diarrhea	Yes	54	44.2
	No	68	55.8
Vomiting	Yes	21	17.2
	No	101	82.8
Tachycardia	Yes	13	10.7
	No	109	89.3
Sudden death	Yes	4	3.3
	NO	118	96.7

n= number of examined animals

DISCUSSION

The overall prevalence of CPV infection was found to be 59.7%. Archana et al. (2010), Roy et al. (2010), and Khare et al. 2019 reported similar higher prevalence 45.30% and 65.04% in Jabalpur and Chhattisgarh, respectively. In contrast to the present findings, Wazir et al. (2013) reported a lower prevalence in Jammu who found a 6.93% prevalence of CPV infection. The present findings indicated the presence of CPV in dogs. An immuno-chromatographic assay-based kit was useful in supporting the diagnosis, and it was also useful particularly in epidemiological studies. The variation in the prevalence of CPV might be due to the diagnostic tests variation among different studies. Due to the wide variation in

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the number of samples tested by the different workers in the different geographically areas, comparison in this regard would be of little value. However, these variations observed in the prevalence were difficult to explain due to the different study areas and differences in the methods of sample analysis. The age prevalence of CPV infection revealed that maximum prevalence in the dogs of 0 to 3 months of age was 65.9%, followed by 4 to 6 months of age was about 53.3%, and > 6 months of age was 20%. These finding agree with Sakulwira et al. (2003) and Al-hosary (2016) that reported a higher prevalence of CPV infection in 3 to 6 months old dogs. However, Roy et al. (2010), Mukhopadhyay et al. (2012), Dongre et al. (2013) and Khare et al. (2019) who stated that, the age wise prevalence of CPV infection revealed maximum prevalence in the dogs of 0-3 months of age i.e. 11.9%, followed by 3-6 months of age i.e. 7.09%, 6-12 months of age i.e. 5.31% and above 12 months of age i.e. 1.11%. The higher prevalence in the dogs of 0 to 3 months of age may be attributed to the higher susceptibility of enterocytes to the viral tropism. Houston et al. (1996) stated that during weaning, enterocytes of the intestinal crypts have a higher mitotic index because of the changes in bacterial flora and diet, and were therefore more susceptible. Thus, the higher prevalence of CPV infection in young dogs (0 to 3 months) was probably because of the close affinity of the virus with rapidly dividing cells of the intestine, which decline with the advancement of age (Banja et al., 2002; Khare et al., 2019). Above one year age, very few incidences was recorded which might be possible due to developing antibodies in the adults either due to vaccination schedule practiced or due to mild exposure to virus leading to build up antibody in the host or some other reasons that need to be explored. Canine Parvovirus infection was reported in the various breeds of the dogs including German shepherds, Doberman, Griffon, and non-descript dogs. The maximum prevalence was noticed in non-descript dogs which was about 48.4% followed by a German shepherd, Doberman, and Griffon in which prevalence was found to be 26.7%, 23.07% and 16.6% respectively. The higher prevalence in non-descript dogs was also reported by Tajpara (2003), Archana et al. (2010) and Wazir et al. (2013), while, Roy et al. (2010), Dongre et al. (2013), Al-hosary (2016) and Khare et al. (2019) reported a higher prevalence in German shepherds breed of dogs. The higher prevalence in non-descript breeds might be due to the higher population density of this breed making their proximity to spread the infection or poor vaccination schedule being followed by the owners of the non-descript breeds due to the lack of awareness among them. No specific comment can be made on breed susceptibility as the population density of the breed varies from one geographical area to another (Archana et al., 2010; Khare et al., 2019). The prevalence was higher in non-vaccinated dogs compared to the vaccinated ones. The finding was in agreement with (Godsall et al., 2010) where unvaccinated puppies aged between six weeks and six months were at greatest risk of developing CPV infection. The higher prevalence of CPV infection in non-vaccinated dogs might be due to a lack of protective immunity. In vaccinated dogs, CPV infection might occur due to incomplete or ineffective primary vaccination course, or a failure of vaccination. The main clinical signs of CPV disease are bloody diarrhea, vomiting, and dehydration. The findings of present study were in agreement with Thomson and Gagnon (2005), Prittie (2004) and Khare et al. (2019). Bloody diarrhea and vomiting were observed in 68.7% and 17.2% of CPV positive dogs respectively. A similar finding was also reported previously by Thomson and Gagnon (2005), Mylonakis et al. (2016). 84.28% of CPV positive dogs had severe dehydration which was supported by the previous study (Laforcade et al., 2003; Mylonakis et al., 2016).

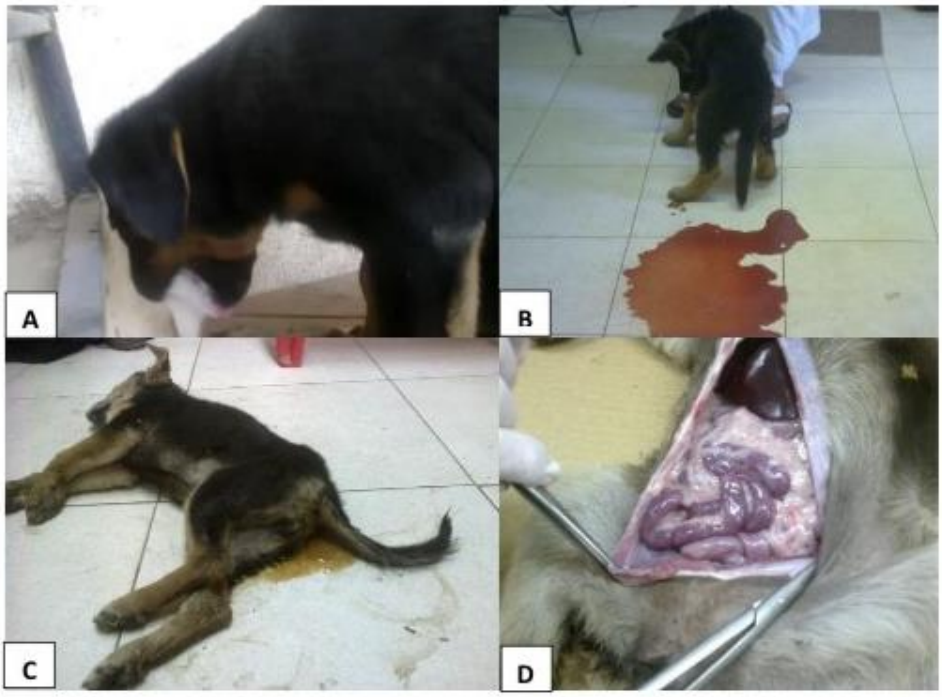

Figure 1. Different clinical signs observed among the dogs infected with canine parvovirus virus. Vomiting (A), bloody diarrhea (B), mucoid diarrhea (C), and hemorrhagic enteritis (D).

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CONCLUSION

Canine Parvovirus is an infective and highly contagious viral disease of dogs. Dogs of all age groups are infected, but puppies in the age less than 3 months were more susceptible than adults. Both non-descript dogs and the breeds like German shepherds, Dobermans and Griffon are susceptible to CPV infection. The rate of the infection was higher in non-vaccinated than vaccinated dogs. So, the identification of the potential risk factors of the disease may be helpful to construct the ideal preventive measures.

DECLARATIONS

Acknowledgments

The authors express their sincere gratitude to Pet Animal Vaccine Department, Vaccine and Serum Research Institute, Abbasia, Cairo, Egypt for all the support provided in laboratory experiments. Funding of this study was on the expense of the authors and did not receive any funds from any agencies. Nonetheless, the authors acknowledged the support and cooperation of the administration and staff of the Department of Internal Medicine and Infectious Diseases, Faculty of veterinary Medicine, Mansoura University, Egypt.

Competing interests

All authors declare no competing interests that might interfere with the data provided in the current manuscript.

Author's contributions

Manuscript preparation, writing-reviewing, editing and statistical analysis were conducted by Mohamed Sayed-Ahmed. All authors have contributed to lab work, and the experimental design. All authors read, revised and approved the final manuscript.

Consent to publish

All the authors approved and agreed to publish the manuscript

REFERENCES

Albaz A, Sayed-Ahmed M, Younis E and Khodier M (2015). Investigation of the antiviral effect of acyclovir on canine parvovirus infection. Pharmacy Pharmacology International Journal, 2(2): 14. DOI: http://dx.doi.org/10.15406/ppij.2015.02.00014

Al-hosary AAT (2016). Prevalence of Parvovirus Infection in Household Dogs with Special Reference to its Effects on Some Blood Parameters. Alexandria Journal of Veterinary Sciences, 51(2): 174-177. DOI: http://dx.doi.org/10.5455/ajvs.236999

Archana S, Gupta DK and Kumar B (2010). Epidemiology of canine parvovirus infection. Indian Journal of Veterinary Research, 18: 42-44. DOI: http://dx.doi.org/10.14202/vetworld.2015.33-37

Banja BK, Sahoo N, Das PK and Ray SK (2002). Clinico-therapeutic aspects of gastroenteritis in dogs. Indian Veterinary Journal, 79: 837-840. Available at: https://agris.fao.org/agris-search/search.do?recordID=IN2004000441

Brown AJ and Otto CM (2008). Fluid therapy in vomiting and diarrhea. Veterinary Clinics of North America: Small Animal Practice, 38(3): 653-75. DOI: http://dx.doi.org/10.1016/j.cvsm.2008.01.008

Burleson FG, Chambers TM; Wiedbrauk DL (2014). Virology: a laboratory manual. 3rd ed. Saint Louis: Elsevier Science pp. 223-233. Available at: https://www.worldcat.org/title/virology-a-laboratory-manual/oclc/1041746460?referer=di&ht=edition

Coles EH (1986). Veterinary clinical pathology 4th edition. W. B. Saunders Company, Philadelphia, London and Toronto, pp. 1-8. Available at: https://openlibrary.org/books/OL2531334M/Veterinary_clinical_pathology

Decaro N, Desario C, Campolo C, Cavalli A, Ricci D, Martella V, Tempesta M and Buonavoglia C (2004). Evaluation of the lactogenic immunity to canine parvovirus in pups. New Microbiologica, 27: 375-379. Available at: https://pubmed.ncbi.nlm.nih.gov/15646052/

Decaro N, Elia G, Martella V, Desario C and Campolo M (2005a). A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 DNA in the feces of dogs. Veterinary Microbiology, 105: 19-28. DOI: http://dx.doi.org/10.1016/j.vetmic.2004.09.018

Decaro N, Desario C, Campolo M. Elia G, Martella V, Ricci D, Lorusso E and Buonavoglia C (2005b). Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. Journal of Veterinary Diagnostic Investigation, 17(2): 133-138. DOI: http://dx.doi.org/10.1177/104063870501700206

Desario C, Decaro N, Campolo M, Cavalli A, Cirone F, Elia G, Martella V, Lorusso E, Camero M and Buonavoglia C (2005). Canine parvovirus infection: which diagnostic test for virus. Journal of Virological Methods, 126(1): 179-185. DOI: http://dx.doi.org/10.1016/j.jviromet.2005.02.006

Dongre J, Mehta H and Maheshwari P (2013). Hematological changes in dogs affected with canine parvo virus Infection, Veterinary Practitioner, 14(2): 235-238. Available at: http://www.vetpract.in/Archives/Dec-2013/VP-14-2--DEC-2013/

Faz M, Martínez JS, Quijano-hernández I and Fajardo R (2017). Reliability of clinical diagnosis and laboratory testing techniques currently used for identification of canine parvovirus enteritis in clinical settings. The Journal of Veterinary Medical Science, 79(1): 213–217. DOI: http://dx.doi.org/10.1292/jvms.16-0227

Goddard A and Leisewitz AL (2010). Canine parvovirus. Veterinary Clinics of North America: Small Animal Practice, 40(6): 1041-1053. DOI: http://dx.doi.org/10.1016/j.cvsm.2010.07.007

Godsall S, Clegg S, Stavisky J, Radford A and Pinchbeck G (2010). Epidemiology of canine parvovirus and coronavirus in dogs presented with severe diarrhoea to PDSA Pet Aid hospitals, Veterinary Record, 167(6): 196-201. DOI: http://dx.doi.org/10.1136/vr.c3095

Hao L, Wang Y, Bai C, Jin Y, Zheng D, Chen L, Li X, Deng J and Tian K (2017). Preparation and Application of two Monoclonal Antibodies against Canine Parvovirus Vaccine and Field Strains. Journal of Vaccines and Immunology, 3(1): 1-4. DOI: https://doi.org/10.17352/jvi.000020

Hong C, Decaro N, Desario C, Tanner P, Camila Pardo M, Sanchez S, Buonavoglia C and Saliki JT (2007). Occurrence of canine parvovirus type 2c in the United States Journal of Veterinary Diagnostic Investigation, 19(5): 35-539. DOI: http://dx.10.1177/104063870701900512

Houston DM, Ribble CS, and Laurie L (1996). Risk factors associated with Parvovirus enteritis in dogs: 283 cases. Journal of American Veterinary Medicine Association, 208: 542-546. Available at: https://pubmed.ncbi.nlm.nih.gov/8603904/

Laforcade AM, Freeman LM, Shaw SP, Brooks MB, Rozanski EA and Rush JE (2003). Hemostatic changes in dogs with naturally occurring sepsis. Journal of Veterinary Internal Medicine, 17: 674-679. DOI: http://dx.10.1111/j.1939-1676.2003.tb02499.x

Lamm CG and Rezabek GB (2008). Parvovirus infection in domestic companion animals. Veterinary Clinics of North America: Small Animal Practice, 38(4): 837-50. DOI: http://dx.10.1016/j.cvsm.2008.03.008

Kalli I, Leontides LS, Mylonakis ME, Adamama-Moraitou K, Rallis T and Koutinas AF (2010). Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. Research in Veterinary Science, 89(2): 174-178. DOI: http://dx.10.1016/j.rvsc.2010.02.013

Kelly WR (1990). Veterinary Clinical Diagnosis. 3rd edit. Bailliere Tindall, London. Available at: https://www.amazon.com/Veterinary-Clinical-Diagnosis-W-R-KELLY/dp/0702009067

Khare DS, Gupta DK, Shukla PC, Das G, Tiwari A, Meena NS and Khare R (2019). Prevalence of canine parvovirus infection in dogs in Jabalpur (M.P.). Journal of Entomology and Zoology Studies, 7(3): 1495-1498. Available at: https://www.entomoljournal.com/archives/?year=2019&vol=7&issue=3&ArticleId=5386

Marcovich JE, Stucker KM, Carr AH, Harbison CE, Scarlett JM and Parrish CR (2012). Effects of canine parvovirus strain variations on diagnostic test results and clinical management of enteritis in dogs. Journal of American Veterinary Medical Association, 241(1): 66-72. DOI: http://dx.10.2460/javma.241.1.66

Martella V, Cavalli A, Decaro N, Elia G, Desario C, Campolo M, Bozzo G, Tarsitano E and Buonavoglia C (2005). Immunogenicity of an intranasally administered modified live canine parvovirus type 2b vaccine in pups with maternally derived antibodies. Clinical and Diagnodstic Laboratory Immunology, 12(10): 1243-1245. DOI: http://dx.10.1128/CDLI.12.10.1243-1245.2005

McAdaragh JP, Eustis SL, Nelson DT, Stotz I and Kenefick K (1982). Experimental infection of conventional dogs with canine parvovirus. American Journal of Veterinary Research, 43: 693-696. Available at: https://europepmc.org/article/med/7073093

McCaw DL and Hoskins JD (2006). Canine viral enteritis. In: Green CE, editor. Infectious Diseases of the Dog and Cat. 4th ed. St Louis, MO: Saunders, pp. 63-73. Available at: https://www.elsevier.com/books/infectious-diseases-of-the-dog-and-cat/sykes/978-1-4160-6130-4

Mukhopadhyay HK, Thanislass J, Pillai RM, Antony PX and Rao VN (2012). Detection of canine distemper by nested PCR. The Indian veterinary journal, 89(11): 9-11. Availabe at: http://www.connectjournals.com/archives.php?bookmark=CJ-000735

Mylonakis EM, Kalli I and Rallis ST (2016). Canine parvoviral enteritis: an update on the clinical diagnosis, treatment, and prevention. Veterinary Medical Research Reports, 7: 91-100. DOI: http://dx.10.2147/VMRR.S80971

Pollock RVH and Carmichael LE (1988). Canine viral enteritis. In: Barlough JE, editor. Manual of small animal infectious diseases. New York: Churchill Livingston, pp. 101-117. Available at: https://www.amazon.com/Manual-Small-Animal-Infectious-Diseases/dp/0443085080

Prittie J (2004). Canine parvoviral enteritis: a review of diagnosis, management, and prevention. Journal of Veterinary Emergency Critical Care, 14: 167-176. DOI: https://doi.org/10.1111/j.1534-6935.2004.04020.x

Rossitter PB, and Jessett DM (1982). Microtiter techniques for the assay of rinderpest virus and neutralizing antibody. Research of Veterinary Sciences, 3(2): 253. DOI: https://doi.org/10.1016/S0034-5288 (18)32424-X

Roy S, Roy M and Sagar KA (2010). Haemato-biochemical and therapeutic studies of canine parvovirus infection. Intas Polivet, 11: 344-347. Available at: https://www.cabdirect.org/cabdirect/abstract/20113131820

Sakulwira K, Vanapongtipagorn PA, Theamboonlers K, Oraveerakulk and Poovorawan Y (2003). Prevalence of canine coronavirus and parvovirus infection with gastroenteritis in Thailand. Veterinární medicína, 48(6): 163-167. DOI: https://doi.org/10.17221/5764-VETMED

Schmitz S, Coenen C, König M, Thiel HJ and Neiger R (2009). Comparison of three rapid commercial canine parvovirus antigen detection tests with electron microscopy and polymerase chain reaction. Journal of Veterinary Diagnostic Investigation, 21(3): 344-345. DOI: https://doi.org/10.1177/104063870902100306

Sykes JE (2014). Canine parvovirus infections and other viral enteritides. In: Sykes JE, editor. Canine and Feline Infectious Diseases. 1st ed. St Louis, MO: Elsevier, PP. 141-151. Available at: https://www.elsevier.com/books/canine-and-feline-infectious-diseases/sykes/978-1-4377-0795-3

To cite this paper: Sayed-Ahmed MZ, Elbaz E, Younis E and Khodier M (2021). Canine Parvovirus Infection in Dogs: Prevalence and Associated Risk Factors in Egypt. *World Vet. J.*, 10 (4): 571-577. DOI: https://dx.doi.org/10.54203/scil.2020.wvj68

Tajpara M (2003). Epidemiological study on canine parvoviral diarrhoea in dogs by Polymerase Chain Reaction and its comparative efficacy with other diagnostic assays. M.V.Sc. and A.H. thesis (Veterinary Medicine). Anand Agricultural University, Anand. Available at: https://krishikosh.egranth.ac.in/handle/1/5810047927

Hasan T, Bairagi N, Hossan MM, Saddam Hossain MD, Nath C, Chowdhury S, and Paul P (2018). Prevalence of canine parvovirus infection in dog in relation to CVASU, Bangladesh and VCRI-MVC, India", International Journal of Current Research, 10(4): 67720-67725. Available at: http://www.journalcra.com/

Thomson G and Gagnon A (2005). Canine gastroenteritis associated with a parvovirus-like agent. The Canadian Veterinary Journal, 19: 346. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1789433/

Wazir VS, Gupta SK, Hussain K and SinghVP (2013). Prevalence of canine parvo virus infection in dogs of Jammu district of Jammu dnd Kashmir. Veterinary Practitioner, 14(2): 296-297. Available at: http://www.vetpract.in/Archives/Dec-2013/VP-14-2--DEC-2013/

Wilkes RP, Lee PA, Tsai YL, Tsai CF, Chang HH, Chang HG and Wang HT (2015). An insulated isothermal PCR method on a field-deployable device for rapid and sensitive detection of canine parvovirus type 2 at points of need. Journal of Virological Methods, 220: 35-38. DOI: http://dx.doi.org/10.1016/j.jviromet.2015.04.007

To cite this paper: Sayed-Ahmed MZ, Elbaz E, Younis E and Khodier M (2021). Canine Parvovirus Infection in Dogs: Prevalence and Associated Risk Factors in Egypt. *World Vet. J.*, 10 (4): 571-577. DOI: https://dx.doi.org/10.54203/scil.2020.wvj68

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 578-586, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj69

Survival and Productivity of Culinary Herb Species in a Nutrient Film Technique-type Aquaponic System with Nile Tilapia

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ORIGINAL ARTICLE
pii: S232245682000069-10
Received: 29 Oct 2020
Accepted: 10 Dec 2020

ABSTRACT

Aquaponics is an evolving technology for producing plants and fish (or other aquatic organisms) in an integrated water recirculating system. However, the survival and productivity of terrestrial plants in aquaponic systems have not been evaluated for most plant species. The present study aimed to analyze the survival rate, growth, and biomass production of eight culinary herbs, commonly used in Guatemala, in a Nutrient Film Technique-type (NFT) aquaponic system with Nile tilapia (*Oreochromis niloticus*). The investigated herbs included coriander (*Coriandrum sativum*), parsley (*Petroselinum crispum*), peppermint (*Mentha spicata*), thyme (*Thymus vulgaris*), samat (*Eryngium foetidum*), oregano (*Plectranthus amboinicus*), dill (*Anethum graveolens*), and basil (*Ocimum basilicum*). A total of 50 individuals of each herb species and 150 juvenile Nile tilapias were distributed in 5 aquaponic modules. The survival rate, growth, and biomass production were measured for herbs and tilapias. All the herb species survived against the NFT aquaponic conditions. The findings indicated that the herb survival was species-dependent and ranged 42-98%. There was a significant effect of the herb species both on height and biomass gains. Post hoc comparison showed interspecific differential abilities to grow biomass in NFT aquaponics conditions. Among the investigated herbs, *M. spicata* and *O. basilicum* were the most productive species. Refinement in the selection of initial plants and aquaponic management could improve plant performance.

Keywords: Ecological production, Hydroponics, *Oreochromis*, Recirculating water, Sustainable aquaculture

INTRODUCTION

Aquaponics is an evolving technique to tackle climate change challenges in the agriculture industry (Tyson et al., 2011; König et al., 2018; Palm et al., 2018). In contrast to traditional agricultural resource management, an aquaponic system saves water and reduces waste, costs, and environmental contamination (Lennard and Leonard, 2006; Rizal et al., 2018). The reutilization of water and the use of waste from aquatic organisms to feed the plants in a closed system makes aquaponics a social, ecological, and healthier alternative to meet the sustainable development goals proposed by United Nations (United Nations, 2015; Li et al., 2018; Rizal et al., 2018). Regardless of the fact that aquaponics has been criticized by organic farmers for the industrial origin of the substrate and the absence of soil (Kledal et al., 2019), this form of producing has also been considered the agriculture of the future (Shafeena, 2016).

Although aquaponics has demonstrated to be effective and efficient for small and large scale productions of lettuce, tomatoes, and other salad greens (Somerville et al., 2014; Love et al., 2015), it seems that it is not possible for all plant species to survive and grow in aquatic conditions (Guerra-Centeno et al., 2016). Therefore, it is necessary to identify plant species that can be grown and produced in aquaponic conditions.

Guatemala is a megadiverse country, where many plant species are traditionally used for feeding, spicing, medicinal, and other cultural purposes (Cáceres, 1996; Villar-Anleu, 1998; Knapp and Davidse, 2006). However, there is a dearth of research addressing the productivity of Guatemalan plant species in aquaponic conditions (Guerra-Centeno et al., 2016). Since biomass production is a function of survival and growth, the aim of the current study was to examine these features in eight culinary herb species mainly used in Guatemala (coriander [*Coriandrum sativum* L.], parsley [*Petroselinum crispum* (Mill.) Fuss], peppermint [*Mentha spicata* L.], thyme [*Thymus vulgaris*, L.], samat [*Eryngium foetidum*, L.], oregano [*Plectranthus amboinicus* (Lour.) Spreng], dill [*Anethum graveolens* L.] and basil [*Ocimum basilicum* L.]) using a Nutrient Film Technique-type (NFT) aquaponic system with Nile tilapia (*Oreochromis niloticus* L.).

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MATERIALS AND METHODS

Ethical approval

This study was reviewed and approved by the Graduate School Bioethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry of the University of San Carlos of Guatemala, Guatemala City.

Study site

The study was performed inside a greenhouse, in the facilities of the *Instituto de Investigación en Ciencia Animal y Ecosalud* (IICAE) at the Veterinary and Animal Husbandry Faculty, University of San Carlos of Guatemala, located in Guatemala City.

Study design

The samples in the current study consisted of eight herb species. Depending on the herb species, seedlings or cuttings were used as propagules (Table 1). The performance of each sample was examined using aquaponic modules (Figure 1). Each module had 8 perforated pipelines and each pipeline had 10 holes. Each pipeline was considered as a block and only housed individuals of a single herb species. Therefore, each aquaponic module held 80 plant individuals (10 per species). This arrangement was replicated in five aquaponic modules. The observation period in aquaponic conditions lasted 90 days, from June to August 2018.

Aquaponic system and modules

The aquaponic modules at the IICAE consisted of a water tank, a pump, a biofilter, and a pipeline circuit. For each module, one plastic 750 L water tank was used. For pumping the water, one submersible 0.5 horsepower pump (BOS Truper®, Mexico) was installed and a hose was coupled to conduct the water flow from the tank to a biofilter (Clear Choice PF-1, Tetra®, Blacksburg, VA, USA). Two 4' PVC pipeline circuits were installed in a zigzag arrangement (Figure 1). Water flow was regulated using a nutrient film technique-type (NFT) design.

Herb management

The herb materials were obtained from commercial suppliers (Superb Superseed S.A and Vivero Botanik, Guatemala City). Prior to the observation period in aquaponic conditions, coriander, parsley, samat, dill, and basil seeds as well as peppermint, thyme, and oregano cuttings were placed into seedbeds with fertilized soil where they remained for up to eight weeks. The herbs were then transplanted to the NFT modules. Only the propagules that had three to five leaflets were selected to be transplanted to the aquaponic conditions.

Tilapia management

While the plant material was in the seedbeds, 300 juvenile Nile tilapia, weighing about 1.5 g each, were obtained from a commercial farm (Cazali Tilapia Farm, Escuintla, Guatemala) and put under quarantine for 30 days in a plastic 1000 L water tank. After that period, 30 tilapia organisms, weighing 2-4 g each, were selected and placed in the water tank of each NFT module (i.e., a total of 150 fish) to start the aquaponic phase. All tilapia organisms were managed following the Food and Agriculture Organization recommendations for small-scale aquaponic food production (Somerville et al., 2014). During the first 30 days of the study, tilapias were fed 45% protein extruded food (Tilapia™, Alcon, Honduras), and from day 31 to day 90, tilapias were fed 32% protein extruded food (Tilapia™, Alcon, Honduras).

Measurements

The height/length and weight of each herb and tilapia organism were measured and recorded at days 0, 45, and 90 of the aquaponic observation period. The height of the plants was measured from the base of the stem to the tip of the apical meristem whilst the length of the tilapia organisms was measured from the tip of the snout, with mouth closed, to the extreme of the tail fin. Heights and lengths were measured in centimeters using a measuring tape and approximated to the nearest tenth. The weight was measured in grams using an electronic balance (iBalance 700™, My Weigh, AZ, USA) and approximated to the nearest tenth. Differences between initial and final measurements were recorded. Growth was considered as both height/length and weight (biomass) gains. The values related to water temperature, pH, electric conductivity, and total dissolved solids were measured every 15 days using a digital tester (HI 991300™, Hanna, RI, USA) and the mean values were calculated.

Statistical analyses

The descriptive statistics included the calculation of the mean and standard deviations for height/length and weight measurements of herbs and tilapia. Since the obtained data did not meet the assumptions of normality, the non-

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parametric test of Kruskal-Wallis was used to compare the means values of plant longitudinal growth and biomass production. In this regard, the post hoc test of Mann-Whitney pairwise was run to determine the specific differences. The correlation between survival rate and herb species was analyzed using the Pearson's chi-squared test. Statistical analyses were performed using Past Program®, version 3.20 (Hammer et al., 2001). The significance level for the statistical tests was $p < 0.05$.

Figure 1. Components of a nutrient film technique (NFT) aquaponic module at the IICAE greenhouse, including (1) water container with a submersible pump; (2) hose to conduct the water from the tank to the biofilter; (3) biofilter; (4) PVC pipe circuits for water circulation and plant hosting.

Table 1. Descriptive statistics of experimental arrays of eight investigated herbs species in five aquaponics modules

Herb species	Number of individuals per pipeline, per module	Total number of individuals	Type of propagule
Coriander (*Coriandrum sativum)*	10	50	Seedling
Parsley (*Petroselinum crispum*)	10	50	Seedling
Peppermint (*Mentha spicata*)	10	50	Cutting
Thyme (*Thymus vulgaris*)	10	50	Cutting
Samat (*Eryngium foetidum*)	10	50	Seedling
Oregano (*Plectranthus amboinicus*)	10	50	Cutting
Dill (*Anethum graveolens*)	10	50	Seedling
Basil (*Ocimum basilicum*)	10	50	Seedling
Total	80	400	---

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RESULTS

The findings of the current study indicated that all herb species survived to the NFT aquaponic conditions. Survival rates were within the range of 0.42-0.98 (Table 2) and were associated with herb species ($p < 0.01$). There was a significant effect of the herb species both on height ($p < 0.01$) and biomass ($p < 0.01$) gains. The obtained results of post hoc comparison were indicative of interspecific differential abilities to grow biomass in NFT aquaponics conditions (Table 3). Peppermint and basil were the most productive species. Regarding biomass production, the growth rate of plants varies according to their species (Figures 2 and 3). For most plant species, growth was marked by a slow initial phase, followed by an accelerated phase (Figure 4).

Survival and productive parameters of Tilapia

Tilapias survived and showed a growing response in the water tanks of the NFT aquaponic modules (Table 4).

Water parameters

The mean values related to water temperature, pH, electric conductivity, and total dissolved solids were 24.9 ± 1.2 °C, 7.8 ± 0.5, 458.1 ± 309.7 µS, 232.7 ± 157.4 ppm, respectively.

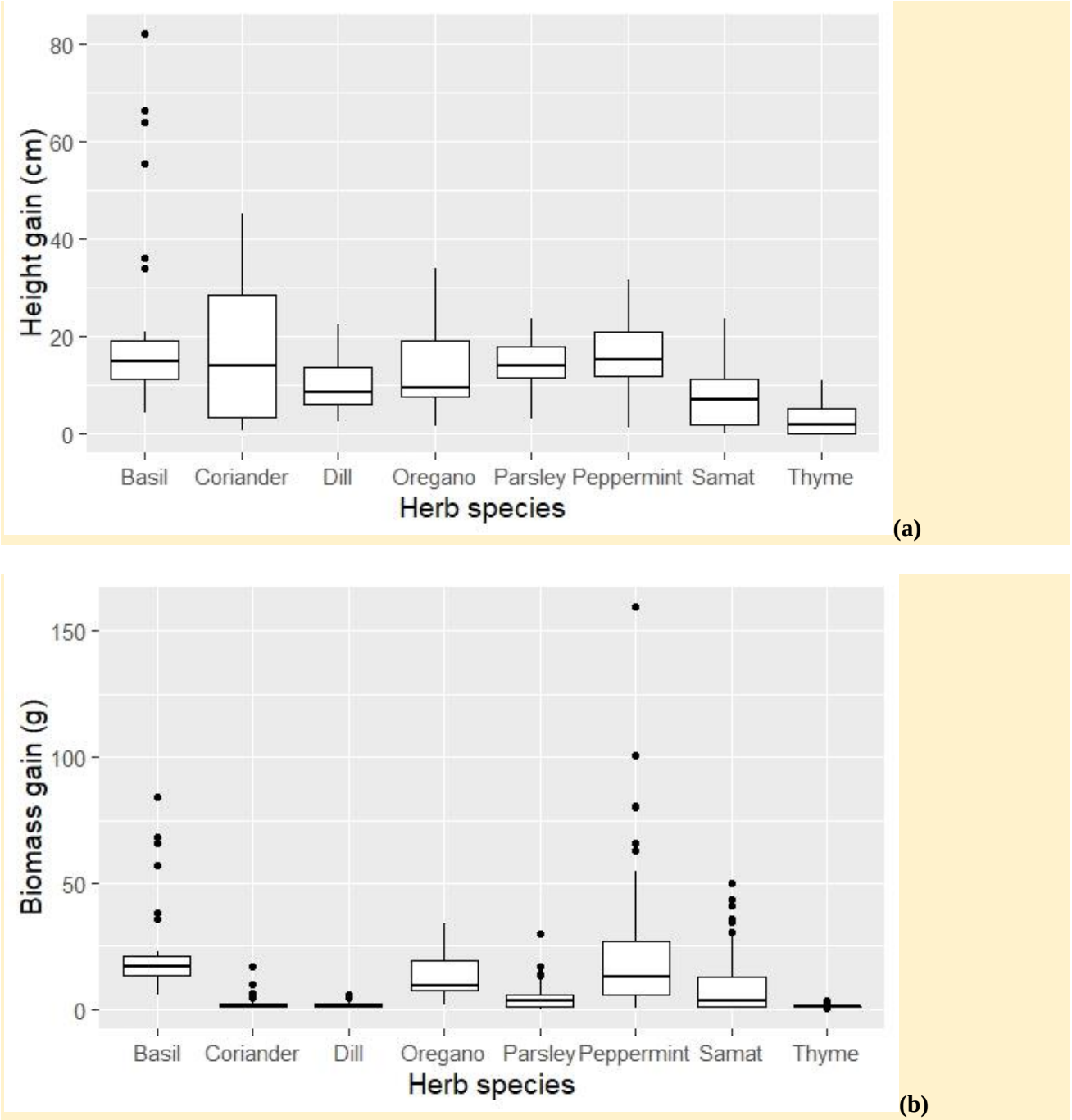

Figure 2. Variation in biomass production for survivors of eight herb species in NFT aquaponic conditions, by day 90 of observation: *a* = height gain; *b* = biomass gain.

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Figure 3. Variation in growth of peppermint individuals from the same block, by day 90 of observation period.

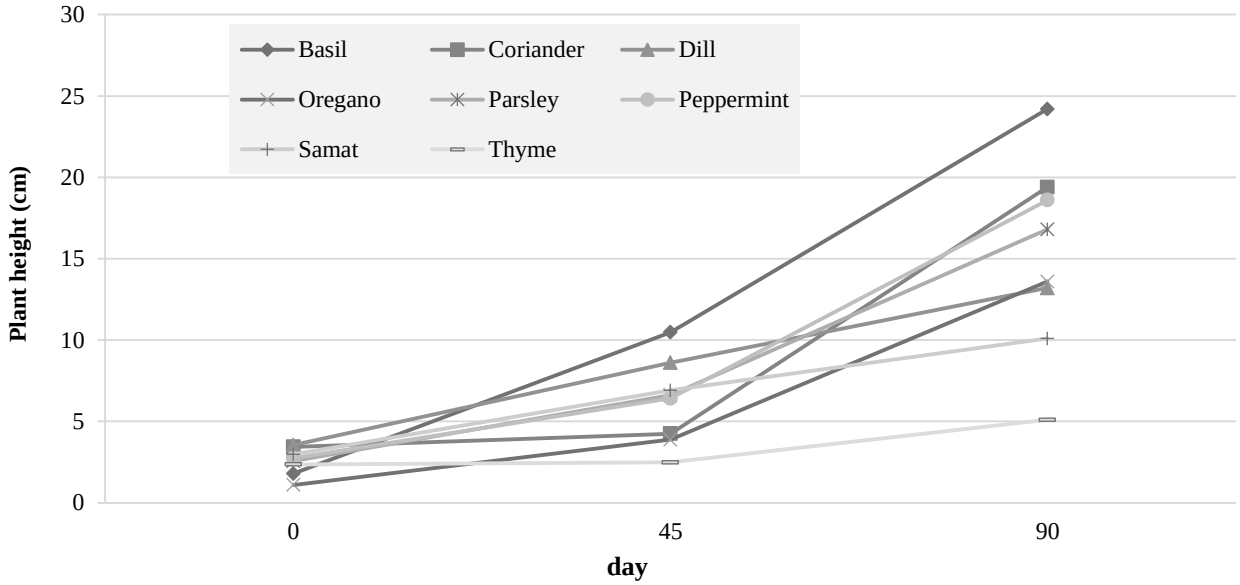

Figure 4. Mean height for eight species of culinary herbs in an NFT aquaponic system from transplantation (day 0) to the end of the observation period (day 90).

Table 2. Survival rate and biomass production of eight herb species in an NFT aquaponic system after 90 days of observation

Herb species	Final number (survival rate)	Height gain median (range) [cm]	Biomass gain median (range) [g]	Total biomass per species at day 90 (g)
Coriander	19 (0.38)	16.0 (4.0-47.0)	1.7 (0.5-16.7)	69.2
Parsley	49 (0.98)	17.0 (5.0-25.0)	3.3 (0.0-29.9)	242.8
Peppermint	49 (0.98)	17.0 (4.0-34.0)	12.7 (0.5-159.5)	1168.9
Thyme	38 (0.76)	4.5 (1.0-13.0)	0.9 (0.5-3.5)	48.7
Samat	44 (0.88)	9.0 (1.0-25.0)	3.1 (0.2-49.8)	418.4
Oregano	21 (0.42)	10.0 (2.0-35.0)	9.2 (1.4-33.8)	265.4
Dill	29 (0.58)	12.0 (2.0-25.0)	1.1 (0.5-5.9)	54.9
Basil	27 (0.54)	17.0 (6.0-84.0)	16.7 (5.9-83.9)	1006.5

The initial number of individuals was 50 for all species. NFT: nutrient film technique

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Table 3. Mann Whitney Pairwise post hoc comparisons for biomass gain values of eight herb species

Items	Coriander	Parsley	Peppermint	Thyme	Samat	Oregano	Dill	Basil
Coriander	-	.011	< .001	.084	.037	< .001	.728	< .001
Parsley	.011	-	< .001	< .001	.991	< .001	.001	< .001
Peppermint	< .001	< .001	-	< .001	< .001	.356	< .001	.129
Thyme	.084	< .001	< .001	-	.002	< .001	.027	< .001
Samat	.037	.991	< .001	.002	-	.005	.045	< .001
Oregano	< .001	< .001	.356	< .001	.005	-	< .001	.007
Dill	.728	.001	< .001	.027	.045	< .001	-	< .001
Basil	< .001	< .001	.129	< .001	< .001	.007	< .001	-

Table 4. Survival and production parameters of Nile tilapia in an NFT aquaponic system at day 90.

Parameters	Mean (SD)
Mean final number per module	27.0 (1.9)
Mean survival rate per module (%)	90.0 (6.2)
Mean final individual length (cm)	17.3 (2.2)
Mean final individual weight (g)	106.2 (37.9)
Mean biomass per tank (g)	2866.2 (660.9)

NFT: nutrient film technique; SD: standard deviation

DISCUSSION

All the evaluated species showed some potential to survive and grow in NFT aquaponics conditions. However, the observed variation in survival rate and biomass production suggested the existence of some factors affecting both individual and species-related abilities for production in aquaponic conditions. The outcomes of the current study underlined the more recent studies on the differential abilities of plant species in terms of biomass adaptation and production in NFT aquaponics (Hu et al., 2015; Guerra-Centeno et al., 2016; Valdez-Sandoval et al., 2017), according to which a large number of factors could contribute to such differences. To name a few, the findings of the present study suggested propagule size (in the early aquaponic stage), hydraulic charge (water flow), and root-covering algae as the most important growth-limiting factors. Life in aquatic conditions might be the most important constrain to earth-evolved plant species. It is known that flooding hinders growth and development for most vascular plants (Jackson and Colmer, 2005). The major constrain in flooding conditions is an inadequate supply of oxygen to the submerged roots. Oxygen diffusion in water is about 10,000 times slower than in air (Colmer, 2003). Some flood-resistant plant species can survive and even grow in flooding conditions due to various evolutive adaptations, including metabolic, hormonal, and morphological responses (e.g., adventitious roots and aerenchyma, Blom and Voesenek, 1996; Visser et al., 2000, Visser et al., 2003). As a result, it can be concluded that although it is impossible for all the plant species to survive in floodplains, some plant species could survive in aquaponic conditions if oxygen is available to plant roots via circulating water (Maucieri et al., 2018).

Regardless of water recirculation and oxygen carriage in aquaponic NFT systems, a hemp wick is required in case of small transplanted propagules to pass water from the nutrient film flow to the plant substrate by capillarity. This issue usually causes water stagnation in the substrate inside the net pots that can, to some extent, decreased oxygen availability for the plant. In the present study, there were instances of water stagnation, death or poor foliage, and root development in some coriander, parsley, thyme, oregano, and dill individuals which neither survived nor showed growth.

Bigger propagules with well-developed roots, such as those of beans and peppers, can easily reach the water flow and survive the water stasis inside the plant substrate (Valdez-Sandoval et al., 2017). Leaves and stems development could favor survival in early aquaponic stages as atmospheric oxygen can be captured via stomas and lenticels through convection (Pardos, 2004). Other aquaponic systems, such as a raft or floating bed, could favor the survival and growth of little propagules, as the tiny roots (i.e., typical of early propagules) directly contact the recirculating water. However, it should be mentioned that a larger amount of water may lead to a lower density of nutrients and less absorption by the propagules (Lennard and Leonard, 2006).

Another issue of consideration in the current study was related to the association between algae and suspended solids accumulation in the roots and delayed plant growth. The algae or solids film that forms on the root system could

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block the diffusion of oxygen and other nutrients from the water film to the roots (Roosta and Afsharipoor, 2012; Hu et al., 2015). However, this possible growth-inhibition factor must be examined more thoroughly in experimental conditions.

A third factor that could decrease survival and growth is the speed of the water flow. It is known that high hydraulic loads cut the contact time between roots and water, and consequently hinder the absorption of nutrients (Wongkiew et al., 2017). Additionally, the mechanical action of the water flow that permanently impacts the root system of the plant can affect the formation of adventitious roots, an important factor for radicular aerenchyma formation (Blom and Voesenek, 1996). The low growth of many thyme individuals (i.e., the size remained almost the same as transplantation size) in the present study could be due to the effect of such mechanical action. However, the obtained results of such low growth may vary according to the plant species and, therefore, this issue should be empirically investigated for all plant species.

Concerning the growing behavior of plants, the generalized slow growth rate observed in the first days is consistent with our earlier observations (Guerra-Centeno et al., 2016; Valdez-Sandoval et al., 2017). This slow growth rate was probably caused by a low nitrogenated waste production when the fish were still small. Once the fish get larger in size, the amount of available nitrogenous waste allows the accelerated growth of the plants, depending on their species (Hu et al., 2015). Additionally, many of the herb individuals exhibited a retarded radicular development during some weeks after transplantation to the NFT modules. Based on the findings of the current study, it can be concluded that the stagnant phase that eventually retarded growth and biomass production could be overcome using more mature propagules with well-developed roots. Direct contact of well-developed roots with the recirculating water not only favors oxygen absorption but also nitrogen uptake (Hu et al., 2015).

Another interesting finding of this study was the divergent ability of individual plants to survive, adapt, and grow in NFT aquaponic conditions. This can suggest that the genetic traits of the fittest individuals can be fixed through selection management leading to the technical and financial feasibility for small or large production. In the current study, peppermint and basil were the only herb species that showed small and large-scale production potential in the NFT system. However, the rest of the species should not be undervalued by these results.

With regard to tilapia, the observed survival rates and growth responses were higher or consistent with earlier studies in similar conditions (Mbahinzireki et al., 2001; Coyle et al., 2004; Bahnasawy, 2009). This confirms the feasibility of tilapia to produce in recirculating systems supporting their sustainability and economical profitability (Blidariu and Grozea, 2011).

In the present study, the plant performance values were, in general, below those reported for aquaponic (Espinosa-Moya et al., 2018) or soil conditions (Telci et al., 2004; Salim et al., 2014); therefore, there is a need to do further studies in order to derive conclusions and decisive decisions in selecting the investigated herb species for production purposes in conditions similar to the IICAE NFT systems.

CONCLUSION

Peppermint (*Mentha spicata*) and basil (*Ocimum basilicum*) were the most productive species in NFT aquaponics conditions. Among the investigated species, the obtained results of these plants showed potential for commercial or domestic production. Based on the findings of the current study, it is, however, believed that the survival and production capabilities of the other herb species in NFT aquaponics conditions could be improved by refinement or modifications in the management techniques, including initial individual plant selection, water flow management, suspended solids management, and algae control.

DECLARATIONS

Acknowledgments

This research was co-financed with public funds administered by Dirección General de Investigación, University of San Carlos of Guatemala (budget line 4.8.63.2.59, year 2018).

Competing interests

The authors declared no conflict of interest.

Authors' contribution

Carlos Valdez-Sandoval designed the plan of the study and managed the funds. Dennis Guerra-Centeno conceived the idea and drafted the manuscript. Manuel Lepe-López analyzed and interpreted the data. Mercedes Díaz-Rodríguez collected the data and reviewed the manuscript. Lariza Pineda-Alvizures processed the data and reviewed the manuscript.

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REFERENCES

Bahnasawy MH (2009). Effect of dietary protein levels on growth performance and body composition of monosex Nile tilapia, *Oreochromis niloticus* L. reared in fertilized tanks. Pakistan Journal of Nutrition, 8(5): 674-678. DOI: https://www.doi.org/10.3923/pjn.2009.674.678

Blidariu F and Grozea A (2011). Increasing the economical efficiency and sustainability of indoor fish farming by means of aquaponics-review. Scientific Papers Animal Science and Biotechnologies, 44(2):1-8. Available at: http://www.spasb.ro/index.php/spasb/article/view/287/168

Blom CWPM and Voesenek LACJ (1996). Flooding: the survival strategies of plants. Trends in Ecology & Evolution, 11(7): 290-295. DOI: https://www.doi.org/10.1016/0169-5347(96)10034-3

Cáceres A (1996). Plantas de uso medicinal en Guatemala. Editorial Universitaria, Guatemala, pp. 401-402.

Colmer TD (2003). Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant, Cell & Environment, 26(1): 17-36. DOI: https://www.doi.org/10.1046/j.1365-3040.2003.00846.x

Coyle SD, Mengel GJ, Tidwell JH and Webster CD (2004). Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquaculture Research, 35(4): 365-370. DOI: https://www,doi.org/10.1111/j.1365-2109.2004.01023.x

Espinosa-Moya A, Alvarez-Gonzalez A, Albertos-Alpuche P, Guzman-Mendoza R and Martínez-Yáñez R (2018). Growth and development of herbaceous plants in aquaponic systems. Acta Universitaria, 28(2):1-8. DOI: https://www.doi.org/10.15174/au.2018.1387

Guerra-Centeno D, Valdez-Sandoval C, Aquino-Sagastume E, Díaz M and Ríos L (2016). Adaptación y rendimiento de plantas autóctonas de Guatemala en un sistema acuapónico. Revista Electrónica de Veterinaria, 17(11): 1-13. Available at: https://www.redalyc.org/pdf/636/63649051013.pdf

Hammer O, Harper DAT and Ryan PD (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. Paleontologia Electronica, 4(1): 1-9. Available at: https://palaeo-electronica.org/2001_1/past/issue1_01.htm#:~:text=The%20program%2C%20called%20PAST%20(PAleontological,plotting%2C%20and%20simple%20phylogenetic%20analysis.

Hu Z, Lee JW, Chandran K, Kim S, Brotto AC and Khanal SK (2015). Effect of plant species on nitrogen recovery in aquaponics. Bioresource Technology, 188: 92-98. DOI: https://www.doi.org/10.1016/j.biortech.2015.01.013

Jackson MB and Colmer TD (2005). Response and adaptation by plants to flooding stress. Annals of Botany, 96(4): 501-505. DOI: https://www.doi.org/10.1093/aob/mci205

Kledal PR, König B and Matulić D (2019). Aquaponics: The Ugly Duckling in Organic Regulation. In Goddek S., Joyce A., Kotzen B., & Burnell G. (Eds.), Aquaponics Food Production Systems, Springer, Cham, pp.487-500 (Ch). DOI: https://www.doi.org/10.1007/978-3-030-15943-6_19

Knapp S and Davidse G (2006). Flora of Guatemala. In E. Cano (Editor), Biodiversidad de Guatemala, Volume I. Universidad del Valle, Guatemala, pp. 24-47.

König B, Janker J, Reinhardt T, Villarroel M and Junge R (2018). Analysis of aquaponics as an emerging technological innovation system. Journal of Cleaner Production, 180: 232-243. DOI: https://www.doi.org/10.1016/j.jclepro.2018.01.037

Lennard WA and Leonard BV (2006). A comparison of three different hydroponic sub-systems (gravel bed, floating and nutrient film technique) in an aquaponic test system. Aquaculture International, 14(6): 539-550. DOI: http://www.dx.doi.org/10.1007/s10499-006-9053-2

Li C, Lee CT, Gao Y, Hashim H, Zhang X, Wu WM and Zhang Z (2018). Prospect of aquaponics for the sustainable development of food production in urban. Chemical Engineering Transactions, 63: 475-480. DOI: https://www.doi.org/10.3303/CET1863080

Love DC, Fry JP, Li X, Hill ES, Genello L, Semmens K and Thompson RE (2015). Commercial aquaponics production and profitability: Findings from an international survey. Aquaculture, 435: 67-74. DOI: https://www.doi.org/10.1016/j.aquaculture.2014.09.023

Maucieri C, Nicoletto C, Junge R, Schmautz Z, Sambo P and Borin M (2018). Hydroponic systems and water management in aquaponics: A review. Italian Journal of Agronomy, 13(1): 1-33. DOI: https://www.doi.org/10.4081/ija.2017.1012

Mbahinzireki GB, Dabrowski K, Lee KJ, El-Saidy D and Wisner ER (2001). Growth, feed utilization and body composition of tilapia (*Oreochromis* sp.) fed cottonseed meal-based diets in a recirculating system. Aquaculture Nutrition, 7(3): 189-200. DOI: https://www.doi.org/10.1046/j.1365-2095.2001.00172.x

Palm HW, Knaus U, Appelbaum S, Goddek S, Strauch SM, Vermeulen T, Haïssam M and Kotzen B (2018). Towards commercial aquaponics: A review of systems, designs, scales and nomenclature. Aquaculture International, 26(3): 813-842. DOI: https://www.doi.org/10.1007/s10499-018-0249-z

Pardos JA (2004). Responses of plants to soil flooding. Forest Systems, 13(4): 101-107. DOI: https://doi.org/10.5424/srf/200413S1-00858

Rizal A, Dhahiyat Y, Andriani Y, Handaka AA and Sahidin A (2018). The economic and social benefits of an aquaponic system for the integrated production of fish and water plants. IOP Publishing, 137: 1-8. DOI: https://www.doi.org/10.1088/1755-1315/137/1/012098

Roosta HR and Afsharipoor S (2012). Effects of different cultivation media on vegetative growth, ecophysiological traits and nutrients concentration in strawberry under hydroponic and aquaponic cultivation systems. Advances in Environmental Biology, 6(2): 543-555. Available at: https://pdfs.semanticscholar.org/3103/f53bf4f771a4bf66b7b6889cdd981e678e80.pdf

Salim EA, El Hassan GM and Khalid HES (2014). Effect of spacing and seasonal variation on growth parameters, yield and oil content of mint plants. Journal of Forest Products & Industries, 3: 71-74. DOI: https://www.doi.org/10.13140/RG.2.1.5166.3600

To cite this paper: Valdez-Sandoval C, Guerra-Centeno D, Lepe-López M, Díaz-Rodríguez M and Pineda-Alvizuris L (2021). Survival and Productivity of Culinary Herb Species in a Nutrient Film Technique-type Aquaponic System with Nile Tilapia. *World Vet. J.*, 10 (4): 578-586. DOI: https://dx.doi.org/10.54203/scil.2020.wvj69

Shafeena T (2016). Smart aquaponics system: challenges and opportunities. European Journal of Advances in Engineering and Technology, 3(2): 52-55. Available at: http://www.ejaet.com/PDF/3-2/EJAET-3-2-52-55.pdf

Somerville C, Cohen M, Pantanella E, Stankus A and Lovatelli A (2014). Small-scale aquaponic food production: integrated fish and plant farming. Food and Agriculture Organization of the United Nations, Rome, Italy 589: 281-288. Available at: http://www.fao.org/3/a-i4021e.pdf

Telci I, Sahbaz NI, Yilmaz G and Tugay ME (2004). Agronomical and chemical characterization of spearmint (*Mentha spicata L.*) originating in Turkey. Economic Botany, 58(4):721. DOI: https://www.doi.org/10.1663/0013-0001(2004)058[0721:AACCOS]2.0.CO;2

Tyson RV, Treadwell DD and Simonne EH (2011). Opportunities and challenges to sustainability in aquaponic systems. Hort Technology, 21(1): 6-13. DOI: https://www.doi.org/10.21273/HORTTECH.21.1.6

United Nations (2015). Transforming our world: The 2030 agenda for sustainable development. Resolution adopted by the General Assembly, New York 70(1):1-35. Available at: https://www.un.org/en/development/desa/population/migration/generalassembly/docs/globalcompact/A_RES_70_1_E.pdf

Valdez-Sandoval C, Guerra-Centeno D, Díaz M and Ríos L (2017). Adaptación, crecimiento y rendimiento de variedades de chile nativos de Guatemala en un sistema acuapónico con tilapia nilótica. Revista Electrónica de Veterinaria, 18(5): 1-11. Available at: https://www.redalyc.org/pdf/636/63651419005.pdf

Villar-Anleu L (1998). La flora silvestre de Guatemala. Editorial Universitaria, Guatemala, pp. 1-63.

Visser EJW, Colmer TD, Blom CWPM and Voesenek LACJ (2000). Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono-and dicotyledonous wetland species with contrasting types of aerenchyma. Plant, Cell & Environment, 23(11): 1237-1245. DOI: https://www.doi.org/10.1046/j.1365-3040.2000.00628.x

Visser EJW, Voesenek LACJ, Vartapetian BB and Jackson MB (2003). Flooding and plant growth. Annals of Botany, 91(2): 107-109. DOI: https://www.doi.org/10.1093/aob/mcg014

Wongkiew S, Hu Z, Chandran K, Lee JW and Khanal SK (2017). Nitrogen transformations in aquaponic systems: A review. Aquacultural Engineering, 76: 9-19. DOI: https://www.doi.org/10.1016/j.aquaeng.2017.01.004

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2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 587-596, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj70

Effect of L-Carnitine and Yeast Chromium Supplementation on Productive Performance in Pekin and Sudani Duckling during Growth Period

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ORIGINAL ARTICLE
pii: S232245682000070-10
Received: 26 Sep 2020
Accepted: 09 Nov 2020

ABSTRACT

The present study aimed to evaluate the effect of L-carnitine and Yeast chromium supplementation on the productive performance of Pekin and Sudani duckling breeds. A total number of 450 both unsexed Pekin and Sudani ducklings (225 per each breed) one-day-old were investigated in the current study. The experimental period lasted 12 weeks of age. Experimental ducklings were randomly divided into the 5 equal treatments with 90 ducklings (45 number from both Pekin and Sudani ducklings per each). Each experimental treatment was randomly divided into 3 equal replicates of 30 ducklings (15 ducklings in each breed). The five experimental treatments were as follows: the first treatment was the control with basal diets, treatments 2 and 3 received basal diets supplemented with 300 and 450 mg/kg diet L-carnitine (LC), respectively, while treatments 4 and 5 received basal diets supplemented with 400 and 600 µg/kg diets Yeast chromium (Cr), respectively. The results indicated that growing duckling fed diets supplemented with LC and Cr were significantly improved in live body weight, body weight gain, feed intake, and feed conversion ratio. The relative weight of carcass quality and weight of lymphoid organs significantly increased with supplemented diets. Therefore, both duckling breeds fed on diets supplemented with 450 mg LC/kg resulted in better performance without any adverse effect on carcass quality as well as economic efficiency.

Keywords: L-carnitine, Pekin ducks, Productive Performance, Sudani ducks, Yeast chromium.

INTRODUCTION

Demand for animal-sourced protein has increased as a result of elevating human population growth leading to rising in animal production especially poultry meat production (Adeola, 2006). Ducks production in Egypt is about 42,000 tons representing 1.7% of total world production in 2006 (Soltan et al., 2014). Duck carcasses contain about 30% fat while broiler carcasses have 15% fat in a marketing age. Therefore, an increase in consumer's desire for leaner meat has stimulated interest in reducing abdominal fat deposition in ducks. Excessive fat in ducks is unattractive for consumers who are concerned about the negative effects of saturated fat intake on diet (Arslan et al., 2003). This fat represents a waste product from ducks, so numerous attempts have been made to minimize this fat accumulation either genetically or by dietary manipulation with different degrees of success (Awad et al., 2014). Therefore, improving growth performance and carcass composition by using natural feed additives is the main target for poultry research (Taklimi et al., 2015). Ducks industry in Egypt is under constant pressure to provide high-quality and more economical products for consumers. Egyptian Sudani ducks is a local breed with low growth performance as a meat type duck. Growth performance is subject to significant factors including genetics, gender, and environmental variables (Awad et al., 2014). The L-carnitine (LC) is synthesized in the body from lysine and methionine, and it is formed with contributions from vitamins B_3, B_6, B_{12}, C and folic acid, as well as iron (Michalczuk et al., 2012). According to Harmeyer (2002), the body cannot produce enough LC to fully cover its own needs because some conditions such as stress, disease, and physical strain may result in LC deficiency. Therefore, LC supplementation resulted in improving growth rate, feed conversion breast, thigh meat yield, and reduced abdominal fat in broilers.

Moreover, LC is used in poultry for a multi-functional purpose that includes promoting growth and improving antioxidant status (Adabi et al., 2011). Animals and birds under any stressors are needed to Chromium (Cr) elements to achieve biological responses (Piva et al., 2003). Several varieties of Chromium (Cr) exist, but Cr from yeast, picolinate, and chloride forms ($CrCl_2$) have been cited mostly for their biological activities (Khan et al., 2014). The Cr is one of the essential minerals which is required for improving productive performance in poultry due to its important functions in metabolism, growth, and reduction of lipid and protein peroxidation (Farag et al., 2017).

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The present study aimed to evaluate the differences of two dietary supplementations that included LC and Cr on growth performance and some physiological parameters in both Pekin (foreign) and Sudani (local) ducklings under the Egyptian environmental conditions.

MATERIALS AND METHODS

Ethical approval

The Institute's ethical rules for animal research were followed and the study plan was approved by the Institute's Research Committee on 1 May 2016 (code no. 020203429).

Experimental design

The current study was carried out at El-Serw Research Station, Water Fowl Research Department, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The experiment was carried out during the summer season from June to August of 2016. A total number of 450 both unsexed Pekin and Sudani ducklings (225 per each breed) one-day-old were used in the present study. The experimental period lasted until 12 weeks of age. Experimental ducklings were randomly divided into the five equal treatments with 90 ducklings (45 numbers from both of Pekin and Sudani ducklings per each). Each experimental treatment was randomly divided into three equal replicates of 30 ducklings (15 ducklings of each breed). The five experimental treatments were as follows: Number one was control treatment received basal diets, treatments 2, 3 received basal diets supplemented with 300 and 450 mg/kg diet L-carnitine (LC), while treatments 4, 5 received basal diets supplemented with 400 and 600 µg/kg diets Yeast chromium (Cr), respectively. Ducklings were maintained in deep-litter floor pens. Natural light system (16 hours was light and 8 hours was dark per day) was used all over the experiment. The experimental ducklings were examined clinically for having diseases, being healthy and receiving vaccines (at 3 days of age ducklings had been vaccinated against duck hepatitis viral disease $(DHVD)_1$, at 7 days of age ducklings had been vaccinated against Bird flow (Avian Influenza), at 10 days of age ducklings had been vaccinated against Duck $plague_1$ (Duck virus Enteritis) and Duck $Cholera_1$, at 30 days of age ducklings had been vaccinated against Duck $plague_2$ (Duck virus Enteritis), at 45 days of age ducklings had been vaccinated against Duck hepatitis viral disease $(DHVD)_2$ and Duck $Cholera_2$. Also, ducklings had been vaccinated against coccidiosis at 3 days of age in food). Fresh water and food were available *ad libitum*. All ducklings were fed on a starter ration from first to 12 weeks of age which contained 19.20% crude protein and 2868 kcal/kg metabolized energy during the first four weeks of age followed by a grower diet contains 15.20% crud protein and 2690 kcal/kg, and also supplemented by adequate levels of nutrients recommended by NRC (2005). Compositions of starter and grower ration were presented in Table 1.

Table 1. Composition of the starter and grower rations that ducklings were fed during the experiment

Ingredients %	Basal diet	
	Starter	Grower
Yellow corn (%)	65.00	63.00
Soya bean meal (44%)	30.45	15.50
Wheat bran	0.65	17.78
DI-Calcium phosphate	1.80	1.25
Calcium carbonate ($Ca\ Co_3$)	1.40	1.80
Vitamin & minerals mixture	0.30	0.30
Sodium chloride (Na Cl)	0.30	0.30
D.L. Methionine	0.10	0.07
Total	100.00	100.00
Crude protein (%)	19.20	15.20
Metabolic energy (Kcal/Kg)	2868.00	2690.00

Each 2.5 kg of vitamins and minerals mixture contain; 12000.000 IU vitamin A acetate; 2000.000 IU vitamin D3; 10.000 mg vitamin E acetate; 2000 mg vitamin K3; 100 mg vitamin B; 4000 mg vitamin B2; 1500 mg vitamin B6; 10 mg vitamin B12; 10.000 mg Pantothenic acid; 20.000 mg Nicotininc acid; 1000 mg Folic acid; 50 mg Bioten; 500.000 mg Chorine; 10.000 mg Copper; 1000 mg Iodine; 30.00 mg Iron; 55.000 mg Manganese; 55.000 mg Zinc; and 100 mg Selnium.

Growth performance

Birds were weighed every four weeks to measure their body weight gain, feed consumption, feed conversion ratio (feed consumed (g)/ weight gain (g)). The mortality of the ducklings was also calculated during the experimental period. By the end of the experiment, three ducklings from each treatment group were weighed and slaughtered by slitting the jugular vein, then scalded and de-feathered and the carcasses were manually eviscerated and weighted. The liver, heart, gizzard, digestive tract, and abdominal fat were removed and their relative percentages of live body weight were estimated. Chemical analyses of breast and thigh muscle meat were determined according to AOAC (2000).

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Economic efficiency

The economic efficiency was calculated based on the price of L-carnitine (68.7%) was traded 50 LE per one kg and Yeast chromium traded was 17 LE per one kg. L-Carnitine and Yeast Chromium were respectively derived from Carniking (Longsha Company in Hong Kong, L-Carnitine in it) and Yeast Chromium (Alltech Biology Company in Beijing). One kg of live body weight coasted 28 and 22 LE for Sudani and Pekin ducklings, respectively, while the prices of one duckling which is one-day-old were 8 LE and 3 LE, respectively for Sudani and Pekin, which were prevailing during 2016.

Statistical analysis

Data were analyzed by the least-squares analysis of variance using the General Linear Models procedure of the statistical analysis model (SAS, 2001), all results were analyzed using two-way ANOVA. The statistical model was as follows: $Y_{ijk}= \mu + T_i + B_j + (TB)_{ij} + E_{ijk}$.

Y_{ijk} = An observation; μ = Overall mean; T_i = Effect of dietary supplementation ($_i$ = 1, 2, 3, 4, 5); B_j = Effect of duck breeds ($_j$ = 1, 2); $(TB)_{ij}$ = Interaction effect between dietary supplementation and breed of ducks and E_{ijk}= Random error component assumed to be normally distributed. The significant differences among means of treatments were compared using Duncan's multiple range tests ($p \leq 0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance

Live body weight and body weight gain

Data in Table 2 indicated that Live Body Weight (LBW) in this study was significantly changed with duckling breeds of all ages. It was significantly ($p \leq 0.01$) affected due to LC or Cr supplementation during all ages except the first week of age. The live body weight of Sudani ducklings was significantly higher by 33.58, 35.16, 45.97, and 26.62% than Pekin ducklings aged 1, 4, 8, and 12 weeks, respectively. The heaviest bodyweight values were recorded for duckling treatment with 450 mg/kg diet L-carnitine (LC) compared to other groups, including the control. These results might be due to Sudani ducklings have a greater growth rate than Pekin and it tends to mature earlier as well as they consumed more feed (Gouda, 2015).

Duckling which received dietary by 300 or 450 (mg LC/kg) LC and 400 or 600 (µg Cr/kg) Cr supplementation had significantly higher ($p \leq 0.01$) LBW by 9.92, 10.40, 6.29 and 6.53% than the control group at 12 weeks of age, respectively. Additionally, Interaction between duckling breeds and fed ration supplementation significantly affected LBW at different ages as LBW of Sudani ducklings were significantly higher ($p \leq 0.01$) than Pekin ducklings with any diet supplementation. A similar trend was obtained for Body Weight Gain (BWG) values (Table 2). Bodyweight of Sudani ducklings was significantly higher ($p \leq 0.01$) by 35.26, 54.70, and 26.49% than Pekin ducklings during (0-4), (4-8), and (0-12) weeks of age, respectively. All duckling which fed ration supplemented with LC and Cr had significantly higher ($p \leq 0.01$) of BWG than control once during (0-4), (8-12), and (0-12) weeks of age. Improvement of BWG values were 10.08, 10.57, 6.39 and 6.67 % for duckling fed ration supplemented with LC (300 or 450 mg/kg) and Cr (400 or 600 µg/kg), compared to those fed the control ration during 0-12 weeks of age, respectively.

These improvements might be due to LC plays a major role by increasing plasma insulin-like growth factor I concentration, which serves as stimulating substances for chicken's growth (Xu et al., 2003). Also, it might be due to the improvement in the utilization of dietary ingredients as a result of LC transfer the long-chain fatty acids across the liner mitochondrial membranes and controls the rates of β-oxidation of long-chain fatty acids as well as it plays a pivotal role in energy metabolism (Arslan et al., 2003). Moreover, improvement in growth performance in Cr treatment might be due to the activity of Cr in controlling the anabolic action of insulin.

These results were in agreement with those of El-Hommosany (2008) who reported that Cr supplementation (125 and 250 µg/kg feed) was associated with a significant increase in body weight and weight gain than in the control group in Japanese quails at 6 weeks of age. Abdel-Fattah et al. (2014) indicated that dietary supplementation of LC (200-400 mg/kg) significantly increased LBW and cumulative BWG for Japanese quails. Awad et al. (2016) reported that ducklings LBW was enhanced by 7.14, 11.20, 12.74 and 9.74% for Domyati ducklings fed a diet supplemented with 150, 300, 450, and 600 mg LC/kg as compared with those fed the control diets at 84 days of age, respectively. BWG was improved by 8.95, 15.11, 16.50, and 12.92% during the periods from 21 to 84 days of age, respectively. El-Kelawy (2019) reported that Cr supplementation improved body weight at 28 and 49 days of age and BWG during periods (14-28), (29-49), and (14-49) days of Japanese quails age compared with the control group.

Interaction between duckling breed and dietary supplementation significantly affected the BWG during different experimental periods; Sudani ducklings had significantly higher BWG than Pekin ducklings with any diet supplementation.

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Table 2. Effects of breed and dietary supplementation on live body weight and body weight gain in Sudani and Pekin ducks during 12 weeks of age.

Age (weeks)	DB	Treatments					AV	Probability		
		Control	L-carnitine		Yeast chromium					
			300	450	400	600		B	T	BT
Live body weight (g)										
0	S	50.11^{a}±0.11	50.17^{a}±0.10	50.01^{a}±0.12	50.12^{a}±0.11	50.19^{a}±0.15	50.12^{a}			
	P	37.51^{b}±0.09	37.41^{b}±0.08	37.57^{b}±0.09	37.58^{b}±0.07	37.55^{b}±0.11	37.52^{b}	*	NS	*
	AV	43.81	43.79	43.79	43.85	43.87				
4	S	817.93^{b}±2.32	952.54^{a}±2.39	955.18^{a}±2.42	890.57^{b}±2.30	895.35^{b}±2.31	902.31^{a}			
	P	590.61^{e}±2.30	714.45^{c}±2.28	720.74^{c}±2.33	650.57^{d}±2.39	661.48^{d}±2.31	667.57^{b}	**	**	**
	AV	704.27^{c}	833.50^{a}	837.96^{a}	770.57^{b}	778.42^{b}				
8	S	2084.21^{c}±7.95	2245.35^{a}±8.15	2270.27^{a}±8.91	2146.74^{b}±7.16	2158.97^{b}±8.92	2181.11^{a}			
	P	1338.69^{f}±7.16	1587.94^{d}±9.11	1592.41^{d}±7.13	1464.72^{e}±7.05	1487.24^{e}±8.13	1494.20^{b}	**	**	**
	AV	1711.45^{c}	1916.65^{a}	1931.34^{a}	1805.73^{b}	1823.11^{b}				
12	S	3131.32^{c}±14.83	3354.41^{a}±14.22	3375.24^{a}±13.81	3257.27^{b}±14.07	3261.25^{b}±13.11	3275.90^{a}			
	P	2367.44^{f}±14.12	2689.74^{d}±14.02	2695.41^{d}±14.18	2587.25^{e}±13.25	2596.46^{e}±14.11	2587.26^{b}	**	**	**
	AV	2749.38^{c}	3022.08^{a}	3035.33^{a}	2922.26^{b}	2928.86^{b}				
Body weight gain (g)										
0-4	S	767.82^{c}±7.12	902.37^{a}±7.36	905.17^{a}±7.23	840.45^{b}±6.15	845.16^{b}±6.55	852.19^{a}			
	P	553.10^{e}±7.14	677.04^{d}±6.11	683.17^{d}±7.21	612.99^{d}±6.28	623.93^{d}±7.30	630.05^{b}	**	**	**
	AV	660.46^{b}	789.71^{a}	794.17^{a}	726.72^{a}	734.55^{a}				
4-8	S	1266.28^{a}±16.26	1292.81^{a}±16.35	1315.09^{a}±15.24	1256.17^{a}±16.31	1263.62^{a}±15.21	1278.79^{a}			
	P	748.08^{c}±14.21	873.49^{b}±15.16	871.67^{b}±16.27	814.15^{b}±16.14	825.76^{b}±15.75	826.63^{b}	**	NS	**
	AV	1007.18	1083.15	1093.38	1035.16	1044.69				
8-12	S	447.11^{d}±27.14	509.06^{c}±27.75	504.97^{c}±28.11	510.53^{c}±26.15	502.28^{c}±27.04	494.79^{b}			
	P	1028.75^{b}±27.39	1101.80^{a}±25.11	1103.00^{a}±26.30	1122.53^{a}±27.14	1109.22^{a}±25.12	1093.06^{a}	**	**	**
	AV	737.93^{b}	805.43^{a}	803.99^{a}	816.53^{a}	805.75^{a}				
0-12	S	3081.21^{c}±31.54	3304.24^{a}±31.71	3325.23^{a}±32.41	3207.14^{b}±31.59	3211.06^{b}±32.54	3225.78^{a}			
	P	2329.93^{f}±28.87	2652.33^{d}±29.52	2657.84^{d}±30.41	2549.67^{e}±28.97	2560.91^{e}±30.45	2550.14^{b}	**	**	**
	AV	2705.57^{c}	2978.29^{a}	2991.54^{a}	2878.41^{b}	2885.99^{b}				

a, b, c, d, e, f: Means with different superscripts in the same row within item differ significantly; NS: Not significant; *: (p≤0.05); **: p≤0.01); DB: Duckling breed; S: Sudani ducks; P: Pekin ducks; AV: Overall mean.

Feed intake

The effect of different treatments and breeds on Feed Intake (FI) has been presented in Table 3. The FI was significantly affected by duckling breeds of all ages; it could be observed that Sudani ducklings had consumed significantly higher amounts of ration than Pekin ducklings during the different experimental periods by about 18.75% during 0-12 weeks of age. These results might be due to Pekin duckling have a poor growth rate so consumed less feed which reflects in decreasing body weights compared to Sudani ducklings (Gouda, 2015). Duckling fed ration supplemented with LC 300 or 450 mg/kg and Cr 400 or 600 µg/kg consumed more feed by 3.76, 4.10, 2.79, and 2.97% than control during 0-12 weeks of age, respectively. This might be due to ducklings can compensate their FI according to their energy requirements as well as the experimental diet had similar metabolizable energy. These results agreed with El-Kelawy (2019) who reported that Cr supplementation improved FI during periods 14-49 days of age of Japanese quails, compared with the control group. Interaction between duckling breed and dietary supplementation significantly affected FI during different experimental periods as the Sudani ducklings had significantly higher FI than Pekin ducklings with any diet supplementation.

Feed conversion ratio

Feed Conversion Ratio (FCR) was significantly affected by duckling breed at all ages as Table 3 indicated that Pekin ducklings were significantly attenuated than Sudani ducklings during 0-4, 4-8, and 0-12 weeks of age. The FCR was improved by 6.33% for Sudani than Pekin ducklings during 0-12 weeks of age. These findings might be due to Sudani ducklings had lighter BWG than Pekin during the growth period.

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Duckling breed fed ration supplemented with LC and Cr had the best FCR compared with the control during 0-4, 4-8, 8-12, and 0-12 weeks of age (Table 3). The improvement of FCR values were 6.87, 7.19, 4.07, and 4.37% for duckling fed ration supplemented with LC and Cr as compared with control during (0-12) weeks of age, respectively. Generally, the improvement in FCR was associated with increasing LBW values which might be attributed to improving BWG of ducklings. Feed conversion improved since LC increases fatty acid burning and improves intestinal mucous membrane by active and passive mechanisms **(**Fathi and Farahzadi, 2014). These findings are in agreement with Debski et al. (2004) and El-Kelawy (2019) who observed a slightly improving feed conversion in the group that received Cr. Parsaeimehr et al. (2014) reported that dietary LC supplementation (200-30 mg/kg diet) resulted in improving FCR of broiler chickens during the growing period (45 days). Also, Abdel-Fattah et al. (2014) found that a significant improvement in the feed conversion ratio of quails occurred as a result of dietary supplementation with LC (200-30 mg LC/kg diet) in comparison with control. However, Awad et al. (2016) reported that FCR was numerically similar for ducklings fed on different LC diets with 150, 300, 450, and 600 mg LC/kg as compared with those fed the control diets during the overall experimental period (21-84 days of age). Interaction between duckling breed and dietary supplementation significantly affected FCR during different experimental periods, Pekin ducklings had significantly worst FCR than Sudani ducklings with any diet supplementation during different all experimental periods except 8-12 weeks of age.

Table 3. Effect of breed and dietary supplementation on feed intake and feed conversion ratio in Sudani and Pekin ducks during 12 weeks of age.

Age (weeks)	DB	Treatments					AV	Probability		
		Control	L-carnitine		Yeast chromium					
			300	450	400	600		B	T	BT
Feed intake (g / duckling)										
0-4	S	2439^{a}±14.67	2580^{a}±12.27	2591^{a}±13.21	2531^{a}±13.69	2557^{a}±14.67	2539.6^{a}			
	P	1895^{c}±13.51	1940^{b} ±14.11	1953^{b} ±12.13	1914^{b}±14.26	1925^{b}±14.26	1925.4^{b}	**	NS	**
	AV	2167	2260	2272	2222.50	2241				
4-8	S	3350^{b}±16.11	3457^{a}±14.29	3486^{a}±16.37	3435^{a}±14.21	3420^{a}±13.37	3430^{a}			
	P	3277^{c}±15.41	3371^{b}±14.34	3356^{b}±13.55	3328^{b}±16.30	3319^{b}±15.22	3330.2^{b}	**	**	**
	AV	3313.5^{b}	3414^{a}	3421^{a}	3382.5^{b}	3369.5^{b}				
8-12	S	4570^{b}±17.74	4772^{a}±18.78	4793^{a}±17.73	4753^{a}±16.37	4761^{a}±17.22	4729.8^{a}			
	P	3655^{d}±18.36	3787^{c}±16.11	3794^{c}±13.51	3761^{c}±17.90	3774^{c}±15.81	3754.2^{b}	**	**	**
	AV	4112.5^{b}	4279.5^{a}	4293.5^{a}	4257^{a}	4267.5^{a}				
0-12	S	10359^{c}±18.52	10809^{a}±19.59	10870^{a}±17.12	10719^{b}±18.66	10738^{b}±16.97	10699^{a}			
	P	8827^{e}±18.36	9098^{d}±17.58	9103^{d}±16.33	9003^{d}±18.77	9018^{d}±17.53	9009.8^{b}	**	**	**
	AV	9593^{c}	9953.5^{a}	9986.5^{a}	9861^{b}	9878^{b}				
Feed conversion ratio (g feed/ g gain)										
0-4	S	3.18^{a}±0.05	2.86^{b}±0.07	2.86^{b}±0.04	3.01^{a}±0.05	3.03^{a}±0.07	2.98^{b}			
	P	3.43^{a}±0.07	2.87^{b}±0.04	2.86^{b}±0.05	3.12^{a}±0.07	3.09^{a}±0.06	3.06^{a}	**	**	**
	AV	3.30^{a}	2.86^{b}	2.86^{b}	3.07^{a}	3.06^{a}				
4-8	S	2.65^{c}±0.07	2.67^{c}±0.12	2.65^{c}±0.05	2.73^{c}±0.13	2.71^{c}±0.09	2.68^{b}			
	P	4.38^{a}±0.13	3.86^{b}±0.17	3.85^{b}±0.09	4.09^{a}±0.07	4.02^{a}±0.06	4.03^{a}	**	**	**
	AV	3.51^{a}	3.27^{b}	3.25^{b}	3.41^{b}	3.37^{b}				
8-12	S	10.22^{a}±0.27	9.37^{b}±0.22	9.49^{b}±0.25	9.31^{b}±0.27	9.48^{b}±0.19	9.56^{a}			
	P	3.55^{c}±0.18	3.44^{c}±0.22	3.44^{c}±0.24	3.35^{c}±0.17	3.40^{c}±0.22	3.43^{b}	**	**	**
	AV	8.36^{a}	6.41^{b}	6.47^{b}	6.33^{b}	6.44^{b}				
0-12	S	3.36^{b}±0.11	3.27^{c}±0.09	3.27^{c}±0.12	3.34^{c}±0.08	3.34^{c}±0.13	3.32^{b}			
	P	3.79^{a}±0.12	3.43^{b}±0.09	3.42^{b}±0.11	3.53^{b}±0.15	3.52^{b}±0.07	3.53^{a}	**	**	**
	AV	3.58^{a}	3.35^{b}	3.34^{b}	3.44^{b}	3.43^{b}				
Mortality%										
0-12	S	2.92^{b}	2.85^{b}	2.86^{b}	2.90^{b}	2.91^{b}	2.89^{b}			
	P	5.21^{a}	5.00^{a}	5.11^{a}	5.13^{a}	5.15^{a}	5.12^{a}	*	NS	*
	AV	4.07	3.93	3.99	4.02	4.03				

a, b, c, d, e, f: Means with different superscripts in the same row within item differ significantly; NS: Not significant; *: ($p \leq 0.05$); **: $p \leq 0.01$); DB: Duckling breed; S: Sudani ducks; P: Pekin ducks; AV: Overall mean.

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Mortality rate

It can be observed from Table 3 that the mortality rate during 12 weeks of age was significantly higher ($p \leq 0.05$) in Pekin (5.12%) than Sudani (2.89%) breeds. It might be due to Pekin was more sensitive and Sudani was more adapted to the Egyptian conditions. These results agreed with Ali (2005) who indicated that the mortality percentage was 4% for Domyati during the periods (0-12) weeks of age. Also, Ali et al. (2017) reported that the mortality rate during 12 weeks of age was significantly higher in Pekin (4.79%) than Domyati (3.07%). Duckling dietary supplemented with different LC and Cr levels significantly recorded a reduction in mortality rate than those fed on control ration from 4.07 to 3.93%. This improvement in mortality could be due to the fact that LC and Cr had played important roles in increasing resistance to stress as well as the immunity system. Interaction between duckling breeds and fed ration supplementation significantly affected on mortality rate during different experimental periods. Pekin ducklings had a significantly higher rate of mortality than Sudani ducklings with any diet supplementation during all different experimental periods.

Carcass traits

The overall means of carcass weight (%) and the proportional weight of some body organs are presented in Table 4. Carcass, total edible parts, and abdominal fat percentages significantly increased ($p \leq 0.05$) based on duckling breed. Carcass and total edible parts percentages were significantly increased ($p \leq 0.01$) by 2.06 and 2.99% for Sudani duckling than Pekin, respectively. All carcass characteristics were significantly affected due to treatments that supplemented with LC and Cr as compared with control at 12 weeks of age, respectively. Carcass percentage significantly increased ($p \leq 0.01$) by 3.03, 2.93, 3.12, and 3.44%, respectively for ducklings Sudani and Pekin dietary supplemented with 300 or 450 mg/kg diet LC and 400 or 600 µg /kg diet Yeast Cr as compared with control. Total edible parts percentage significantly increased ($p \leq 0.01$) by 3.48, 3.49, 3.21, and 3.62%, respectively for duckling breed fed ration supplemented with 300 or 450 mg/kg diet LC and 400 or 600 µg /kg diet Yeast Cr as compared with control. Carcass percentage follows the same trend of LBW and BWG, where body weight and carcass characteristics have a high positive phenotypic and genetic correlation. (Pramod et al., 2002).

Generally, the improvement of eviscerated carcass and edible parts percentage might be due to improvement in final live weight and decreasing un-edible parts as a result of supplementing LC and Cr addition to the diet. The current finding is in agreement with those obtained by Wang et al. (2003) who reported that adding Cr (400-600 µg/kg) alone decreased abdominal fat percentage by 22.92% and 22.54% and adding LC (30, 50, and 100 mg/kg) alone decreased abdominal fat percentage by 33.33%, 28.16%, and 31.61% for broiler chickens at 7 weeks of age. Ibrahiem et al. (2011) reported that the carcass percentage of geese was significantly improved by supplementing 150 mg LC/kg, compared to the control group. Oladele et al. (2011) found that dressing carcass percentage significantly increased by increasing inclusion levels of LC in broilers diets. Also, Abdel-Fattah et al. (2014) presented that supplemental LC (400 mg/kg diet) significantly increased the dressing percent of quail.

The abdominal fat % was significantly ($p \leq 0.05$) decreased by supplemented the LC (300 or 450 mg/kg) and Cr (400 or 600 µg/kg) as compared with control. The decrease in abdominal fat might be due to LC prevents fatty tissues buildup, decreased the calorie requirement, and increased tolerance to effort because it may play major roles in facilitating the removal of short and medium-chain fatty acids from the mitochondria that accumulate as a result of normal and abnormal metabolism and promotes the β-oxidation of these fatty acids to generate Adenosine triphosphate (ATP) energy and improved energy utilization by reducing the amount of long-chain fatty acids availability for etherification to triacylglycerols and storage in the adipose tissue (Xu et al., 2003).

Parsaeimehr et al. (2014) and Huang et al. (2016) reported that supplementing LC (300 mg/kg) and Cr enhanced dressing percentage and decreased abdominal fat percentage of broiler chickens that had been exposed to heat stress in comparison with control groups. Also, Awad et al. (2016) reported that the improvement of eviscerated carcass and edible parts reached from 3.54 to 4.57% and 3.94 to 4.47%, respectively, for Domyati ducklings fed the diet supplemented with different LC levels (150, 300, 450 and 600 mg/kg diet) as compared to control, while the abdominal fat percentage was decreased by 36.14 to 45.18%. Ali et al. (2017) confirmed that carcass and total edible parts percentages were significantly increased due to duckling breed by 3.35 and 4.00% for Pekin ducklings than Domyati, respectively. El-Kelawy (2019) reported that Cr supplementation improved the percentage of carcass weight and decreased abdominal fat at 49 days of the age of Japanese quails compared with control groups. However, there were no significant effects of different Cr levels on inner body organs including liver, gizzard, and heart.

Lymphoid organs

Table 4 has been presented the effect of dietary supplementation on some relative lymphoid organ weights. The LC and Cr levels significantly improved ($p \leq 0.05$) the relative weights of lymphoid organs compared with the control group as reflected by immune status. This result agrees with Ali et al. (2017) who reported that spleen and thymus percentage were significantly increased due to duckling breeds by 33.33 and 60.0% for Domyati ducklings than Pekin, respectively.

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Table 4. Effect of breed and dietary supplementation on carcass traits and some lymphoid organs in Sudani and Pekin ducks during 12 weeks of age.

Items	DB	Treatments					AV	Probability		
		Control	L-carnitine		Yeast chromium					
			300	450	400	600		B	T	BT
Carcass traits										
Carcass%	S	72.41^{b} ±0.87	74.58^{a} ±0.77	74.28^{a} ±0.81	73.57^{ab} ±0.79	73.84^{ab}±0.77	73.74^{a}	**	**	**
	P	70.11^{c} ±0.56	72.25^{b} ±0.64	72.41^{b} ±0.67	73.39^{ab} ±0.88	73.57^{ab}±0.88	72.35^{b}			
	AV	71.26^{b}	73.42^{a}	73.35^{a}	73.48^{a}	73.71^{a}				
Gizzard%	S	3.15^{a}±0.08	3.32^{a}±0.08	3.54^{a}±0.09	3.19^{a}±0.09	3.25^{a}±0.06	3.29^{a}	*	*	*
	P	2.81^{b}±0.08	3.01^{ab}±0.06	3.05^{ab}±0.07	2.86^{b}±0.08	2.95^{b}±0.05	2.94^{b}			
	AV	2.98^{b}	3.17^{a}	3.30^{a}	3.03^{a}	3.10^{a}				
Liver%	S	2.09^{ab}±0.07	2.55^{a}±0.08	2.41^{a}±0.07	2.35^{a}±0.07	2.40^{a}±0.08	2.36^{a}	*	*	*
	P	1.89^{c}±0.08	1.99^{b}±0.09	2.00^{b}±0.06	1.95^{b}±0.06	1.98^{b}±0.09	1.96^{b}			
	AV	1.99^{b}	2.27^{a}	2.21^{a}	2.15^{a}	2.19^{a}				
Heart%	S	0.79±0.04	0.86±0.05	0.88±0.03	0.85±0.04	0.82±0.06	0.84	NS	NS	NS
	P	0.71±0.06	0.75±0.05	0.77±0.05	0.73±0.03	0.72±0.05	0.74			
	AV	0.75	0.81	0.83	0.79	0.77				
Total Giblets%	S	6.03^{ab}±0.15	6.73^{a}±0.24	6.83^{a}±0.16	6.39^{a}±0.20	6.47^{a}±0.34	6.49^{a}	*	*	*
	P	5.41^{b}±0.22	5.75^{b}±0.22	5.82^{b}±0.18	5.54^{b}±0.22	5.65^{b}±0.15	5.63^{b}			
	AV	5.72^{b}	6.24^{a}	6.33^{a}	5.97^{b}	6. 06^{a}				
Edible parts%	S	78.^{44b}±0.98	81.31^{a}±0.95	81.11^{a}±1.05	79.96^{b}±0.95	80.31^{a}±1.06	80.23^{a}	*	**	**
	P	75.52^{c}±1.02	78.00^{b}±0.94	78.23^{b}±0.92	79.93^{b}±1.01	79.22^{b}±0.91	77.98^{b}			
	AV	76.98	79.66	79.67	79.45	79.77				
Abdominal fat%	S	1.17^{b}±0.05	0.92^{c} ±0.04	0.91^{c}±0.03	0.95^{c}±0.02	0.93^{c}±0.02	0.98^{b}	*	*	*
	P	1.79^{a} ±0.04	0.99^{c} ±0.02	0.97^{c} ±0.04	1.09^{ab}±0.05	1.08^{ab}±0.06	1.18^{a}			
	AV	1.48^{a}	0.96^{b}	0.94^{b}	1.02^{ab}	1.01^{ab}				
Lymphoid Organs										
Spleen%	S	0.07^{b} ±0.03	0.08^{a} ±0.02	0.09^{a} ±0.02	0.08^{a} ±0.03	0.08^{a} ±0.03	0.08^{a}	*	*	*
	P	0.05^{c} ±0.02	0.07^{b} ±0.01	0.07^{b} ±0.03	0.07^{b} ±0.01	0.06^{c} ±0.01	0.06^{b}			
	AV	0.06^{c}	0.08^{a}	0.08^{a}	0.08^{a}	0.07^{b}				
Thymus%	S	0.006^{b} ±0.02	0.008^{a} ±0.03	0.009^{a} ±0.01	0.008^{a} ±0.01	0.008^{a} ±0.02	0.008^{a}	*	*	*
	P	0.003^{d} ±0.01	0.005^{c} ±0.01	0.006^{b} ±0.02	0.005^{b} ±0.03	0.005^{b} ±0.03	0.005^{b}			
	AV	0.005^{b}	0.007^{a}	0.008^{a}	0.007^{a}	0.007^{a}				

a, b, c, d, e, f: Means with different superscripts in the same row within item differ significantly; NS: Not significant; *: (p≤0.05); **: p≤0.01); DB: Duckling breed; S: Sudani ducks; P: Pekin ducks; AV: Overall mean.

Chemical analysis of breast and thigh

Chemical compositions of duckling meat at 12 weeks of age are presented in Table 5. Dietary LC and Cr supplementation had significant effects on moisture; crude protein and ether extract content percentage of breast and thigh meat, while ash% was not significantly affected. Crude protein content% was significantly increased (p≤0.01) by increasing both LC and Cr supplementation levels for breast and thigh meat than the control group. However, ether extract content% was significantly decreased (p≤0.01). Crude protein% was significantly higher (p≤0.01) by 24.82-35.04 and 6.05-14.64%, respectively in breast and thigh meat for duckling breed fed ration supplemented with LC and Cr as compared with control. The reduced of ether extract content in breast and thigh muscles by supplementing LC diet might be because LC acts by reducing the total activities of glucose-6-phosphate dehydrogenize, malic dehydrogenize, isocitrate dehydrogenize, lipoprotein lipase, and total activities of carnitine palmitoyltransferase-I in breast muscles (Xu et al., 2003). On the other hand, it may accelerate lipid flux into oxidative metabolism, and consequently reduces the body lipid accumulation **(**Shuenn et al., 2012). These results were partially in agreement with Zhang et al. (2005) who indicated that enrichment of diet with yeast could favorably improve the quality of edible meat from broiler chickens. Awad et al. (2016) reported that crude protein percentage was significantly increased by 8.13, 11.91, 22.54, and 22.62 in breast muscles, whereas, ether extract percentage was significantly decreased by 16.73, 21.10, 52.77 and 50.72% in breast muscles for Domyati ducklings fed diets supplemented with different LC levels (150, 300, 450 and 600 mg/kg of diet), respectively as compared to control diet. El-Fiky et al. (2019) reported that moisture and crude protein percentage

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were significantly increased in the meat of the Mulard ducks (74.33 and 19.45%) compared with Muscovy ducks (73.72 and 18.90%), while, the ether extract and ash percentage were significantly high (3.82 and 1.50%) in the meat of the Muscovy ducks, compared with the Mulard ducks (1.35 and 1.35%). On the other hand, these results were in contrast with the results obtained by Younis (2015) who reported that the chemical composition of breast muscles were not affected due to the addition of LC supplementation by 500 mg/kg.

Economic efficiency

Calculations of Economic Efficiency (EE) have been listed in Table 6. Different levels of LC and Cr supplementation had effects on total coast and returns as well as economic efficiency values. Generally, net return and EE values were higher by feeding diet supplemented with different LC and Cr levels than the control diet. Economic efficiency values for Sudani ducklings were improved by 3.97, 1.77, 1.28 and 0.16% for ducklings fed the diet supplemented with both LC and YC, respectively than those fed the control diet. However, Economic efficiency values for Pekin ducklings were improved by 56.85, 50.88, 45.61 and 44.15% for ducklings fed the diet supplemented with both LC and Cr, respectively than those fed the control diet. These results agreed with Awad et al. (2016) who conferred that economic efficiency value was significantly higher for Domyati breed which fed ratio supplementing with different levels of LC than those fed the control diets. Awad et al. (2017) observed that total cost had insignificantly improved by supplementing different levels of LC to the diet. The total return had significantly improved for Sudani breeds which fed ratio supplemented with 150, 300, 450, 600, and 750 mg LC/kg, respectively than those fed the control diets. El-Kelawy (2019) found that economic efficiency was improved by Cr supplementation during 14-49 days of Japanese quail's age.

Table 5. Effect of breed and dietary supplementation on the chemical composition of meat in Sudani and Pekin ducks during 12 weeks of age

Age (weeks)	DB	Treatments					AV	Probability		
		Control	L-carnitine		Yeast chromium					
			300	450	400	600		B	T	BT
Breast meat										
Mo (%)	S	70.11^{c}±0.31	74.52^{a}±0.28	74.97^{a}±0.37	72.58^{b}±0.30	72.87^{b}±0.29	73.01^{a}	*	*	*
	P	70.02^{c}±0.18	74.50^{a}±0.22	74.81^{a}±0.19	72.50^{b}±0.20	72.71b±0.21	72.91^{b}			
	AV	70.07^{c}	74.51^{a}	74.89^{a}	72.54^{b}	72.79^{b}				
CP (%)	S	15.45e±0.22	20.39^{ab} ±0.17	21.15^{a}±0.19	19.87^{b}±0.21	20.11^{ab}±0.18	19.39^{a}	**	**	**
	P	14.68^{f}±0.15	18.91^{c}±0.12	19.55^{b}±0.17	17.75^{d}±0.11	18.45^{c}±0.16	17.87^{b}			
	AV	15.07^{d}	19.65^{b}	20.35^{a}	18.81^{c}	19.28^{b}				
EE (%)	S	5.57^{b}±0.08	2.84^{e} ±0.07	2.45^{e}±0.09	3.45^{d}±0.11	3.16^{d}±0.15	3.49^{b}	**	**	**
	P	8.64^{a}±0.17	4.48^{c}±0.13	4.17^{c}±0.19	5.57^{b}±0.21	5.24^{b}±0.16	5.62^{a}			
	AV	7.11^{a}	3.66^{c}	3.31^{c}	4.51^{b}	4.20^{b}				
Ash (%)	S	1.12±0.05	1.96 ±0.06	1.42±0.07	1.75±0.09	1.35±0.11	1.52	NS	NS	NS
	P	1.00±0.08	1.92±0.09	1.40±0.07	1.65±0.6	1.31±0.07	1.46			
	AV	1.06	1.94	1.41	1.70	1.33				
Thigh meat										
Mo (%)	S	72.64^{d}±0.23	75.30^{a}±0.25	75.61^{a}±0.29	74.74^{b}±0.22	74.93^{b}±0.23	74.64^{a}	*	*	*
	P	71.67^{e}±0.25	74.60^{b} ±0.23	74.96^{b}±0.21	73.67^{c}±0.22	73.97^{c}±0.21	73.77^{b}			
	AV	72.16^{c}	74.95^{b}	75.29^{a}	74.21^{b}	74.45^{b}				
CP (%)	S	16.74^{d}±0.22	18.19^{b} ±0.21	19.01^{a}±0.23	18.15^{b}±0.25	18.17^{b}±0.26	18.05^{a}	**	**	**
	P	14.67^{f}±0.15	16.80^{d}±0.13	17.01^{c}±0.16	15.16^{e}±0.11	15·87^{e}±0.19	15.90^{b}			
	AV	15.71^{d}	17.50^{b}	18.01^{a}	16.66^{c}	17.02^{b}				
EE (%)	S	7.47^{b}±0.09	4.57^{e} ±0.08	4.15^{e}±0.11	5.65^{d}±0.07	5.47^{d}±0.08	5.46^{b}	**	**	**
	P	9.11^{a}±0.13	6.40^{c}±0.11	6.02^{c}±0.18	7.61^{b}±0.21	7.00^{b}±0.151	7.23^{a}			
	AV	8.29^{a}	5.49^{c}	5.09^{c}	6.63^{b}	6.24^{b}				
Ash (%)	S	1.11±0.08	1.41±0.06	1.33±0.09	1.31±0.07	1.24±0.08	1.28	NS	NS	NS
	P	1.00±0.07	1.15±0.09	1.29±0.11	1.12±0.08	1.02±0.12	1.12			
	AV	1.06	1.28	1.31	1.22	1.13				

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Table 6. Effect of dietary treatments on the economic efficiency of Sudani and Pekin ducks during 12 weeks of age

Items	Sudani					Pekin				
	Control	L-carnitine (mg/kg diet)		Yeast chromium (µg/kg diet)		Control	L-carnitine (mg/kg diet)		Yeast chromium (µg/kg diet)	
		300	450	400	600		300	450	400	600
Price diet/kg (LE)	4.7	4.8	4.85	4.73	4.75	4.7	4.8	4.85	4.73	4.75
Total feed coast (LE)	56.69	59.88	60.72	58.70	59.01	44.49	46.67	47.15	45.58	45.84
Total return (LE)	87.68	93.92	94.51	91.20	91.32	52.08	59.17	59.30	56.92	57.12
Net return (LE)	30.99	34.04	33.78	32.50	32.31	7.60	12.50	12.15	11.34	11.29
Economic efficiency (EE)	54.67	56.84	55.64	55.37	54.76	17.08	26.79	25.77	24.87	24.62

Total cost: feed cost + LC or YC cost + duckling price; LE: Egyptian pound; Net Revenue: Total Revenue - Total feed cost; Economic efficiency: (Net Revenue / Total feed cost) ×100.

CONCLUSION

It could be concluded that both duckling breeds that received dietary supplemented with L-carnitine by 450 mg LC/kg diets resulted in better performance without any adverse effects on physiological responses and carcass quality as well as economic efficiency.

DECLARATIONS

Author's contributions

Conceptualization done by Sobhy Hassan HM and Ahmed Ali KhAE-M; formal analysis done by Sobhy Hassan HM and Ahmed Ali KhAE-M; data curation done by Sobhy Hassan HM, Ahmed Ali KhAE-M and Gouda ARAE-H; methodology done by Sobhy Hassan HM, Ahmed Ali KhAE-M and Gouda ARAE-H; supervision done by project Gouda ARAE-H; administration done by Sobhy Hassan HM, Ahmed Ali KhAE-M and Gouda ARAE-H; writing done by review and editing done by Sobhy Hassan HM, Ahmed Ali KhAE-M and Gouda ARAE-H. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declared no conflict of interest.

REFERENCES

Abdel-Fattah SA, El-Daly EF and Ali NGM (2014). Growth performance, immune response, serum metabolites and digestive enzyme activates of Japanese quail fed supplemental L-carnitine. Global, Veterinaria, 12: 277-286. DOI: https://www.doi.org/10.5829/idosi.gv.2014.12.02.82224

Adabi GSH, Cooper RG, Ceylan N and Corduk M (2011). L-carnitine and its functional effects in poultry nutrition. World Poultry Sciences journal, 67: 277-296. DOI: https://www.doi.org/10.1017/S0043933911000304

Adeola O (2006). Review of research in duck nutrient utilization. International Journal of Poultry Science, 5: 201-218.

Ali WAH, Kh A.A.Ali Amal M Hekal, Fadila M Easa, EL-AIK MAA and Reham AM Ali (2017). Effect of dietary Iodine supplementation on productive performance of Pekin and Domyati ducks during Growth Period. journal of Animal and Poultry Production Mansoura University, (9): 381- 389. DOI: https://www.doi.org/10.21608/JAPPMU.2017.46013

Ali WAH (2005). Productive and genetical studies on ducks. Ph.D Thesis, Faculty of Agriculture Cairo University Egypt.

Association of Official Agricultural Chemists (AOAC) (2000). Official Methods of Analysis 15th ed. Association of Official Analytical Chemists. Washington, DC., USA.

Arslan C, Citil M and Saatci M (2003). Effects of L-carnitine adminstration on growth performance, carcass traits, blood serum parameters and abdominal fatty acid composition of ducks. Archives of Animal Nutrition, 57: 381-388. DOI: https://www.doi.org//10.1080/00039420310001607734

Awad AL, Fahim HN, Beshara MM and El-Shhat AM (2017). Effects of sex and L-carnitine addition on growth performance and carcass quality of Sudani ducklings. Poultry Sciences, 37: 1013-1032. DOI: https://www.doi.org/10.21608/EPSJ.2017.5377

Awad AL, Fahim HN and Beshara MM (2016). Effects of dietary L-carnitine supplementation on productive performance and carcass quality of local duck breeds in summer season. Egypt Poultry Sciences, 36: 11-27. DOI: https://www.doi.org/10.21608/epsj.2016.11372

Awad AL, Ibrahim AF, Fahim HN and Beshara MM (2014). Effects of dietary betaine supplementation on growth performance and carcass traits of domyati ducklings under summer conditions. Egypt Poultry Sciences, 34: 1019-1038. DOI: https://www.doi.org/10.3390/ani9080506

Debski B, Zalewski W, Gralak MA and Kosla T (2004). Cr-yeast supplementation of chicken broilers in an industrial farming system. Journal of Trace Elements in Medicine and Biology, 18: 47-51. DOI: https://doi.org/10.1016/j.jtemb.2004.02.003

Duncan DB (1955). Multiple range and multiple F- tests. Biometrics, 11: 1-42.

El-Fiky AA, Zanaty GA, Ali WAH and Dahrouj AAF (2019). Study on the ducks fatty liver production under the Egyptian conditions. Menoufia Journal of Animal, Poultry and Fish production, 3: 13-27.

To cite this paper: Sobhy Hassan HM, Ahmed Ali KhAE-M and Gouda ARAE-H (2021). Effect of L-Carnitine and Yeast Chromium Supplementation on Productive Performance in Pekin and Sudani Duckling during Growth Period. *World Vet. J.,* 10 (4): 587-596. DOI: https://dx.doi.org/10.54203/scil.2020.wvj70

El-Hommosany YM (2008). Study of the physiological changes in blood chemistry, humeral immune response and performance of quail chicks fed supplemental chromium. International Journal of Poultry Science, 7 (1): 40-44. DOI: https://www.doi.org/10.3923/ijps.2008.40.44

El-Kelawy MI (2019). Effect of different sources of chromium on productive performance of Japanese quail under heat stress conditions. Egypt Poultry Science, (39) (I): 99-115. DOI: https://www.doi.org/10.21608/EPSJ.2019.28827

Farag MR, Alagawany M, Abd El-Hack ME, Arif M, Ayasan T, Dhama K, Patra A and Karthik K (2017). Role of Chromium in Poultry Nutrition and Health: Beneficial Applications and Toxic Effects. International Journal of Pharmacology, 13 (7): 907-915. DOI: https://www.doi.org/10.3923/ijp.2017.907.915

Fathi E and Farahzadi R (2014). Application of L-carnitine as nutritional supplement in veterinary medicine. Romanian Journal of Biochemistry, 51: 31-41.

Gouda AR (2015). Genetic characterization of local and exotic duck strains and evaluation of their productive and reproductive traits under Egyptian condition. M.Sc. Thesis, Institute of African Research and Studies, Egypt.

Harmeyer J (2002). The physiological role of L-carnitine. Lohman Information, 27: 15-21. http:// www.Anasi.okstate.edu/research/2001rr/35/35.htm

Huang Y, Yang J, Xiao F, Lloyd K and Lin X (2016). Effects of supplemental chromium source and concentration on growth performance, carcass traits and meat quality of broilers under heat stress conditions. Biological Trace Element Research, 170: 216-223. DOI: https://www.doi.org/10.1007/s12011-015-0443-z

Ibrahiem ZA, Ezzat W, El-Zaiat AA and Shoeib MS (2011). Effect of L-carnitine on the growth, serum components, carcass traits and histological stricture of muscles in the growing geese. Egyptian Journal of Animal Production, 48: 91-104.

Khan RU, Naz S and Dhama K (2014). Cr Pharmacological applications in heat-stressed poultry. International Journal of Pharmacology 10: 213-217. Available at: http://scialert.net/qredirect.php?

Michalczuk M, Lukasiewiez M, Niemice j, Wnuk A and Sosnowska NM (2012). Effect of L-carnitine on performance and dressing percentage of broiler chickens. Animal Science, 51: 89-99.

National Research Council (NRC) (2005). Mineral tolerance of animals. Second revised edition, Committee on minerals and toxic substances in diets and water for animals, Board on agriculture and natural resources, Division on earth and life studies. National Academy Press, Washington DC.

Oladele OA, Adeboye F, Richard S and Zainob H (2011). Growth response, carcass yield and serum biochemistry of broiler chicken feds with supplemental L-carnitine in feed or drinking water. Journal of Poultry Science, 48:223-228. DOI: https://www.doi.org/10.2141/jpsa.011016

Parsaeimehr K, Afrouziyeh M and Hoseinzadeh S (2014). The effects of L-carnitine and different levels of anim al fat on performance, carcass characteristics, some blood parameters and immune response in broiler chicks. Iranian Journal of Applied Aminal Science, 4: 561-566. Received on: 11 Sep 2013 Revised on: 19 Jan 2014 Accepted on: 30 Jan 2014 Online Published on Sep 2014

Piva A, Meola E, Gatta PP, Biagi G, Castellani G, Mordenti AL, Luchansky JB, Silva S and Mordenti A (2003). The effect of dietary supplementation with trivalent on production performance of laying hens and the chromium content in the yolk. Animal Feed Sciences Technology, 106:149–163. DOI: https://www.doi.org/10.1016/S0377-8401(03)00006-3

Pramod K, Verma SB, Akhtar KGSM and Kumar P (2002). Influence of sex on body weight and conformation traits in a random bred population of Japanese quail. Indian Jpurnal of Animal Health, 41: 1, 61-63.

SAS (2001). SAS User's Guide: Statistics. 9th, Edition SAS Institute., Inc., Cary. NC., USA.

Shuenn DY, Fu GL and Chyng (2012). Effects of dietary L-carnitine, plant proteins and lipid level on growth performance, body composition, blood traits and muscular carnitine status in juvenile silver perch (Bidyanusbidyanus). Aquaculture, 342-343. DOI: https://www.doi.org/10.1016/j.aquaculture.2012.02.002

Soltan ME, Enab AA, Gebriel GM and Kandil MS (2014). Meat production efficiency of two lines of Pekin ducks under two different feeding systems. Minufiya Journl of Argicultural Research, 39:521-535.

Taklimi SM, Ghazvinian K and Kasgri MRA (2015). Effect of L-carnitine on performance and carcass quality of broiler chickens. Academia Journal of Scientific Research, 3: 50-54.

Wang J, Du R, Qin J, Wang S, Wang W, Li H, Pang Q (2003). Effect of yeast chromium and L-carnitine on lipid metabolism of broiler chickens. Asian-Australasian Journal of Animal Science, 16(12): 1809-1815. DOI: https://www.doi.org/10.5713/ajas.2003.1809

Xu ZR, Wang MQ, Mao HX, Zhan XA and Hu CH (2003). Effects of L-carnitine on growth performance, carcass composition and metabolism of lipids in male broilers. Poultry Science, 82: 408-413. DOI: https://www.doi.org/10.1093/ps/82.3.408

Younis MEM (2015). Influence of dietary L-carnitine on productive performance, internal organs and carcass characters of two duck breeds reared for foiegras production. Alexanderia Journal of Veterinary Science, 44: 159-168. DOI: https://www.doi.org/10.5455/ajvs.178704

Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB and Lee CH (2005). Effects of yeast (Saccharomyces cerevisiae) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Science, 84:1015–1021.DOI: https://www.doi.org/10.1093/ps/84.7.1015

To cite this paper: Sobhy Hassan HM, Ahmed Ali KhAE-M and Gouda ARAE-H (2021). Effect of L-Carnitine and Yeast Chromium Supplementation on Productive Performance in Pekin and Sudani Duckling during Growth Period. *World Vet. J.,* 10 (4): 587-596. DOI: https://dx.doi.org/10.54203/scil.2020.wvj70

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 597-601, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj71

Epidemiological Assessment of Contagious Bovine Pleuropneumonia in Central Tanzania

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ORGINAL ARTICLE
pii: S232245682000071-10
Received: 09 Sept 2020
Accepted: 05 Nov 2020

ABSTRACT

A retrospective study was conducted to determine the epidemiology of Contagious Bovine Pleuropneumonia (CBPP) in the Central Zone of Tanzania. The present study used data from archived information of Central Zone Veterinary Centre (CZVC) for the past five years in the forms of weekly, monthly, and slaughterhouse reports, as well as Event Mobile Application (EMA-i) reports submitted to the zone. The present study found that out of 14 Local Government Authorities (LGAs) in the Central Zone,10 reported the disease in the past five years. Moreover, 56, 426, and 11147cases were reported as deaths, and the cattle at risk respectively. Therefore, 3.8%, 13%, and 0.5%were reported as CBPP prevalence, case fatality rate, and mortality rate, respectively. It was also revealed that there was a clear temporal pattern of CBPP occurrence, with more cases being reported between August to December. In conclusion, CBPP was a seasonal problem in Central Tanzania. Therefore, the present research recommended the strengthening of control measures against this disease in the central zone of Tanzania. In order to be able to assess the actual burden of the disease on-site, this extensive study must be carried out, since the parameters obtained during the study were lower compared to the situation on-site.

Keywords: Central zone, Contagious bovine pleuropneumonia, Prevalence and distribution

INTRODUCTION

Contagious Bovine Pleuropneumonia (CBPP), is a significant constraint to cattle production throughout sub-Saharan Africa (Wakgari et al., 2018). The causative agent of CBPP is *Mycoplasma mycoides* subspecies mycoides (small colony), which is transmitted through direct contact. Clinically, CBPP is characterized by three forms, including pre-acute, acute, and chronic forms. Temporary, obvious infections and the presence of persistently infected animals with the encapsulated infected sequestra are features of chronic CBPP that promote further spread of the disease (Di Provvido et al., 2017). In 2015, 45 Local Government Authorities (LGAs) reported CBPP in mainland Tanzania, in which a total of 3795 cattle were infected and 706 of them died. Since 2013, the government has spent 1,835,166,910.00 Tanzanian shillings (over USD 1,000,000) to purchase 15,185,800 doses of CBPP vaccines, resulting in national and economic losses (Matthew et al., 2016). The present study aimed to describe the status and seasonality of CBPP in the Central Zone of Tanzania, and necessary information is needed to combat the disease in the zone and the country in general.

MATERIALS AND METHODS

Ethical approval

The present research has been conducted in accordance with ethical principles issued by the Ministry of Livestock and Fisheries in Tanzania, under the Animal Welfare Act (No. 19, 2008).

Study area

The study was conducted in central Tanzania, the zone that consisted of two administrative regions, Dodoma and Singida, which have 14 LGAs. The geographical coverage of the zone was approximately 48653.3 km^2 (Dodoma 41,310 km^{2}and Singida 7,343.3 km^2) with a cattle population of 3.6 Million (Figure 1).

Study design

In this retrospective study design, archived data from CZVC, which were collected and used in the study in the last five years (From January 2014 to December 2018). Weekly, monthly, abattoir, and Event Mobile Application (EMA-i) reports were used. The key variables retrieved were case reports, the population at risk, death reports, region, LGA name, village names, geo-coordinates, and observation date.

To cite this paper: Mngumi S, Makungu S and Mdetele D (2021). Epidemiological Assessment of Contagious Bovine Pleuropneumonia in Central Tanzania. *World Vet. J.*, 10 (4): 597-601. DOI: https://dx.doi.org/10.54203/scil.2020.wvj71

Data analysis and management

The data retrieved were entered and managed in Microsoft Excel sheets, and checked for completeness before analysis (Data cleaning). Descriptive and analytical statistics were done using Microsoft Excel and Quantum GIS software, which were used for mapping using recorded coordinates of LGAs with which CBPP was reported.

Prevalence, mortality and case fatality analysis

The prevalence of CBPP was calculated using the Prevalence formula (P: n//N), where P is the prevalence, n is the number of sick animals in a specific place and at a specific time, and N is the population at risk at the same time (Joffe et al., 2011). Mortality (crude) was calculated by dividing the number of cattle that died during the referenced period by the population at risk over the same period (Chaudhary et al., 2013). Case fatality was estimated by dividing the number of cattle died from CBPP by the number of cases (Ghani et al., 2005).

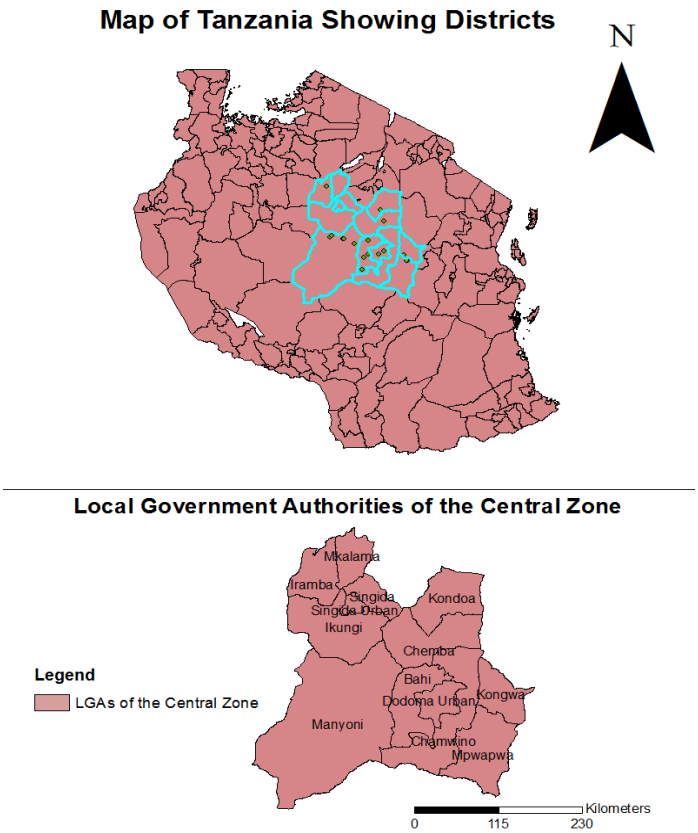

Figure 1. Districts of the study area in Tanzania consisted of two administrative regions (Dodoma and Singida)

RESULTS

During the five-year period from 2014 to 2018, a total of 426 CBPP cases and 56 deaths were reported in both regions. There was a variable number of CBPP cases each year. A total of 56 deaths due to CBPP were reported during the same period. Table 1 indicates that the maximum number of cases per year was 128, and the minimum was 23 cases.

Prevalence of contagious bovine pleoropneumonia

In total of10 out of 14 LGAs reported the disease in the past five years. The overall prevalence of CBPP was 3.8% (426/11147). In contrast, mortality was 0.5% (56/11147), while case fatality was 13% (56/426). The number of affected districts, cases, deaths and population at risk for each year from 2014 to2018 is shown in Table 1.

Mortality and case fatality from contagious bovine pleuropneumonia

The mortality rate of CBPP was 0.5% (56/11147) reported from eight districts, while two districts reported no mortality despite reporting CBPP cases (Table 1). The case fatality of the disease was estimated at 13% (56/426).

Distribution of contagious bovine pleuropneumonia in central Tanzania

The temporal and spatial pattern of the CBPP over the five years is shown in Figure 2 andFigure 3. The disease appeared to be distributed in many parts of the zone with the exception of Singida urban, Mkalama, and Ikungi. Further outbreaks were observed between August and December.

To cite this paper: Mngumi S, Makungu S and Mdetele D (2021). Epidemiological Assessment of Contagious Bovine Pleuropneumonia in Central Tanzania. *World Vet. J.*, 10 (4): 597-601. DOI: https://dx.doi.org/10.54203/scil.2020.wvj71

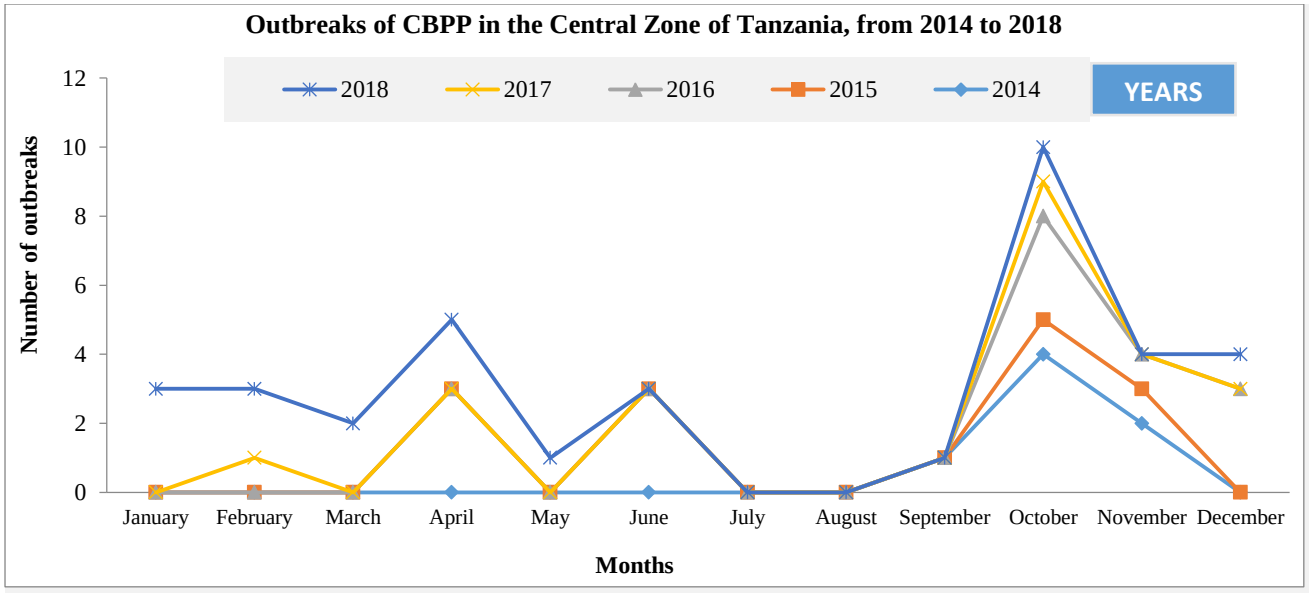

Figure 2. Time distribution of contagious bovine pleuropneumonia cases reported in Central Tanzania from 2014 to 2018

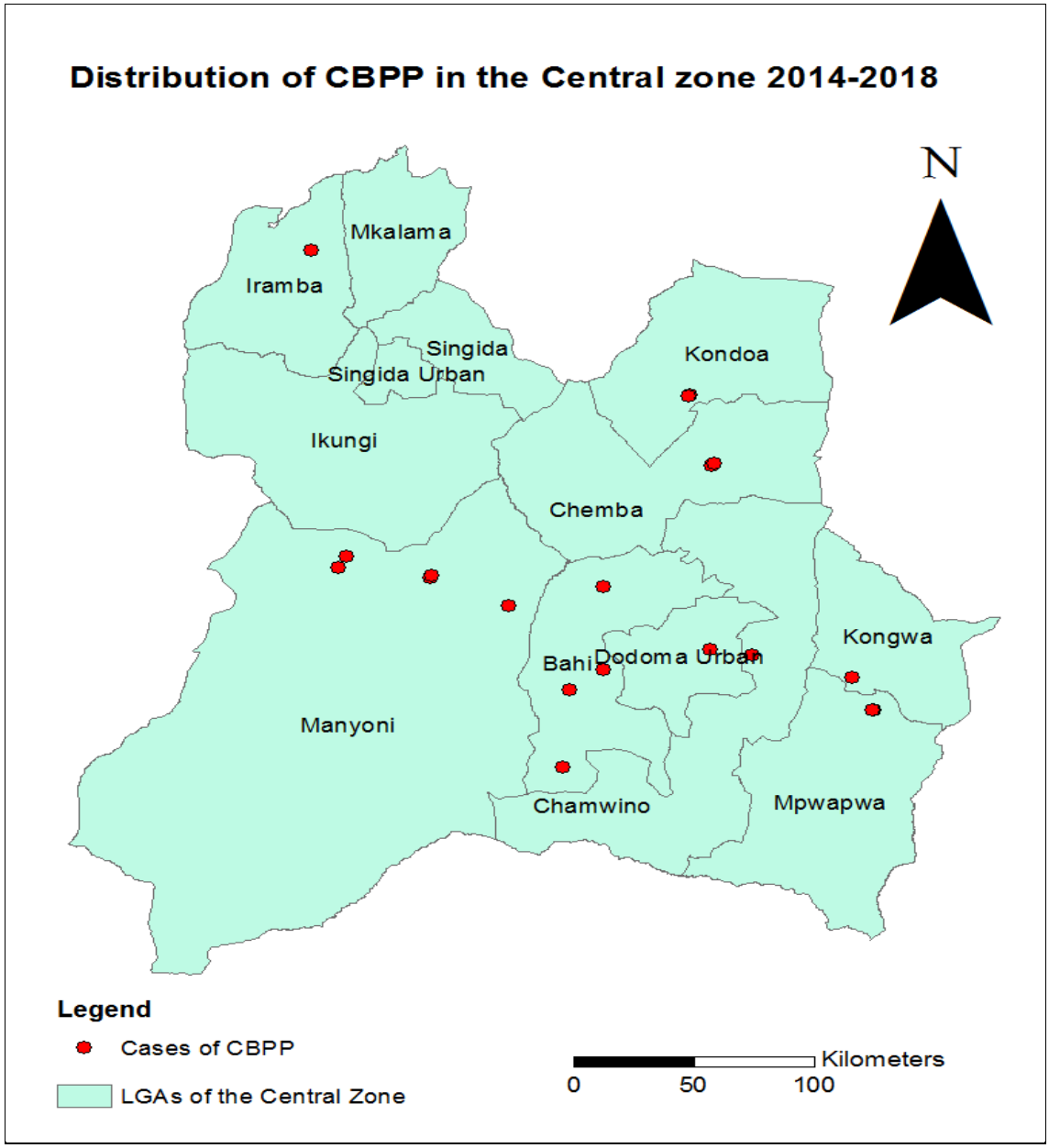

Figure 3. Spatial distribution of contagious bovine pleuropneumonia cases in central Tanzania from 2014 to 2018

To cite this paper: Mngumi S, Makungu S and Mdetele D (2021). Epidemiological Assessment of Contagious Bovine Pleuropneumonia in Central Tanzania. *World Vet. J.*, 10 (4): 597-601. DOI: https://dx.doi.org/10.54203/scil.2020.wvj71

Table 1. The number of contagious bovine pleuropneumonia cases and deaths reported in Central Tanzania from 2014 to 2018

Year	Affected districts	Population at risk	Cases (%)	Deaths (%)
2014	4	1646	113 (6.8)	22 (1.3)
2015	4	748	100 (13)	10 (1.3)
2016	6	2508	62 (2.5)	13 (0.5)
2017	2	2454	23 (0.9)	2 (0.1)
2018	5	3791	128 (3.4)	9 (0.2)
Total		11147	426 (3.8)	56 (0.5)

Table 1. Distribution of contagious bovine pleuropneumonia cases in districts of the Central Tanzania from 2014 to 2018

District	Population at risk	Cases (%)	Deaths (%)
Bahi	358	68 (19)	10 (2.8)
Chamwino	105	14(13)	3 (2.9)
Chemba	288	35 (12)	3 (1)
Dodoma urban	211	3 (1.4)	1 (0.5)
Kondoa	915	19 (2.1)	0 (0)
Kongwa	104	5 (4.8)	1(1)
Mpwapwa	7463	184 (2.5)	9 (0.1)
Iramba	19	1 (5.3)	0 (0)
Itigi	183	7 (3.8)	1 (0.5)
Manyoni	1501	90 (6)	28 (1.9)
Total	11147	426 (3.8)	56 (0.5)

DISCUSSION

The overall prevalence, mortality, and case fatality of CBPP in the present study was3.8%, 0.5%, and 13%, respectively, which were lower compared to other studies. In a fully susceptible population, morbidity and mortality due to CBPP are always higher (Abdela and Yune, 2017). This low prevalence, mortality, and case fatality rate could be due to the endemicity of the disease or under-reporting of LGAs. Hence, few CBPP cases and deaths were reported to the central zone office. The present study demonstrated that a total of ten LGAs reported CBPP in the central Tanzania in the past five years, including Iramba, Itigi, Manyoni, Bahi, Chamwino, Kondoa, Kongwa, Mpwapwa, Chemba, and Dodoma. On the other hand, the remaining four LGAs of central zone did not report this disease for the entire period of the past five years; including Singida Municipal, Singida rural, Mkalama, and Ikungi. The possible explanation for some LGAs reporting CBPP while others didnot, could be that some LGAs with good technical veterinary professionals were effective compared to others, that they had good reporting tendencies than others. Therefore, it seemed like some LGAs have more CBPP cases than others. These differences in CBPP cases between LGAs in Central Tanzania could also be due to understaffing or inability of farmers to recognize CBPP cases which were compounded by syndromic nature of this disease, as many cases go unnoticed (Swai et al., 2013). The results indicated that there was a clear temporal pattern of CBPP outbreaks in the central Tanzania, with more CBPP outbreaks reported between August and December compared to other seasons (Figure 2). These results were in consistent with the results of a study conducted in Uganda in 2017, in which most CBPP cases were reported during November (Ssemadaali et al., 2017). The reason could be that at that time of the year, due to droughts that cause pasture and water shortages, animals gather in areas where it is possible to keep pasture and water. The close contacts between healthy and carrier animals in these areas enhanced the transmission of infection. It is worth noting that market points and pasture sharing contributed to animal contact and facilitate the transmission of the disease.

CONCLUSION

The study determined the prevalence of 4% for CBPP in central Tanzania based on passive surveillance at local government authority level in central Tanzania. The onset of the disease was also found to have a specific temporal pattern, revealing that duration of August to December was the peak time for CBPP in the central zone. Since CBPP remains a problem in central Tanzania, control measures for this disease across entire central Tanzania have to be strengthened, including vaccination and transmission control. Serological and meat inspection report studies will be much more important to get comprehensive results with laboratory-confirmed disease results for a proper evaluation of the disease status in the study area.

To cite this paper: Mngumi S, Makungu S and Mdetele D (2021). Epidemiological Assessment of Contagious Bovine Pleuropneumonia in Central Tanzania. *World Vet. J.*, 10 (4): 597-601. DOI: https://dx.doi.org/10.54203/scil.2020.wvj71

DECLARATIONS

Authors' contribution

Makungu Selemani designed the study, Samwel Mngumi played a role in study design, data retrieval and analysis, and manuscript writing, Daniel Mdetele contributed in manuscript writing. Finally, all authors revised the manuscript and approved it for publication.

Competing interests

The authors declared that they have no competing interests.

Acknowledgements

This study was supported by Food and Agriculture Organization of the United Nations (FAO) including FAO-Tanzania, the Institute for Infectious Animal Diseases (IIAD), and Texas A&M University through In-Service Applied Veterinary Epidemiology Training (ISAVET) Programme. The authors would also like to thank The Ministry of Livestock and Fisheries of Tanzania, through the Directorate of Veterinary Services, for authorizing this research. Great thanks are extended to Professor Esron Karimuribo, Dr. Emmanuel Swai, Dr. Erick Komba and Dr.Benezeth Lutege for their guidance. Special thanks go to the District Veterinary and Livestock Field Officers in Central Tanzania for reporting disease conditions to the Central Zone.

REFERENCES

Abdela N and Yune N (2017). Seroprevalence and distribution of contagious bovine pleuropneumonia in Ethiopia: Update and Critical Analysis of 20 Years (1996-2016) Reports. Frontiers in veterinary science, 4: 100. DOI: https://www.doi.org/10.3389/fvets.2017.00100

Chaudhary JK, Singh B, Shiv P and Verma MR (2013). Analysis of morbidity and mortality rates in bovine in Himachal Pradesh. Veterinary World, 6(9): 614-619. Availabe at: http://www.veterinaryworld.org/Vol.6/Sept-2013/6.pdf

Di Provvido A, Di Teodoro G, Muuka G , Marruchella G and Scacchia M (2017). Lung lesion score system in cattle: proposal for contagious bovine pleuropneumonia. Tropical Animal Health and Production, 50: 223–228. DOI: https://www.doi.org/10.1007/s11250-017-1409-2

Ghani AC, Donnelly CA, Cox DR, Griffin JT, Fraser C, Lam TH Ho LM, Chan WS, Anderson RM, Hedley AJ et al. (2005). Methods for estimating the case fatality ratio for a novel, emerging infectious disease. American Journal of Epidemiology, 162(5): 479-486. DOI: https://www.doi.org/10.1093/aje/kwi230

Joffe D, Van Niekerk D, Gagne F, Gilleard J, Kutz S and Lobingier R (2011). The prevalence of intestinal parasites in dogs and cats in Calgary, Alberta. Canadian Veterinary Journal-Revue Veterinaire Canadienne, 52(12):1323-1328.Availabe at:: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3215466/

Matthew M, Mruttu H and Gebru G (2016). Animal health strategy and vision forTanzania. Available at: https://www.semanticscholar.org/paper/Animal-health-strategy-and-vision-for-Tanzania-Matthew-Mruttu/a21c6fc3ea91a7cb12f06e7580e85a2f8e3066d5

Ssemadaali MA, Majalija S, Mwebe R, Olet S and Khaitsa ML (2017). Spatial and temporal distribution of contagious bovine pleuro-pneumonia in Uganda (1956-2011). Pan African Medical Journal (ARTISSUE). Availabe at:: https://www.panafrican-med-journal.com/content/series/27/4/2/full/

Swai E, Mwezimpya I, Ulicky E, Mbise A and Moshy W (2013). An abattoir survey of contagious bovine pleuropneumonia lesions in slaughtered cattle in selected districts in Northern Tanzania. Asian Pacific Journal of Tropical Biomedicine, 3(4): 303-306. DOI: https://www.doi.org/10.1016/S2221-1691(13)60067-2

Wakgari M, Kitila G, Chali I, Merdasa D, Guta D, Gezahegn E and Kebede B (2018). Sero-prevalence of contagious bovine pleuropneumonia (CBPP) and its associated potential risk factors in selected districts of east Wollega zone, Oromia region, Ethiopia Austin Journal of VeterinaryScience andAnimal Husbandry, 5(2): id1043. Availabe at: https://austinpublishinggroup.com/veterinary-science-research/fulltext/avsah-v5-id1043.php

To cite this paper: Mngumi S, Makungu S and Mdetele D (2021). Epidemiological Assessment of Contagious Bovine Pleuropneumonia in Central Tanzania. *World Vet. J.*, 10 (4): 597-601. DOI: https://dx.doi.org/10.54203/scil.2020.wvj71

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 602-608, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj72

Coxiella Burnetii in Horses of Algeria: Seroprevalence and Associated Risk Factors

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ORGINAL ARTICLE
pii: S232245682000072-10
Received: 05 Oct 2020
Accepted: 16 Dec 2020

ABSTRACT

The Q fever is a worldwide zoonotic disease caused by *Coxiella burnetii* (an obligate intracellular bacterium). This pathogen affects humans, ruminants, equines, carnivores, rodents, and birds. A cross-sectional study was carried out from March 2017 to May 2018 to assess the seroprevalence and identify the risk factors of *C. burnetii* infection in horses (*Equus Caballus*) residing in three districts of Algerian, namely Tiaret, El-Bayadh, and Ghardaia. Serum samples collected from 182 horses were analyzed via enzyme-linked immunosorbent assay (ELISA). Association of seropositivity with potential risk factors related to animals (e.g., age, gender, breed, housing, and presence of ticks), breeding characteristics (e.g., geographical localization, contact with animals), and environmental characteristics (i.e., presence of water source) was analyzed by univariate and multivariate logistic regression. An overall seroprevalence of 9.9% (18/182) was obtained. The univariate analysis of risk factors for *C. burnetii* seroprevalence demonstrated higher seropositivity in horses that had contact with small ruminants (p=0.004) and dromedaries (p=0.002) as well as in those living near a water source (p=0.036) and in El-Bayadh district (p=0.005). The multivariate logistic regression analysis indicated that the risk of *C. burnetii* infection was significantly higher in horses that were in contact with small ruminants (RR: 15.6). Algeria is endemic for Q fever in horses and prophylactic measures must be taken to reduce /prevent its transmission to animals and humans.

Keywords: Algeria, *Coxiella burnetii*, ELISA, Horses, Q fever, Seroprevalence

INTRODUCTION

Coxiella burnetii is a Gram-negative obligate intracellular bacterium that causes a zoonotic disease called Q fever, which affects humans, mammalians, and non-mammalians animals. Recently, this bacterium was classified in the order Legionellales, family *Coxiellaceae* (Bielawska-Drózd et al., 2013). Affected animals excrete *C. burnetii* in their body secretions, which could directly or indirectly contaminate the environment, animals, and humans. Parturient fluids from infected animals can contaminate the environment (Tissot Dupont et al., 1992). Although one of the most important sources of this pathogen is the birth products (i.e., placenta, amniotic fluid) of infected animals, dairy products of animals (e.g., milk and cheese) have been reported to transmit *C. burnetii* (Maurin and Raoult, 1999). The disease is transmitted to humans and animals not only through inhalation of infected particles from goats, sheep, and cattle but also by ticks that have been strongly implicated as vectors (Duron et al., 2015). In humans, Q fever can be asymptomatic, but acute forms with pneumonia or hepatitis have been also reported (Raoult et al., 1989; Angelakis and Raoult, 2010; Eldin et al., 2017). Additionally, endocarditis, vascular, and osteoarticular infections are associated with chronic Q fever (Raoult et al., 1989; Angelakis and Raoult, 2010). Since domestic ruminants are mostly incriminated in the transmission of *C. burnetii* to humans, many epidemiological studies have been conducted throughout the world. Infected ruminants with *C. burnetii* are mainly asymptomatic and the only detectable clinical signs include abortions at the end of gestation, usually 15 days before term (Rodolakis, 2004), stillbirth, premature delivery, birth of weak offspring, agalactia, and infertility (Cetinkaya et al., 2000).

To date, the role of horses in the Q fever epidemiology has not been deeply investigated. In experimental conditions, horses infected with *C. burnetii* developed fever, depression, enteritis, and/ or bronchopneumonia (Zotov et al., 1956). Moreover, the isolation of *C. burnetii* from the placenta of aborted horses using molecular tests indicates a

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real involvement of the pathogen in reproductive disorders in this species (Runge et al., 2012; Roest et al., 2013). The role of horses in the transmission of *C. burnetii* to humans has been investigated. Some studies reported that people in close contact with horses, such as veterinarians and horseback riders, have more chances to be infected (Karagiannis et al., 2009; Palmela et al., 2012).

In Algeria, the zoonotic risk of *C. burnetii* has not been extensively investigated. In a study conducted by Lacheheb and Raoult (2009), the implementation of the immunofluorescence antibody test (IFAT) indicated a seroprevalence of 15.5% in humans, in Setif (northern Algeria). Recently, Ghaoui et al. (2018) identified 4 placentas affected with *C. brunettii* via IS1111 qPCR and IS30 qPCR as well as 3 seropositive individuals using IFAT out of 745 febrile spontaneous abortions in women. The findings of several studies on animals using serological tests indicated a seroprevalence range of 10-75% in ruminants and dromedaries (Yahiaoui et al., 2013; Agag et al., 2016; Khaled et al., 2016; Benaissa et al., 2017; Djellata et al., 2019; Bellabidi et al., 2020; Menadi et al., 2020). To our knowledge, there is no report about Q fever infection in horses in Algeria. Therefore, the present study aimed to assess the seroprevalence of *C. burnetii* in horses in three Algerian districts (i.e., Tiaret, El-Bayadh, and Ghardaia) and to identify the potential risk factors associated with *C. burnetii* infection in horses.

MATERIALS AND METHODS

Ethical approval

Risk assessment was submitted to and approved by the ethics committee of the Algerian Ministry of the Interior, the Local Government and the Algerian Ministry of Agriculture and Rural Development. Blood sampling was performed by a qualified veterinarian according to the guidelines for the care and use of the animal.

Study area and sample collection

The study was carried out in the three following Algerian districts (called Wilayas), namely Tiaret (North West), El-Bayadh (Southwest), and Ghardaïa (South) as indicated in Figure 1. The study covers an area of 185648 km^2 spreading out between 28°54'30.87" and 35°38'50.86" North - 0°30'10.88" West and 4°58'54.03" East. These regions were chosen for their high concentration in horse breeding used for traditional events and production. The samples in the current study consisted of 182 horses randomly chosen from March 2017 to May 2018. The blood samples (approximately 5 ml) were taken through the jugular vein using vacutainer tubes. Sera were obtained by centrifugation at 3000 rpm for 10 min and stored at -20 °C until serological analysis.

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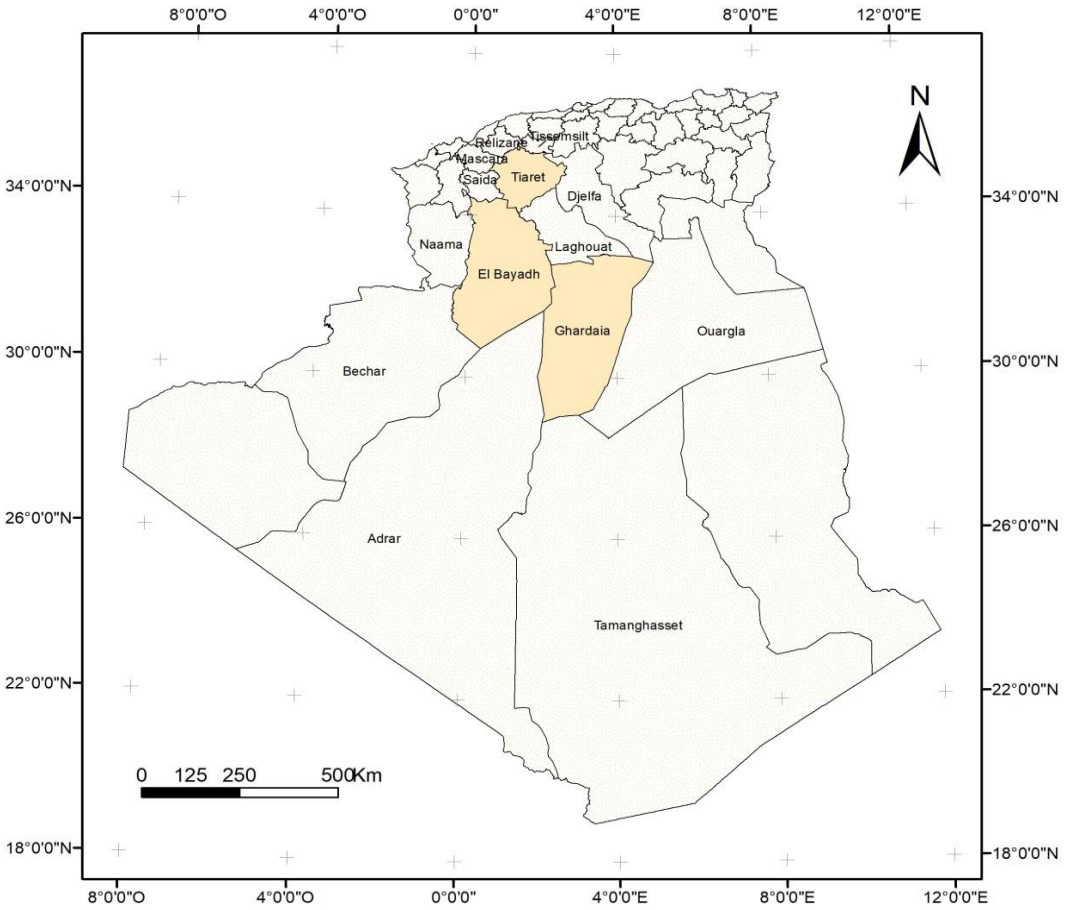

Figure 1. Location of Algerian districts for sample collection

Serologic test

The collected serum samples were tested for the presence of anti-*C. burnetii* antibodies via enzyme-linked immunosorbent assays (ELISA) using a commercial kit (ELISA-ID Screen® Q Fever Indirect Multi-species, IDVet, Montpellier, France) according to the manufacturer's instructions. The reaction was quantified by a spectrophotometer reading at 450 nm (Biotek Instruments Inc., USA). The results were reported in the optical density (OD) value. The interpretation of the results provided by the supplier (IDVet) is based on the value index. Samples were considered negative in case the OD was lesser than 40%, questionable when OD was between 40% and 50%, positive in case OD was higher than 50%. Two control serums (positive and negative) delivered by the manufacturer were used to validate the test.

Questionnaire

The recorded data was collected directly from the owners of the horses through questionnaire and included the demographic information of animals (age, gender, breed, housing, and presence of ticks), their breeding characteristics (geographical localization, contact with small ruminants and dromedaries), and environmental characteristics (presence of water source up to 1 km away).

Statistical analysis

The data were analyzed using R Studio (version 1.1.383, R Studio Inc., Boston, MA, USA). Seroprevalence was calculated by dividing the number of animals possessing anti-*C. burnetii* antibodies by the total number of the investigated animals. The explanatory variables were categorized as age (< 5, 5-10, > 10 years), gender (male, female), breed (Arabian, barb, Arab/barb, thoroughbred English), housing (box, stable), presence of ticks on animal body (yes, no), contact with ruminants (yes, no), contact with dromedaries (yes, no), presence of water source (yes, no), geographic location (Tiaret, El-Bayadh, Ghardaïa). Univariate analysis was used to analyze risk factors associated with seroprevalence. Multivariate logistic regression analysis was used to test the strength of association between the risk factors and *C. burnetii* infection using the generalized linear models (GLM). The best-fitting model to the dataset was constructed with a backward stepwise approach. The Akaike information criterion (AIC) was recruited to assess the multivariate model and the lowest AIC was used to select the best model. *P*-value less than 0.05 was considered statistically significant.

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RESULTS

Seroprevalence

Out of 182 horses' sera examined, 18 (9.9%) samples were seropositive for *C. burneti* antibodies.

Risk factors

Results of the univariate analysis of risk factors for *C. burnetii* seroprevalence in horses in the study area are summarized in Table 1. Proximity of horses with dromedaries and small ruminants was significantly associated with seropositivity ($p<0.05$). Horses in contact with small ruminants and dromedaries had a higher risk of *C. burnetii* infection (15.4% and 17%, respectively), compared to those that had no contact with these two species. A higher risk of *C. burnetii* infection (14.1%) occurred in horses raised near watering points, compared to those that were farther. The close contact between watering points and horses significantly increased the seropositivity ($p=0.036$). Seroprevalence was significantly different from one Wilaya to another ($p=0.005$). This value was the highest in El-Bayadh (18.6%), followed by Ghardaïa (10.5%) and Tiaret (3.2%). Table 2 tabulates the obtained results of the logistic regression analysis. The potential risk factor related to the proximity with small ruminants indicated that *C. burnetii* infection was higher in horses that were in contact with small ruminants with a relative risk of 15.6% (95% CI = 1.87-130.02).

Table 2. The factors influencing the risk of *Coxiella burnetii* seropositivity in horses.

Risk factors	Category	RR	95% confidence interval	p-value
Contact with small ruminants	Yes No	15.6 Ref	1.87-130.02 Ref	0.006
Watering points	Yes No	0.35 Ref	0.1 - 2.25 Ref	0.17

RR: Risk ratio

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Table 1. Univariable analysis of risk factors of *Coxiella burnetii* seropositivity in horses.

Risk factors	Category	Total No.	Seropositive No.	Seroprevalence % (95% confidence interval)	p-value
Age	<5	59	7	11.9 (3.6 - 20.1)	0.660
	[5-10]	79	6	7.6 (1.8 - 13.4)	
	>10	44	5	11.4 (2 - 20.7)	
Gender	Female	173	18	10.4 (5.8 - 14.9)	0.308
	Male	9	0	0	
Breed	Arab	56	2	3.6 (0 - 8.4)	0.129
	Barb	72	8	11.1 (3.9 - 18.4)	
	Arabe-Barbe	54	8	14.8 (5.3 - 24.3)	
Housing	Box	76	5	6.6 (1 - 12.2)	0.205
	Stable	106	13	12.3 (6 - 18.5)	
Contact with dromedaries	Yes	88	15	17 (9.2 - 24.9)	0.002
	No	94	3	3.2 (0 - 6.7)	
Contact with small ruminants	Yes	104	16	15.4 (8.5 - 22.3)	0.004
	No	78	2	2.6 (0 - 6.1)	
Presence of watering points	Yes	99	14	14.1 (7.3 - 21)	0.036
	No	83	4	4.8 (2 - 9.4)	
Presence of ticks	Yes	106	13	12.3 (6 - 18.5)	0.205
	No	76	5	6.6 (1 - 12.2)	
Geographic localization (Algeria district)	Tiaret	93	3	3.2 (0 - 6.8)	0.005
	El-Bayadh	70	13	18.6 (9.5 - 27.7)	
	Ghardaïa	19	2	10.5 (0 - 24.3)	

No: Number

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DISCUSSION

The *C. burnetii* has been reported in domestic animals, including sheep, goats, bovines, and dromedaries, (Dechicha et al., 2010; Yahiaoui et al., 2013; Agag et al., 2016; Khaled et al., 2016; Benaissa et al., 2017; Bellabidi et al., 2020; Menadi et al., 2020) as well as humans (Benslimani et al., 2005; Ghaoui et al., 2018) residing in Algeria. However, the present study was the first report of *C. burnetii* in horses and risk factors associated with *C. burnetii* infection in horses. Among 182 tested horses, a seroprevalence of 9.9% was obtained. This value is comparable to the pooled seroprevalence of 16% estimated throughout different areas of the word (Marenzoni et al., 2013). Higher rates of *C. burnetii* infection in horses were reported using molecular tests in different countries. Using PCR, a 42.2% rate of prevalence following abortions in horses was recorded in Croatia (Račić et al., 2014) and 7.7% was recorded in the aborted or non-aborted placenta in the Netherlands (Roest et al., 2013). Using loop-mediated isothermal amplification, a prevalence of 22.2% was observed in horse blood samples in China (Pan et al., 2013). Lower rates were observed via the use of complement fixation test in the horses' sera (0%) in Denmark (Agerholm et al., 2015) and by real-time PCR in aborted fetuses in France (1.5%) (Leon et al., 2012).

In Algeria, the observed seroprevalence in horses was lower, compared to the reported values in Cattle (10-29%, Dechicha et al., 2010; Agag et al., 2016; Khaled et al., 2016; Menadi et al., 2020), in dromedaries 71-75%, (Benaissa et al., 2017; Bellabidi et al., 2020), and in small ruminants 14-23%, (Yahiaoui et al., 2013; Khaled et al., 2016). The present study indicated that horses from El-Bayadh had a significantly higher risk of being infected (18.6%), compared to those of Ghardaïa (10.5%), and Tiaret (3.2 %). This discrepancy in the obtained results of various studies on the prevalence of *C. burnetii* can be due to many risk factors, such as the presence of tick vectors, climatic conditions, management practices, control measures, presence of infected animals in the horses' vicinity, and size of samples across different study areas. The prevalence differences were observed in small ruminants residing in different regions of other countries (Ullah et al., 2019; Aljafar et al., 2020).

Horses living near watering points were among the ones with the highest rate of infection. As reported, water proximity in Algeria allowed the proliferation of vectors, such as ticks, to transmit *C. burnetii* (Bessas et al., 2016; Leulmi et al., 2016; Aouadi et al., 2017). The contact of horses with small ruminants was a real risk factor for being seropositive to *C. burnetii*. The obtained result of the current study was indicative of the relative risk of 15.6% (95% CI = 1.87-130.02) for contracting the disease when horses were in contact with small ruminants. Small ruminants were considered as a reservoir and shedders of *C. burnetii* (Mertens et al., 2017). Since *C. burnetii* is resistant in the environment (Marrie and Raoult, 1997; Tissot Dupont et al., 1999), common spaces to horses and small ruminants become an important source of contamination. Our findings were in agreement with those of Menadi et al. (2020) and Keshavamurthy et al. (2020) investigating cattle, Maurin and Raoult (1999) and Rizzo et al. (2016) examining small ruminants. However, Benaissa et al. (2017) and Selim and Ali (2020) reported no significant relationship between Q fever infection in dromedaries and contact with small ruminants. In the current study, the contact of horses with dromedaries was identified as a risk factor for *C. burnetii* infection. Previous studies reported that dromedaries were important reservoirs of *C. burnetii* infection in the investigated areas (Benaissa et al., 2017; Bellabidi et al., 2020), and therefore, their proximity with horses increased the risk of contamination.

The findings of the current study indicated no significant association between the presence of ticks and Q fever infection which was supported by other researchers (Menadi et al., 2020; Selim and Ali, 2020). Previous studies, however, indicated that the presence of ticks increased the risk of infection by *C. burnetii* (Toledo et al., 2008; Benaissa et al., 2017). The role of ticks in the epidemiology of Q fever has been well documented, *C. burnetii* DNA was identified in about 40 species of ticks (Porter et al., 2011). It is well established that ticks play an important role in the sylvatic cycle of *C. burnetii* and in the contamination of humans and animals (Maurin and Raoult, 1999).

Age and gender in the current study were other insignificant risk factors, which were also reported in an earlier study on horses in South Korea (Seo et al., 2016). In ruminants, females were shown to be more infected *with C. burnetii* than males due to hormonal differences (Cetinkaya et al., 2000; Mazeri et al., 2013; Aljafar et al., 2020). Considering ruminants and dromedaries, aged animals are the most infected (McCaughey et al., 2010; Muskens et al., 2011), which could be due to the long duration of exposure to the pathogen. Also, there was no significant relationship between horse breed and Q fever infection. Although no exact explanation can be given, genetic predisposition is postulated as a reason. The type of horse sheltering was not associated with the infection of horses although keeping them in individual boxes could decrease the risk of tick-borne infections.

In this study, ELISA detected only IgG, thus it is difficult to differentiate between current and old infections, the use of more adequate tests in horses as molecular tests is highly recommended.

CONCLUSION

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The obtained results of the current study indicated that *C. burnetii* was endemic in horses in the study areas. El-Bayadh with a seroprevalence of 18.6% was the most infected area. The contact with small ruminants and dromedaries, living near water sources, and geographical location were identified as risk factors for *C. burnetii* seropositivity in horses. It is supported that horses can play an important role in the transmission of the pathogen to other animals and humans. Therefore, molecular characterization of *C. burnetii* strains is recommended to compare strains isolated from horses with those from humans.

DECLARATIONS

Author's contributions

Ansel Samir conceived the study design, carried out laboratory work, participated in data analysis and interpretation, drafted the manuscript. Benfodil Karima conceived the study design, participated in data analysis and interpretation, revised the manuscript. Miroud Kamel revised the manuscript. Mohamed-Cherif Abdellah carried out laboratory work, participated in data analysis and interpretation. Abdelli Amine participated in statistical analysis and interpretation and revised the manuscript. Kaidi Rachid carried out laboratory work and revised the manuscript. Ait-Oudhia Khatima conceived the study design, took part in the coordination and management as well as field studies, participated in data analysis and interpretation, and revised the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgments

The authors wish to thank the Algerian Ministry of Higher Education and Scientific Research for their contribution to my Ph.D. training and Dr. Benbouras Mohamed Amin of the high national polytechnic School of Algiers for making up the geographical maps.

REFERENCES

Agag S, Kaidi R, and Khelef D (2016). Séroprévalence de la fièvre Q chez les bovins de la région de Bejaïa (Algérie). Revue d'élevage et de médecine vétérinaire des pays tropicaux, 69: 155-159. DOI: https://doi.org/10.1007/s11250-020-02295-6

Agerholm JS, Svejstrup CA, Christoffersen A-B, and Agger JFG (2015). Apparent seroprevalence for *Coxiella burnetii* in the Danish horse population. Dansk Veterinaertidsskrift, 98:26–29. Available at: https://www.forskningsdatabasen.dk/en/catalog/2468265634

Aljafar A, Salem M, Housawi F, Zaghawa A, and Hegazy Y (2020). Seroprevalence and risk factors of Q-fever (*C. burnetii* infection) among ruminants reared in the eastern region of the Kingdom of Saudi Arabia. Tropical Animal Health Production, 52: 2631-2638. DOI: https://doi.org/10.1007/s11250-020-02295-6.

Angelakis E, and Raoult D (2010). Q fever. Veterinary Microbiology, 140:297-309. DOI: https://doi.org/10.1016/j.vetmic.2009.07.016

Aouadi A, Leulmi H, Boucheikhchoukh M, Benakhla A, Raoult D, and Parola P (2017). Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis* and *Babesia ovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. Comparative Immunology, Microbiology and Infectious Diseases, 50: 34-39. DOI: https://doi.org/10.1016/j.cimid.2016.11.008

Bellabidi M, Benaissa MH, Bissati-Bouafia S, Harrat Z, Brahmi K, and Kernif T (2020). *Coxiella burnetii* in camels (*Camelus dromedarius*) from Algeria: Seroprevalence, molecular characterization, and ticks (Acari: Ixodidae) vectors. Acta Tropica, 206:105443. DOI: https://doi.org/10.1016/j.actatropica.2020.105443

Benaissa MH, Ansel S, Mohamed-Cherif A, Benfodil K, Khelef D, Youngs, CR, Kaidi R, and Ait-Oudhia K (2017). Seroprevalence and risk factors for *Coxiella burnetii*, the causative agent of Q fever in the dromedary camel (*Camelus dromedarius*) population in Algeria. Onderstepoort Journal of Veterinary Research, 84:1-7. DOI: https://doi.org/10.4102/ojvr.v84i1.1461

Benslimani A, Fenollar F, Lepidi H, and Raoult D (2005). Bacterial Zoonoses and Infective Endocarditis, Algeria. Volume 11, Number 2 February, Emerging Infectious Diseases journal, CDC. DOI: https://doi.org/10.3201/eid1102.040668

Bessas A, Leulmi H, Bitam I, Zaidi S, Ait-Oudhia K, Raoult D, and Parola P (2016). Molecular evidence of vector-borne pathogens in dogs and cats and their ectoparasites in Algiers, Algeria. Comparative Immunology, Microbiology and Infectious Diseases, 45:23–28. DOI: https://doi.org/10.1016/j.cimid.2016.01.002

Bielawska-Drózd A, Cieślik P, Mirski T, Bartoszcze M, Knap JP, Gaweł J, and Żakowska D (2013). Q fever--selected issues. Annuals of Agricultural and Environmental Medicine, 20: 222–232. Available at:http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.agro-eac740fc-d15e-439d-8343-6bb91e70d111

Cetinkaya B, Kalender H, Ertas HB, Muz A, Arslan N, Ongor H, and Gurcay M (2000). Seroprevalence of coxiellosis in cattle, sheep and people in the east of Turkey. Veterinary Record, 146: 131–136. DOI: https://doi.org/10.1136/vr.146.5.131

Dechicha A, Gharbi S, Kebbal S, Chatagnon G, Tainturier D, Ouzrout R, and Guetarni D (2010). Serological survey of etiological agents associated with abortion in two Algerian dairy cattle breeding farms. Journal of Veterinary Medicine and Animal Health, 2: 1-5. DOI: https://doi.org/10.5897/JVMAH.9000024

Djellata N, Yahimi A, Hanzen C, Saegerman C, and Kaidi R (2019). Prevalence and factors associated with a higher or lower risk of exposure to *Coxiella burnetii*, *Chlamydia abortus* and *Toxoplasma gondii* in dairy cows that have aborted in Algeria. Revue Scientifique Et Technique (International Office of Epizootics), 38: 761-786. DOI: https://doi.org/10.20506/rst.38.3.3025

Duron O, Noël V, McCoy KD, Bonazzi M, Sidi-Boumedine K, Morel O, Vavre F, Zenner L, Jourdain E, Durand P et al. (2015). The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q Fever pathogen, *Coxiella burnetii*. PLOS Pathogens, 11:e1004892. DOI: https://doi.org/10.1371/journal.ppat.1004892

Eldin C, Mélenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, Mege J-L, Maurin M, and Raoult D (2017). From Q fever to *Coxiella burnetii* infection: a Paradigm change. Clinical Microbiology Reviews, 30:115-190. DOI: https://doi.org/10.1128/CMR.00045-16

To cite this paper: Ansel S, Benfodil K, Miroud K, Cherif AM, Abdelli A, Kaidi R, and Ait-Oudhia Kh (2021). *Coxiella Burnetii* in Horses of Algeria: Seroprevalence and Associated Risk Factors. *World Vet. J.*, 10 (4): 602-608. DOI: https://dx.doi.org/10.54203/scil.2020.wvj72

Ghaoui H, Bitam I, Ait-Oudhia K, Achour N, Saad-Djaballah A, Saadnia FZ, Kedjour S, and Fournier P-E (2018). *Coxiella burnetii* infection with women's febrile spontaneous abortion reported in Algiers. New Microbes and New Infections, 26: 8-14. DOI: https://doi.org/10.1016/j.nmni.2018.08.003

Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, Van Rotterdam B, De Bruin A, Wijkmans C, Rietveld A, and Van Duynhoven Y (2009). Investigation of a Q fever outbreak in a rural area of The Netherlands. Epidemiology & Infection, 137: 1283-1294. DOI: https://doi.org/10.1017/S0950268808001908

Keshavamurthy R, Singh BB, Kalambhe DG, Aulakh RS, and Dhand NK (2020). Identification of risk factors associated with *Coxiella burnetii* infection in cattle and buffaloes in India. Preventive Veterinary Medicine, 181:105081. DOI: https://doi.org/10.1016/j.prevetmed.2020.105081

Khaled H, Sidi-Boumedine K, Merdja S, Dufour P, Dahmani A, Thiéry R, Rousset E, and Bouyoucef A (2016). Serological and molecular evidence of Q fever among small ruminant flocks in Algeria. Comparative Immunology, Microbiology and Infectious Diseases, 47:19-25. DOI: https://doi.org/10.1016/j.cimid.2016.05.002

Lacheheb A, and Raoult D (2009). Seroprevalence of Q-fever in Algeria. Clinical Microbiology and Infection, 15:167-168. DOI: https://doi.org/10.1111/j.1469-0691.2008.02211.x

Leon A, Richard E, Fortier C, Laugier C, Fortier G, and Pronost S (2012). Molecular detection of *Coxiella burnetii* and *Neospora caninum* in equine aborted foetuses and neonates. Preventive Veterinary Medicine, 104:179-183. DOI: https://doi.org/10.1016/j.prevetmed.2011.11.001

Leulmi H, Aouadi A, Bitam I, Bessas A, Benakhla A, Raoult D, and Parola P (2016). Detection of *Bartonella tamiae*, *Coxiella burnetii* and rickettsiae in arthropods and tissues from wild and domestic animals in northeastern Algeria. Parasites & Vectors, 9:27. DOI: https://doi.org/10.1186/s13071-016-1316-9

Marenzoni ML, Stefanetti V, Papa P, Casagrande Proietti P, Bietta A, Coletti M, Passamonti F, and Henning K (2013). Is the horse a reservoir or an indicator of *Coxiella burnetii* infection? Systematic review and biomolecular investigation. Veterinary Microbiology, 167: 662-669. DOI: https://doi.org/10.1016/j.vetmic.2013.09.027

Marrie TJ, and Raoult D (1997). Q fever--a review and issues for the next century. International Journal of Antimicrobial Agents, 8:145–161. DOI: https://doi.org/10.1016/s0924-8579(96)00369-x

Maurin M and Raoult D (1999). Q fever. Clinical Microbiology Reviews, 12:518–553. DOI: https://doi.org/10.1128/CMR.12.4.518

Mazeri S, Scolamacchia F, Handel IG, Morgan KL, Tanya VN, and Bronsvoort BM dec (2013). Risk factor analysis for antibodies to *Brucella*, *Leptospira* and *C. burnetii* among cattle in the Adamawa Region of Cameroon: a cross-sectional study. Tropical Animal Health and Production, 45: 617–623. DOI: https://doi.org/10.1007/s11250-012-0268-0

McCaughey C, Murray LJ, McKenna JP, Menzies FD, McCullough SJ, O'Neill HJ, Wyatt DE, Cardwell CR, and Coyle PV (2010). *Coxiella burnetii* (Q fever) seroprevalence in cattle. Epidemiology and Infection, 138: 21-27. DOI: https://doi.org/10.1017/S0950268809002854

Menadi SE, Mura A, Santucciu C, Ghalmi F, Hafsi F and Masala G (2020). Seroprevalence and risk factors of *Coxiella burnetii* infection in cattle in northeast Algeria. Tropical Animal Health and Production, 52: 935-942. DOI: https://doi.org/10.1007/s11250-019-02083-x

Mertens K, Gerlach C, Neubauer H, and Henning K (2017). Q fever – An update. Current Clinical Microbiology Reports, 4:61–70. DOI: https://doi.org/10.1007/s40588-017-0059-5

Muskens J, Engelen E van, Maanen C van, Bartels C, and Lam TJGM (2011). Prevalence of *Coxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. Veterinary Record, 168:79. DOI: https://doi.org/10.1136/vr.c6106

Palmela C, Badura R, and Valadas E (2012). Acute Q fever in Portugal. Epidemiological and clinical features of 32 hospitalized patients. Germs, 2: 43-59. DOI: https://doi.org/10.11599/germs.2012.1013

Pan L, Zhang L, Fan D, Zhang X, Liu H, Lu Q, and Xu Q (2013). Rapid, simple and sensitive detection of Q fever by loop-mediated isothermal amplification of the htpAB gene. PLoS neglected Tropical Diseases, 7: e2231. DOI: https://doi.org/10.1371/journal.pntd.0002231

Porter SR, Czaplicki G, Mainil J, Guattéo R, and Saegerman C (2011). Q fever: current state of knowledge and perspectives of research of a neglected zoonosis. International Journal of Microbiology, 2011: 248418. DOI: https://doi.org/10.1155/2011/248418

Račić I, Spičić S, Galov A, Duvnjak S, Zdelar-Tuk M, Vujnović A, Habrun B, and Cvetnić Z (2014). Identification of *Coxiella burnetii* genotypes in Croatia using multi-locus VNTR analysis. Veterinary Microbiology, 173:340-347. DOI: https://doi.org/10.1016/j.vetmic.2014.08.016

Raoult D, Bollini G, and Gallais H (1989). Osteoarticular infection due to *Coxiella burnetii*. Journal of Infectious Disease, 159:1159-1160. DOI: https://doi.org/10.1093/infdis/159.6.1159

Rizzo F, Vitale N, Ballardini M, Borromeo V, Luzzago C, Chiavacci L, and Mandola ML (2016). Q fever seroprevalence and risk factors in sheep and goats in northwest Italy. Preventive Veterinary Medicine, 130: 10-17. DOI: https://doi.org/10.1016/j.prevetmed.2016.05.014

Rodolakis A (2004). Agents abortifs des ruminants et santé publique : Un vaccin en phase I protégerait mieux contre la fièvre Q. Point vétérinaire, pp. 12-13.

Roest HIJ, Solt CB Van, Tilburg JJHC, Klaassen CHW, Hovius EK, Roest FTF, Vellema P, Brom R Van den, and Zijderveld FG Van (2013). Search for possible additional reservoirs for human Q fever, The Netherlands. Emerging Infectious Diseases, 19: 834-835. DOI: https://doi.org/10.3201/eid1905.121489

Runge M, Hilbert A, and Henning K (2012). Contribution to the occurrence of Coxiella burnetii-infection in horses. Praktische Tierarzt, 93: 220-222. Available at: https://www.cabdirect.org/globalhealth/abstract/20123094548

Selim A, and Ali AF (2020). Seroprevalence and risk factors for *C. burentii* infection in camels in Egypt. Comparative Immunology, Microbiology and Infectious Diseases, 68:101402. DOI: https://doi.org/10.1016/j.cimid.2019.101402

Seo M-G, Lee S-H, VanBik D, Ouh I-O, Yun S-H, Choi E, Park Y-S, Lee S-E, Kim JW, Cho G-J et al. (2016). Detection and Genotyping of *Coxiella burnetii* and Coxiella-Like Bacteria in Horses in South Korea. PLoS ONE, 11: e0156710. DOI: https://doi.org/10.1371/journal.pone.0156710

Tissot Dupont H, Raoult D, Brouqui P, Janbon F, Peyramond D, Weiller PJ, Chicheportiche C, Nezri M, and Poirier R (1992). Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. The American Journal of Medicine, 93:427-434. DOI: https://doi.org/10.1016/0002-9343(92)90173-9

Tissot Dupont H, Torres S, Nezri M, and Raoult D (1999). Hyperendemic focus of Q fever related to sheep and wind. American Journal of Epidemiology, 150: 67-74. DOI: https://doi.org/10.1093/oxfordjournals.aje.a009920

Toledo A, Jado I, Olmeda AS, Casado-Nistal MA, Gil H, Escudero R, and Anda P (2008). Detection of *Coxiella burnetii* in ticks collected from central Spain. Vector-Borne and Zoonotic Diseases, 9: 465–468. DOI: https://doi.org/10.1089/vbz.2008.0070

Ullah Q, El-Adawy H, Jamil T, Jamil H, Qureshi ZI, Saqib M, Ullah S, Shah MK, Khan AZ, Zubair M et al. (2019). Serological and molecular investigation of *Coxiella burnetii* in small ruminants and ticks in Punjab, Pakistan. International Journal of Environmental Research and Public Health, 16: 4271. DOI: https://doi.org/10.3390/ijerph16214271

Yahiaoui WI, Afri-Bouzebda F, Bouzebda Z, and Dahmani A (2013). Sondage sérologique de la fièvre Q chez les ovins par la méthode ELISA et prévalence des avortements dans la région de Ksar El Boukhari (Algérie). Tropicultura, 32: 22-27. Available at: http://www.tropicultura.org/text/v32n1/22.pdf

To cite this paper: Ansel S, Benfodil K, Miroud K, Cherif AM, Abdelli A, Kaidi R, and Ait-Oudhia Kh (2021). *Coxiella Burnetii* in Horses of Algeria: Seroprevalence and Associated Risk Factors. *World Vet. J.*, 10 (4): 602-608. DOI: https://dx.doi.org/10.54203/scil.2020.wvj72

Zotov AP, Chumakov MP, Markov AA, Stepanova NI, and Petrov AN (1956). The experimental reproduction of Q-fever and serological studies. Veterinariya, 1956: 44-53.

To cite this paper: Ansel S, Benfodil K, Miroud K, Cherif AM, Abdelli A, Kaidi R, and Ait-Oudhia Kh (2021). *Coxiella Burnetii* in Horses of Algeria: Seroprevalence and Associated Risk Factors. *World Vet. J.*, 10 (4): 602-608. DOI: https://dx.doi.org/10.54203/scil.2020.wvj72

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 609-616, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj73

Application of Natural Antimicrobial Additives and Protective Culture to Control Aerobic Spore Forming Bacteria in Low Salt Soft Cheese

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ORIGINAL ARTICLE
pii: S232245682000073-10
Received: 28 Oct 2020
Accepted: 16 Dec 2020

ABSTRACT

There is an increasing interest in the application of natural antimicrobials instead of chemical ones to enhance the microbiological quality of dairy products. The objective of this study was to assess the effect of some natural antimicrobial additives and protective culture for reducing the usage of chemical preservatives, shelf-life extension, retarding microbial spoilage in low-salt soft cheese. The antimicrobial agents (protective culture, nisin, lysozyme, and natamycin**)** were studied on the activity of 28 isolates of spore-forming bacteria. Inhibitory effect of different natural antimicrobial additives as protective culture (*Lactobacillus rhamnosus*, 40 mg kg $^{-1}$), nisin (25 mg kg $^{-1}$), lysozyme (100 mg kg $^{-1}$), combination of nisin and lysozyme (25 mg kg $^{-1}$/100 mg kg $^{-1}$), and combination of protective culture and natamycin (40 mg kg $^{-1}$/25 mg kg $^{-1}$) were studied on the growth of aerobic spore-forming bacteria in low-salt soft cheese during the storage period (30 days) at 4±1°C. The results revealed that the addition of different natural antibacterial additives with various concentrations had a significant effect on aerobic spore-forming bacteria, compared to other treatments and control. The growth pattern of aerobic spore-forming bacteria gradually decreased in all treatments along the storage period with variable reduction percentages in comparison with control cheese which was in continuous increment. The application of a combination of nisin and lysozyme had the most significant reduction of aerobic spore-forming bacteria, compared to control and other treatments.

Keywords: Aerobic spore-forming bacteria, Lysozyme, Nisin, Natamycin, Protective culture

INTRODUCTION

Soft cheese is one of the oldest dairy products with the best nutritive value and health keeping function. It is popular in many countries worldwide with palatable taste and various flavor (Awad et al., 2012). Cheese consumption has greatly increased during the past decade in the world (Elsamani et al., 2014). Considering the common consumption of cheeses, there is a growing concern regarding its safety and microbiological quality (Al-Gamal et al., 2019). Soft cheese manufacturing procedures include standardization and in many cases pasteurization of milk, acidification mainly through indigenous production of lactic acid by specific bacteria, coagulation of milk by rennet, dehydration of the curd by transforming coagulum into specific characteristic shapes; the resultant cheese could be consumed fresh or matured (After ripening of the curd, Fox et al., 2017). Salt is a vital component as it affects many aspects of cheese including shelf-life, activity of enzymes, flavor, casein hydration, and microbial proliferation during ripening. Salt is applied during cheese making for several purposes including acquiring the desirable flavor and texture, preservative action, and as a taste enhancer. Many consumers not prefer cheese with elevated salt percentage, mainly due to health risks such as hypertension, cardiovascular disease (CVD), stroke and heart attacks (Bae et al., 2017).

Aerobic spore-forming bacteria mainly Bacillus are of great importance in the dairy industry as the spores of these bacteria in raw milk can survive during pasteurization as well as other processing treatments and finally existed into final products (Coorevits et al., 2008). They are considered one of the most important genera encountered in the spoilage of milk and dairy derivatives. In addition, Bacillus is also involved in the formation of biofilms on stainless steel surface lines of dairy plant equipment (Lopez-Brea et al., 2018). The presence of Bacillus enzymes such as proteases in milk may decrease yield and shorten the required time for coagulation during cheese manufacturing due to the elevated concentration of amino acids that enhance the growth of starter culture quickly (Kumari and Sarkar, 2016). Furthermore, their germination leads to food spoilage as they threaten dairy products manufacturing due to high economic losses, impairment of equipment, and reputational damage of food companies or pathogenic cases resulted from food borne illness (Egan et al., 2016). Some Bacillus species cause two types of foodborne diseases, an emetic (vomiting) intoxication and diarrheal infection. The former has resulted from the ingestion of a preformed toxin in food, and the latter originates from eating bacterial cells/spores that release enterotoxins in the small intestine (Budka et al., 2005).

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Contamination of milk and dairy derivatives with spore-forming bacteria involves two routes of the entrance, namely the raw milk route and the post pasteurization route. Raw milk in the farm tank is contaminated through cattle's teats and inefficiently cleaned milking facilities contaminated by soil, faeces and bedding material. Soil is the primary and direct contamination origin of spore-forming bacteria into foods, since it is a major source of these microorganisms. On the other hand, the post pasteurization contamination of milk with spores is associated with the dairy industry or to the biofilms of spore-forming bacteria growing in processing lines that can finally be widely spread by release into the system of milk production (Heyndrickx, 2011). Improperly cleaned facilities and poor handling within the dairy plant is also considered as main sources and shedders of the contamination of spore formers at dairy processing equipment (Faille et al., 2014).

There is an increasing interest in the application of bio-preservation methods that utilize natural antimicrobial compounds such as nisin, lysozyme and protective culture (Sudagidan and Yemenicioğlu, 2012). Nisin has been evidenced as (Generally Regarded as Safe) by the US Food and Drug Administration (FDA) since 1988 as it has been long applied in food protection (E234) without being associated with health problems (Gharsallaoui et al., 2016). Application of nisin alone, or combined with other treatments as lysozyme, could provide a desirable advance for the microbiological safety and keeping of sensory criteria in milk and dairy products (Sobrino-López and Martín-Belloso, 2008). The functional characteristics of lysozyme as a natural antiviral and antibacterial substance have been implicated in industrial applications, especially in the food, pharmaceutical and medical industries (Arabski et al., 2015). Most food fermentation processes include mixed cultures in which various microbial species interact with each other. These interactions may have a neutral, positive or negative impact on the activity of the strains performing the fermentation (Arioli et al., 2016). Protective cultures have strong antagonistic activity against food-spoilage and pathogenic aerobic spore-forming bacteria as a result of the production of bacteriocins, inhibitory enzymes, organic acids and hydrogen peroxide (Ibrahim and Awad, 2018). Consumers have an increasing knowledge of health risks caused by the usage of chemical preservatives. Therefore, there is an increasing requirement in the dairy industry to prolong product shelf-life and inhibit spoilage by natural preservatives and/or new methods of conservation (Silva et al., 2018). Therefore, this research aimed to study the application of some natural antimicrobial additives (Nisin, lysozyme, protective culture and natamycin) to enhance the quality, shelf-life and safety of low-salt soft cheese.

MATERIALS AND METHODS

Materials

The employed materials in the current study included fresh buffalo milk obtained from a small dairy farm at Abis, Alexandria governorate. Microbial rennet: Fromase, France, Nisin: manufactured in the Netherlands by Siveele B.V, imported by AWA Food Solution. 4th Industrial area, New Borg Al Arab City. Alexandria, found in the market in form of powder with commercial name Nisaplin® and has the EU food additive number E234. Natamycin: from Spain, Imported by AWA Food Solution. 4th Industrial area, New Borg Al Arab City. Alexandria. Lysozyme: Lysozyme from chicken egg white was from Merck, Germany. Protective culture (Lyofast LRB), SACCO Co, Italy. It consists of a selected strain of *lactobacillus rhamnosus* (Used in dairy derivatives as non-starter lactic acid bacterial culture, which provides a slight acidity and smell from slow fermentation). Spore forming isolates included 28 strains isolated from commercial low-salt soft cheese collected from Egyptian markets. These isolates were purified and classified as aerobic spore-forming bacteria (study under publication).

Methods

Low-salt soft cheese manufacture

Low-salt soft cheese was made according to the method adopted by Fahmi and Sharara (1950). Fresh buffalo`s milk was used for manufacturing six treatments of low-salt soft white cheese. Raw milk was pasteurized at 63ºC for 30 min, cooled to 37 ºC, then calcium chloride and sodium chloride was added in 0.02% and 3% (w/v). Cheese milk was divided into six portions, then, the additives for each treatment were added individually. The commercial rennet was added to the milk and incubated at 37 ºC for coagulation within 90 min. The treatments of low-salt soft white cheeses using pasteurized milk were as following: pasteurized milk with no additives as a control (Treatment 1), pasteurized milk with adding protective culture (40 mg/kg, Treatment 2), pasteurized milk with adding lysozyme (100 mg/kg, Treatment 3), pasteurized milk with adding nisin (25 mg/kg, Treatment 4), pasteurized milk with adding nisin and lysozyme (25 mg/kg +100 mg/kg, Treatment 5), pasteurized milk with adding protective culture and natamycin (40 mg/kg + 25 mg/kg, Treatment 6). In treatments of protective culture (T2 and T6), the protective culture (lyofast LRB) 200 mg were added to 5 kg milk according to the recommendation of suppliers followed by incubation at 37 °C for 1 hour then adding NaCl (3 %), $CaCl_2$(0.02%) and finally the addition of rennet (0.002%(w/v)) to coagulate milk within 90 min using the same procedure as in other treatments.

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Microbiological evaluation of laboratory manufactured low-salt soft cheese

Using aseptic technique, 5 grams of low salt laboratory manufactured soft cheese were transferred by sterile spatula to a sterile polyethylene bag then adding 45 ml sterilized sodium citrate 2%, bags were placed in a stomacher for shaking at 160 rpm for 5 min, then serial dilutions using sterilized sodium citrate 2% were performed (Wehr and Frank, 2012).

Enumeration of aerobic spore-forming bacteria

All previous prepared serial dilutions were heated in a water bath at 80 ºC for 10 min then cooled suddenly to the 30 ºC before transferring one ml aliquots into sterilized Petri dishes containing nutrient agar. The duplicate plates were incubated at 32 ºC for 48 hours. Mesophilic aerobic spore-forming bacteria were enumerated on nutrient agar after incubation (Wehr and Frank, 2012).

Isolation of aerobic spore-forming bacteria

Colonies suspected as Bacillus species based on colony morphology, spread with usual features e.g slimy, crusty, dry, embedded or forming skin-like pellicles, were sub-cultured in nutrient broth at 32°C for 48 hours, then purified on non-selective medium nutrient agar plates for another 48 hours at 32°C. The isolates were inoculated into the nutrient broth and incubated at 32°C for 48 hours and then stored in Eppendorf tubes containing nutrient broth with 15-20% glycerol at -20 °C for further examinations (Wehr and Frank, 2012).

Studying the antibacterial activity of natural additives against isolated spore-forming bacteria

Natural antimicrobial agents such as nisin, lysozyme, protective culture and natamycin were evaluated to inhibit the growth of all isolates of spore-forming bacteria. Each of the tested isolated strains was inoculated in nutrient agar medium in Petri plates, After solidification of agar, four-wells in each plate were performed, each natural antibacterial additives such as nisin, lysozyme, protective culture and natamycin with identified concentration and quantity as follow 100 µl of nisin (25% concentration),100 µl of natamycin (concentration 25%), 100 µl of protective culture (concentration 0.04%) lysozyme 100 µl (0.1% concentration) was placed in the wells .The plates were incubated at 37 ºC for 24 hours and then recording of inhibition zone to determine their effectiveness against isolated aerobic spore-forming bacteria. This was carried out according to Performance Standards for Antimicrobial Susceptibility Testing (CLSI, 2018).

Statistical analysis

Statistical analysis of the data was performed using ANOVA, F-test, and LSD procedures available within the SAS software package (version 9.13 2008). Means with a significant difference were compared by Duncan's multiple range tests according to Steel and Torrie (1980). All physicochemical and microbiological analyses were performed in duplicate.

RESULTS

As can be seen in Table (1), the reduction percentage of aerobic spore-forming bacteria in laboratory manufactured low-salt soft cheese treated with different concentrations of natural antimicrobial additives during the storage period (30 days) at 4±1°C. The addition of protective culture (40 mg/kg) reduced aerobic spore- forming bacteria on first day of analysis by 7.41 %, reduction percentage increased on the day 14 of storage to reach 83.10% then the reduction percentage was at a high rate (90.94 %) on day 30 of storage. Aerobic spore- forming bacteria in cheese treated with lysozyme (100 mg/kg) reduced by 33.68% on first day of manufacturing then reached 97.82% on the day 30 of storage. Losing percent of aerobic spore-forming bacteria in cheese treated with nisin (25 mg/kg) was 31.60%, 90.32%, 96.54% on days 1, 14 and 30 of storage, respectively. Reduction percentage of aerobic spore-forming bacteria in cheese treated with combined nisin and lysozyme (25 mg/kg and 100 mg/kg) was at the highest rate among other additives on first day of analysis as was 51.50% then increased to 93.97% on the day 14 of storage then reduction percentage elevated sharply on the day 30 of storage to reach 97.82%. The addition of a combination of protective culture plus natamycin (40 mg/kg and 25 mg/kg) to cheese led to a reduction percentage of 21.18% at the first analysis then elevated to 92.78% at the second analysis then finally increased greatly on the day 30 of storage to reach 93.08% (Figure 1). Results recorded in Table 2 showed that the mean value of inhibition zone (mm) of nisin against isolated spore-forming bacteria was 25.30 ± 11.9 mm, while the mean value of inhibition zone diameter of lysozyme against isolated spore-forming bacteria was 32.90±19.30. The mean value of inhibition zone diameter of protective culture against isolated aerobic spore-forming bacteria was 26.10 ± 10.20. Natamycin had no inhibitory effect against spore-forming bacteria.

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Table 1. Growth pattern and reduction percentage of aerobic spore-forming bacteria (cfu/g) in laboratory manufactured soft cheese treated with different concentrations of natural additives during storage period (30 days) at 4±1°C.

Storage / Period time (day)	Control		Natural additives									
			Protective culture (40 mg kg $^{-1}$)		Lysozyme (100 mg kg $^{-1}$)		Nisin (25 mg kg $^{-1}$)		Nisin (25 mg kg $^{-1}$) + lysozyme (100 mg kg $^{-1}$)		Protective culture (40 mg kg $^{-1}$) + Natamycin (25 mg kg $^{-1}$)	
	Mean ± SD	R%	Mean ± SD	R%	Mean± SD	R%	Mean ±SD	R%	Mean ± SD	R%	Mean ± SD	R%
1	864 ± 0.71^{a}	0	800 ± 0.71^{b}	7.41	573 ± 0.71^{e}	33.68	591 ± 0.71^{d}	31.60	419 ± 0.71^{f}	51.50	681 ± 1.41 ^{c}	21.18
14	1509 ± 0.71^{a}	0	255 ± 0.71^{b}	83.10	119 ± 0.71^{e}	92.11	146 ± 0.71^{d}	90.32	91 ± 0.71^{f}	93.97	109 ± 0.71^{c}	92.78
30	2109 ± 0.71 ^{a}	0	191 ± 0.71^{b}	90.94	46 ± 0.71^{e}	97.82	73 ± 0.71^{d}	96.54	46 ± 0.71^{f}	97.82	146 ± 0.71 ^{c}	93.08

SD= Standard deviation, R%=Reduction percentage, *Means carrying a different superscript small letter on the same row are significantly different (P≤0.05).

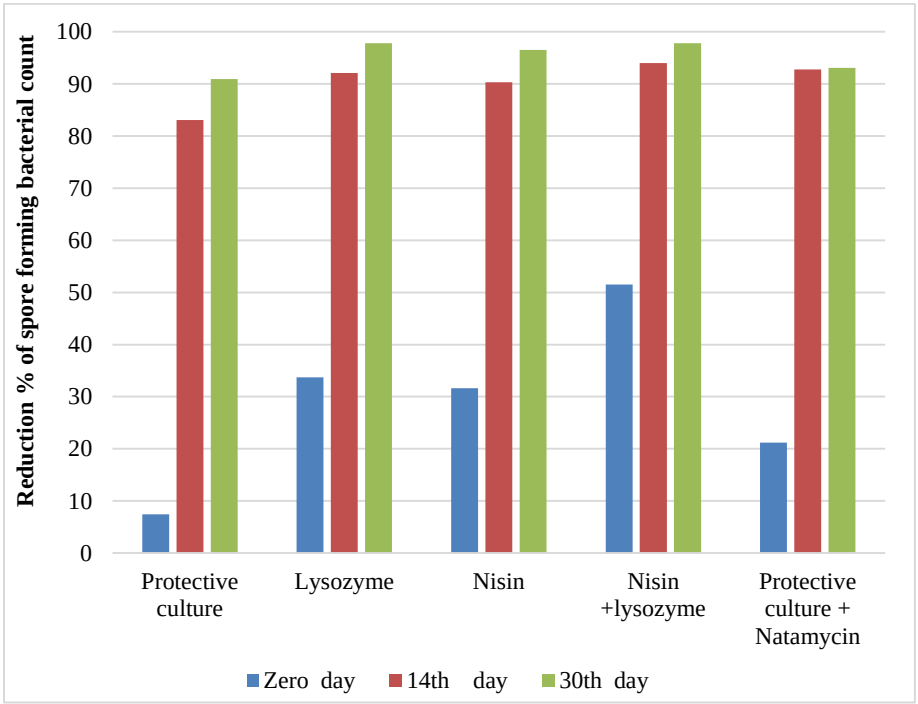

Figure 1. Effect of protective culture and some Natural antimicrobial additives on the reduction count of spore- forming bacteria in low-salt soft cheese

Table 2. Antibacterial activity of natural additives against isolated aerobic spore-forming bacteria (inhibition zone mm)

Number of Tested Isolates	Nisin	Lysozyme	Protective culture	Natamycin
	Inhibition Zone (mm)			
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
28	25.30±11.9	32.90±19.30	26.10±10.20	Not Detected

SD=Standard deviation

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DISCUSSION

Reduction percentage of aerobic spore forming bacteria in laboratory manufactured soft cheese treated with different concentrations of natural additives during storage period (30 days) at 4±1°C

Natural antimicrobial additives are a magic substitute for chemical preservatives for the maintenance of dairy product safety and microbiological quality (Lopes et al., 2019). Spore-forming bacteria cause significant economic losses and health problems in the dairy industries (Coorevits et al., 2010). They are commonly distributed in the environment and are associated with varieties of dairy products. Their existence in foods constitutes great problematic issues because the formation of their spores enables them to be resistant to thermal processing, freezing, chemical agents, and other difficult conditions that the food faces during processing operations. However, the vegetative cells are destroyed by these factors, traditionally these microorganisms have been related to the spoilage of dairy products; recently they have been associated with potential food poisoning cases (Rodriguez-Lozano et al., 2010). Multiplication of aerobic spore formers in raw milk liberates extracellular lipase enzyme, which is attached to milk fat globules and concentrated in the manufactured cheese in the course of making, the enzyme adversely causes bitter flavor by hydrolysis of fat into fatty acids and glycerides. This enzyme could be inactivated by pasteurization but thermal treatment could not destroy it (Beresford et al., 1998). The reduction percentage of aerobic spore-forming bacteria exerted by protective culture LAB strains reached 90.94% at the last analysis since some types of *lactobacillus* (such as *lactobacillus rhamnosus*) can produce antimicrobial factors such as organic acids (lactic acid and acetic acid), ethanol, hydrogen peroxide, diacetyl, acetoin, acetaldehyde, carbon dioxide and bacteriocins that could develop in various ways a potent inhibitory action against many microorganisms involving pathogenic and spoilage ones of aerobic spore formers (Al-Gamal et al., 2019). Lysozyme hydrolyzes 1,4-β-bond between N-acetylmuramic acid, and N-acetylglucosamine were found in the peptidoglycan leading to the suppression of growth, multiplication and prevalence of aerobic spore-forming bacteria which finally decreased from 573±0.71 on the first day of analysis to 46±0.71 on the day 30 of storage (Abdou et al., 2013). With the final reduction percentage of 97.82%, lysozyme showed strong bactericidal action against aerobic spore formers mainly Bacillus species (Abdou et al., 2007). Cheese is considered one of the well-known food applications of lysozyme where it governs spoilage microorganism's growth. Application of lysozyme in soft cheese can result in extending shelf-life and prevention of total bacteria including spore formers, yeast and mold (Doosh and Abdul-Rahman, 2014). Lysozyme content in cheese usually ranged between 50 and 350 mg/kg of cheese weight (Ávila et al., 2014). Nisin exerts its effect by attacking the cell wall so destroy the target microorganisms (Gharsallaoui et al., 2016). Loss of potassium ion of the bacterial cell, depolarization of the cytoplasmic membrane, depression of respiration, and partial efflux of cellular ATP are the mechanisms of action of nisin (Omardien et al., 2016). Nisin exhibited its inhibitory action after the initiation of germination, where nisin attached to lipid II in the spore after outgrowth and inhibited it from becoming metabolically active by interfering with the formation of membrane potential and oxidative metabolism. Germination initiation was required for this lipid II binding to happen as nisin cannot bind to the dormant spore due to the absence of lipid II on the external of the spore (Gut et al., 2011). The reduction percentage of aerobic spore formers in cheese treated with nisin elevated from 31.60 % at first analysis to 96.54 % on the day 30 of storage. It has been noticed that the action of nisin is elevated when it is in combination with other additives such as lysozyme (Chung and Hancock, 2000). Application of lysozyme and nisin in soft cheese can lead to the extension of shelf-life and prevention of spoilage (Zottola et al., 1994; Doosh and Abdul-Rahman, 2014). Application of combination between nisin and lysozyme has the most significant reduction of spore-forming microorganisms in comparison with control and other treatments, which decreased from 419±0.71 on the first day of analysis to 46±0.71 on the day 30 of storage. These findings are in line with those reported by López-Pedemonte et al. (2003), Meruvu et al. (2011) and Ávila et al. (2014) who found that nisin and lysozyme combination were the most effective against gram-positive aerobic spore formers such as Bacillus species.

Studying the antibacterial activity of natural additives against isolated spore-forming bacteria

The mean value of inhibition zone of nisin against isolated spore-forming bacteria was 25.30 ± 11.9 mm, which was in the same range as those reported by Pirttijärvi et al. (2001) showing that the application of diluted nisin led to an inhibition zone around 3-30 mm against many species of aerobic spore-forming bacteria. Nisin effectively suppresses the counts of aerobic spore-forming bacteria during 24hour incubation in the lab that is sufficient for industrial application. Additionally, our results are higher than the data mentioned by Morsy et al. (2018) reporting that the inhibition zone of nisin against aerobic spore-forming bacteria was 15±1.32. Nisin has been used to prevent spore out growth in many types of cheese especially soft cheese also is useful for inhibiting different types of aerobic spore formers (Komitopoulou et al., 1999). The mean value of inhibition zone diameter of lysozyme against isolated spore-forming bacteria was 32.90±19.30 that is higher than results obtained by Morsy et al. (2018) who illustrated that the inhibition zone of lysozyme against aerobic spore formers was 14±1.11 and lower than the data presented by Ramanauskiene et al. (2009) who found that lysozyme produced a clear zone of inhibition of 38.52 ± 0.17 against aerobic spore formers. The mean

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value of the inhibition zone diameter of the protective culture against isolated aerobic spore- forming bacteria was 26.10 ± 10.20. These results are higher than data reported by Tharmaraj and Shah (2009) who found that *lactobacillus rhamnosus* produced an inhibition zone of 0-15 mm against aerobic spore-forming bacteria and also higher than data mentioned by Coman et al. (2014) who illustrated that *lactobacillus rhamnosus* showed inhibition zone of 11.80 ± 0.71 against aerobic spore-forming bacteria. The findings did not support the study conducted with Hawaz (2014) who reviewed that the addition of protective culture *(lactobacillus rhamnosus)* produced no inhibition zone against aerobic spore-forming bacteria. Spore-forming bacteria were reduced by the protective culture (*lactobacillus rhamnosus*) organisms to a greater extent than the non-spore formers, the inhibitory action of protective culture was strongest against aerobic spore-formers (Tharmaraj and Shah, 2009). Natamycin had no inhibitory effect against spore-forming bacteria. It destroys yeasts by specifically attaching to ergosterol and without permeabilizing the plasma membrane. It prevents vacuolar fusion by the definite interaction with ergosterol so, it is active against fungi but not against bacteria (Te Welscher et al., 2010).

CONCLUSION

The low-salt soft cheese has short shelf life due to the presence of aerobic spore-forming bacteria which are resistant to pasteurization temperature, therefore, the producers may use some chemical preservatives. From the above mentioned results and discussion, it could be concluded that some natural antibacterial agents could be used to inhibit the growth of aerobic spore-forming bacteria, which among them addition of lysozyme (100mg/kg) in combination with nisin (25mg/kg) had a great inhibitory effect on aerobic spore-forming bacteria followed by application of lysozyme alone.

DECLARATIONS

Acknowledgements

Great thanks are extended to Prof Dr Ashraf M. Nazem, Department of Food Hygiene, Faculty of Veterinary medicine, Alexandria University, Egypt and Prof Dr Sameh A. Awad, Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Egypt.

Competing interests

The authors declare that they have no competing interests.

Consent to publish

All authors agree on article publication.

REFERENCES

Abdou AM, Higashiguchi S, Aboueleinin AM, Kim M, and Ibrahim HR (2007). Antimicrobial peptides derived from hen egg lysozyme with inhibitory effect against Bacillus species. Food Control, 18(2): 173-178. DOI: http://www.dx.doi.org/10.1016/j.foodcont.2005.09.010

Abdou AM, Kim M, and Sato K (2013). Functional proteins and peptides of hen's egg origin. Bioactive food peptides in health and disease, pp.115-144. Available at: https://www.intechopen.com/books/bioactive-food-peptides-in-health-and-disease/functional-proteins-and-peptides-of-hen-s-egg-origin

Al-Gamal MS, Ibrahim GA, Sharaf OM, Radwan AA, Dabiza NM, Youssef AM, and El-ssayad MF (2019). The protective potential of selected lactic acid bacteria against the most common contaminants in various types of cheese in Egypt. Heliyon, 5(3): e01362. Available at: https://www.sciencedirect.com/science/article/pii/S240584401838589X

Arabski M, Konieczna I, Tusińska E, Wąsik S, Relich I, Zając K, and Kaca W (2015). The use of lysozyme modified with fluorescein for the detection of Gram-positive bacteria. Microbiological research, 170: 242-247. Available at: https://www.sciencedirect.com/science/article/pii/S0944501314000640

Arioli S, Scala GD, Remagni MC, Stuknyte M, Colombo S, Guglielmetti S, Noni ID, Ragg E, and Mora D (2016). Streptococcus thermophilus urease activity boosts *Lactobacillus delbrueckii* subsp. bulgaricus homo lactic fermentation. International journal of food microbiology, 247: 55-64. DOI: http://www.dx.doi.org/10.1016/j.ijfoodmicro.2016.01.006

Ávila M, Gómez-Torres N, Hernández M, and Garde S (2014). Inhibitory activity of reuterin, nisin, lysozyme and nitrite against vegetative cells and spores of dairy-related Clostridium species. International journal of food microbiology, 172: 70-75. DOI: http://www.doi.org/10.1016/j.ijfoodmicro.2013.12.002

Awad RA, Farahat AM, and Salama WM (2012). Production and in vivo Nutritional Evaluation of Functional Soft Cheese Supplemented with Broccoli. World Journal of Dairy and Food Sciences, 7(2): 150-159. Available at: https://www.semanticscholar.org/paper/Production-and-in-vivo-nutritional-evaluation-of-Awad-Farahat/5ebd51f4bfa4626a3076436b29a6b585cb1ce276

Bae I, Park JH, Choi HY, and Jung HK (2017). Emerging innovations to reduce the salt content in cheese; effects of salt on flavor, texture, and shelf life of cheese; and current salt usage: A review. Korean journal for food science of animal resources, 37(6): 793-798. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5932950/

Beresford T, Fitzsimons N, Cogan T, and Condon S (1998). Non-starter lactic acid bacteria: Growth in cheese, contribution to flavor development. In Proceedings of the International Dairy Symposium, 19: 98-134. Available at: https://hal.archives-ouvertes.fr/hal-00895622/document

Budka H, Buncic S, and Colin P (2005). Opinion of the scientific panel on biological hazards on Bacillus cereus and other Bacillus spp. in foodstuffs. European Food Safety Authority Journal, 175: 1-48. Available at: https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2005.175

Chung W, and Hancock RE (2000). Action of lysozyme and nisin mixtures against lactic acid bacteria. International journal of food microbiology, 60(1): 25-32. Available at: http://www.cmdr.ubc.ca/bobh/wp-content/uploads/2016/10/214.-Chung-2000.pdf

To cite this paper: Abo Shaala EK, Awad SA and Nazem AM (2020). Application of Natural Antimicrobial Additives and Protective Culture to Control Aerobic Spore Forming Bacteria in Low Salt Soft Cheese. *World Vet. J.*, 10 (4): 609-616. DOI: https://dx.doi.org/10.54203/scil.2020.wvj73

Clinical, and Laboratory Standards Institute (CLSI) (2018). Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Clinical and Laboratory Standards Institute (CLSI). Pp.100-121. Available at: http://file.qums.ac.ir/repository/mmrc/CLSI-2018-M100-S28.pdf

Coman MM, Verdenelli MC, Cecchini C, Silvi S, Orpianesi C, Boyko N, and Cresci A (2014). In vitro evaluation of antimicrobial activity of L actobacillus rhamnosus IMC 501®, L actobacillus paracasei IMC 502® and SYNBIO® against pathogens. Journal of applied microbiology, 117(2): 518-527. Available at: https://sfamjournals.onlinelibrary.wiley.com/doi/pdf/10.1111/jam.12544

Coorevits A, De Jonghe V, Vandroemme J, Van Landschoot A, Heyndrickx M, and De Vos P (2010). How can the type of dairy farming influence the bacterial flora in milk?.In Organic farming and peanut crops, pp. 123-136. Available at: http://www.hdl.handle.net/1854/LU-949761

Coorevits A, De Jonghe V, Vandroemme J, Reekmans R, Heyrman J, Messens W, De Vos P, and Heyndrickx M (2008). Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms. Systematic and Applied Microbiology, 31(2): 126-140. Available at: https://www.jmbfs.org/wp-content/uploads/2013/03/jmbfs_0290_gonzales.pdf

Doosh KS, and Abdul-Rahman SM (2014). Effect of Lysozyme Isolated from Hen Egg White in Elongation the Shelf Life of Iraqi Soft Cheese Made from Buffalo Milk. Pakistan Journal of Nutrition, 13(11): 635-641. DOI: http://www.doi.org/10.3923/pjn.2014.635.641

Egan K, Field D, Rea MC, Ross RP, Hill C, and Cotter PD (2016). Bacteriocins: novel solutions to age old spore-related problems?. Frontiers in microbiology, 7: 461. Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2016.00461/full

Elsamani MO, Habbani SS, Babiker EE, and Ahmed IA (2014). Biochemical, microbial and sensory evaluation of white soft cheese made from cow and lupin milk. LWT-Food Science and Technology, 59(1): 553-559. Available at: http://khartoumspace.uofk.edu/bitstream/handle/123456789/22216/Pages%20from%20Biochemical,%20microbial%20and%20sensory%20evaluation%20of%20white%20soft%20cheese.pdf?sequence=1

Fahmi AH, and Sharara HA (1950). Studies on Egyptian Domiati cheese. Journal of Dairy Research, 17(3): 312-328. DOI: https://www.doi.org/10.1017/S0022029900005860

Faille C, Benezech T, Midelet-Bourdin G, Lequette Y, Clarisse M, Ronse G, and Slomianny C (2014). Sporulation of Bacillus spp. within biofilms: a potential source of contamination in food processing environments. Food microbiology, 40: 64-74. Available at: http://ssu.ac.ir/cms/fileadmin/user_upload/Mtahghighat/tfood/asil-article/q-z2/Sporulation-of-Bacillus-spp-within-biofilms-A-potential-source-of-contamination-in-food-processing-environments_2014_Food-Microbiology.pdf

Fox PF, Guinee TP, Cogan TM, and McSweeney PL (2017). Fundamentals of cheese science, pp. 185-229. Available at: https://www.springer.com/gp/book/9781489976796

Gharsallaoui A, Oulahal N, Joly C, and Degraeve P (2016). Nisin as a Food Preservative:Part 1: Physicochemical Properties, Antimicrobial Activity, and Main Uses, Critical Reviews in Food Science and Nutrition, 56 (8): 1262-1274. DOI: https://www.doi.org/10.1080/10408398.2013.763765

Gut IM, Blanke SR, and Van der Donk WA (2011). Mechanism of inhibition of Bacillus anthracis spore outgrowth by the lantibiotic nisin. ACS chemical biology, 6(7): 744-752. Available at: https://pubs.acs.org/doi/pdfplus/10.1021/cb1004178

Hawaz E (2014). Isolation and identification of probiotic lactic acid bacteria from curd and in vitro evaluation of its growth inhibition activities against pathogenic bacteria. African Journal of Microbiology Research, 8(13): 1419-1425. Available at: https://academicjournals.org/journal/AJMR/article-full-text-pdf/D80ED0B46925.pdf

Heyndrickx M (2011). The importance of endospore-forming bacteria originating from soil for contamination of industrial food processing. Applied and Environmental Soil Science, pp.1-11. Available at: https://www.hindawi.com/journals/aess/2011/561975/abs/

Komitopoulou E, Boziaris IS, Davies EA, Delves-Broughton J, and Adams MR (1999). Alicyclobacillus acidoterrestris in fruit juices and its control by nisin. International journal of food science and technology, 34(1): 81-85. DOI: https://www.doi.org/10.1046/j.1365-2621.1999.00243.x

Kumari S, and Sarkar PK (2016). Bacillus cereus hazard and control in industrial dairy processing environment. Food Control, 69: 20-29. DOI: https://www.doi.org/10.1016/j.foodcont.2016.04.012

Ibrahim A, and Awad S (2018). Selection and identification of protective culture for controlling *Staphylococcus aureus* in fresh Domiati like cheese. Journal of food safety, pp.1-7. DOI: https://www.doi.org/10.1111/jfs.12418

Lopes NA, Pinilla CMB, and Brandelli A (2019). Antimicrobial activity of lysozyme-nisin co-encapsulated in liposomes coated with polysaccharides. Food Hydrocolloids, 93: 1-9. DOI: http://www.doi.org/10.1016/j.foodhyd.2019.02.009

Lopez-Brea SG, Gomez-Torres N, and Arribas MA (2018). Spore-forming bacteria in dairy products. Microbiology in Dairy Processing: Challenges and Opportunities, First Edition Edited by Palmiro Poltronieri. © 2018 John Wiley and Sons Ltd and the Institute of Food Technologists. Published 2018 by John Wiley and Sons Ltd and the Institute of Food Technologists,pp.11-32. Available at: http://ndl.ethernet.edu.et/bitstream/123456789/36457/1/37.pdf.pdf#page=33

López-Pedemonte TJ, Roig-Sagués AX, Trujillo AJ, Capellas M, and Guamis B (2003). Inactivation of spores of Bacillus cereus in cheese by high hydrostatic pressure with the addition of nisin or lysozyme. Journal of Dairy Science, 86(10): 3075-3081. Available at: https://www.sciencedirect.com/science/article/pii/S0022030203739071

Meruvu H, Vangalapati M, Chippada SC, and Bammidi SR (2011). Synthesis and characterization of zinc oxide nanoparticles and its antimicrobial activity against Bacillus subtilis and Escherichia coli. Journal of Rasayan Chemistry, 4(1): 217-222. Available at: https://translateyar.ir/wp-content/uploads/2018/12/8726-English.pdf

Morsy MK, Elsabagh R, and Trinetta V (2018). Evaluation of novel synergistic antimicrobial activity of nisin, lysozyme, EDTA nanoparticles, and/or ZnO nanoparticles to control foodborne pathogens on minced beef. Food Control, 92: 249-254. Available at: https://webcache.googleusercontent.com/search?q=cache:abw5tjc8HvQJ:https://pubag.nal.usda.gov/catalog/5974877+&cd=1&hl=en&ct=clnk&gl=eg

Omardien S, Brul S, and Zaat SA (2016). Antimicrobial activity of cationic antimicrobial peptides against gram-positives: current progress made in understanding the mode of action and the response of bacteria. Frontiers in cell and developmental biology, 4: 111. Available at: https://www.frontiersin.org/articles/10.3389/fcell.2016.00111/full

Pirttijärvi TSM, Wahlström G, Rainey FA, Saris PEJ, and Salkinoja-Salonen MS (2001). Inhibition of bacilli in industrial starches by nisin. Journal of Industrial Microbiology and Biotechnology, 26(3): 107-114. Available at: https://link.springer.com/content/pdf/10.1038%252Fsj.jim.7000078.pdf

Ramanauskiene K, Inkeniene AM, Savickas A, Masteikova R, and Brusokas V (2009). Analysis of the antimicrobial activity of propolis and lysozyme in semisolid emulsion systems. Acta Poloniae Pharmaceutica, 66(6): 681-688. Available at: https://pubmed.ncbi.nlm.nih.gov/20050532/

Rodriguez-Lozano A, Campagnoli M, Jewel K, Monadjemi F, and Gaze JE (2010). Bacillus spp. thermal resistance and validation in soups. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, 1: 537-544. Available at: http://www.innocua.net/web/download-626/537-544.pdf

Silva CC, Silva SP, and Ribeiro SC (2018). Application of bacteriocins and protective cultures in dairy food preservation. Frontiers in microbiology, 9: 594. Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.00594/full

To cite this paper: Abo Shaala EK, Awad SA and Nazem AM (2020). Application of Natural Antimicrobial Additives and Protective Culture to Control Aerobic Spore Forming Bacteria in Low Salt Soft Cheese. *World Vet. J.*, 10 (4): 609-616. DOI: https://dx.doi.org/10.54203/scil.2020.wvj73

Sobrino-López A, and Martín-Belloso O (2008). Use of nisin and other bacteriocins for preservation of dairy products. International Dairy Journal, 18(4): 329-343. DOI: http://www.doi.org/10.1016/j.idairyj.2007.11.009

Steel RG, and Torrie JH (1980). Principles and procedures of statistics 2nd ed. McGraw Hill, New York, USA, pp.1-633. DOI:

https://www.doi.org/10.1002/bimj.19620040313

Sudagidan M, and Yemenicioğlu A (2012). Effects of nisin and lysozyme on growth inhibition and biofilm formation capacity of Staphylococcus aureus strains isolated from raw milk and cheese samples. Journal of food protection, 75(9): 1627-1633. Available at: http://openaccess.iyte.edu.tr:8080/xmlui/bitstream/handle/11147/5630/56230pdf?sequence=1

Tharmaraj N, and Shah NP (2009). Antimicrobial effects of probiotics against selected pathogenic and spoilage bacteria in cheese-based dips. International Food Research Journal, 16(1): 261-276. Available at: http://www.ifrj.upm.edu.my/16%20(3)%202009/1[1]%20Shah.pdf

Te Welscher YM, Jones L, Van Leeuwen MR, Dijksterhuis J, De Kruijff B, Eitzen G, and Breukink E (2010). Natamycin inhibits vacuole fusion at the priming phase via a specific interaction with ergosterol. Antimicrobial agents and chemotherapy, 54(6): 2618-2625 Available at: https://scholar.google.com/scholar?output=instlink&q=info:SK8KVXIH3WcJ:scholar.google.com/&hl=en&as_sdt=0,5&scillfp=10914247204865706296&oi=lle

Wehr HM, and Frank JF (2012). Standard methods for the examination of dairy products (327-404). Washington, DC: American Public Health Association. DOI: http://doi.org/10.2105/9780875530024

Zottola EA, Yezzi TL, Ajao DB, and Roberts RF (1994). Utilization of cheddar cheese containing nisin as an antimicrobial agent in other foods. International journal of food microbiology, 24(1-2): 227-238. DOI: https://doi.org/10.1016/0168-1605(94)90121-X

To cite this paper: Abo Shaala EK, Awad SA and Nazem AM (2020). Application of Natural Antimicrobial Additives and Protective Culture to Control Aerobic Spore Forming Bacteria in Low Salt Soft Cheese. *World Vet. J.*, 10 (4): 609-616. DOI: https://dx.doi.org/10.54203/scil.2020.wvj73

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 617-624, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj74

Identifying the Virulent Factors of *Clostridium perfringens* Locally Isolated from Different Species

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ORIGINAL ARTICLE
pii: S232245682000074-10
Received: 07 Nov 2020
Accepted: 22 Dec 2020

ABSTRACT

Clostridium perfringens incriminated in many diseases among different species of animals due to its ability to produce many virulence factors. In the current study, 135 intestinal samples were collected from different animal species of different localities in Egypt. Samples were subjected to isolation and identification (morphologically and biochemically) for obtaining *Clostridium perfringens* isolates (n=26, 19.25%). The PCR was carried out to elucidate the virulence factors. It was indicated that all the 26 *Clostridium perfringens* isolates had CPA gene and *Clostridium perfringens* enterotoxin (CPE gene), whereas 23% of isolates of chicken and cattle intestinal samples contained CPA, Net B, and CPE genes as virulence factors. Consequently, those isolates are highly recommended to be used in the preparation of enterotoxemia and necrotic enteritis vaccines as they are more virulent strains.

Keywords: *Clostridium perfringens*, CPA gene, CPE gene, Net B gene

INTRODUCTION

Clostridium perfringens (*C. perfringens*) is considered to be one of the most pathogen that is widely distributed in nature (Uzal et al., 2014), two key features that cause wide pathogenicity are the potential of production many virulence factors and the ability to form resistant spores. Virulent *C. perfringens* isolates produce 16 protein toxins that are important for the development of different diseases, such as food poisoning, antibiotic associated diarrhea, fatal gas gangrene, enterotoxaemia, and hemorrhagic gastroenteritis (Jihong et al., 2016). *C. perfringens* is a Gram-positive, spore forming anaerobic bacterium that causes serious infections in humans and animals by producing different toxins (Mariele et al., 2020). *C. perfringens* isclassified into five toxin types (A, B, C, D, and E) according to the production of four major toxins namely alpha (CPA), beta (CPB), epsilon (ETX) and iota (ITX) (Ferreiraet al., 2016). *C. perfringens* type A strains are defined by producing alpha toxin, while type B produces alpha, beta and epsilon toxins, Alpha and beta toxins produced by type C while type D produces alpha and epsilon toxins, whereas type E produces alpha and iota toxins (Garmory et al., 2000).

The alpha toxin of *C. perfringens* (CPA) is a 43 k Da protein consists of 370 amino acids. It has been formed by two domains, an alpha-helical N-terminal domain harbouring the phospholipase C active site, and C-terminal domain which is involved in membrane binding α-toxin and has lethal, haemolytic, phosphatidylcholine Phospholipase C(PLC) and sphingomyelinase activities (Yang et al., 2018). The four major lethal toxins are not the only biomedically important toxins as some of the *C. perfringens* isolates produce *C. perfringens* enterotoxin (CPE), and necrotic enteritis B-like toxin (Net B) (Gibert et al., 1997). Enterotoxin (CPE) is a 35 k Da polypeptide and it consists of three domains. Domain I which is C-terminal, responsible for binding of receptors while domain II is responsible for oligomerization and membrane insertion, and domain III takes part in physical changes in course of insertion into membranes (Kitadokoro et al., 2011). Net B has been associated with enteric diseases in a wide range of animals (Boujon et al., 2005). It is an accessory toxin in *C. perfringens* mediated antibiotic associated diarrhoea (Fisher et al., 2005). Net B gene distinguishes virulent strains of *C. perfringens* that are capable of inducing necrotic enteritis in poultry from strains that do not cause this syndrome (Keyburn et al., 2006).

This study was aimed to evaluate the virulence factors among *C. perfringens* and consequently use the most virulent isolates in preparing the vaccines to control necrotic enteritis and enterotoxaemia among animals.

MATERIALS AND METHODS

Ethical approval

All procedures were performed according to Egyptian ethical standards of the National Research Committee.

To cite this paper: El-Helw HA, Taha MM, EF El-Sergany, EEZ Kotb, Hussein AS and Abdalla YA (2020). Identifying the Virulent Factors of *Clostridium perfringens* Locally Isolated from Different Species. *World Vet. J.,* 10 (4): 617-624. DOI: https://dx.doi.org/10.54203/scil.2020.wvj74

Sample collection

A total number of 135 intestinal samples were collected from different animal species (20 cattle samples, 20 sheep samples,40 rabbit samples, 30 chicken samples, 20 turkey samples, and 5 ostrich samples) during September and October of 2019 in Anaerobic Research Department, Veterinary Serum and Vaccine Research Inst., Cairo, Egypt. These samples were collected from different localities in El-Giza, El-Fayoum and Beni-Suef governorates in Egypt. Samples were collected just recently after death of sick animals and the parts of intestine (duodenum, ileum, caecum, and proximal colon) were tied from two sides and transfer to laboratory for isolation. These animals before death were suffer from sudden onset of diarrhoea with offensive odour specially in cattle and sheep samples, rabbit suffer from sever distension and diarrhoea prior to death and post mortem examination revealed that there was severe inflammation and haemorrhage in walls of small intestine and caecum.

Bacteriological isolation

Samples were diluted in PBS (1/10), incubated at 80°C for 10 minutes (Ahsani et al., 2010) and subsequently a loopful from each sample was cultivated on 5% sheep blood agar and anaerobically incubated using Gas pack system (Oxoid Ltd., England) at 37°C for 24 hours. Selected colonies were inoculated into cooked meat medium and incubated anaerobically at 37°C for 24 hours (Willis, 1977).

Identification of the isolates

Microscopic examination

Prepared smears from suspected colonies were stained by Gram staining and examined under oil immersion microscope (magnified 100x) according to Wilson and Miles (1975).

Colonial morphology

The Size and morphology (visual appearance of bacterial colonies on blood agar) as well as the haemolytic activity (zone of haemolysis) of the suspected colonies were examined according to Vaikosen and Muller (2001).

Biochemical and sugar fermentation tests

The catalase (by adding hydrogen peroxide and examine the formation of gas bubbles), oxidase (oxidase reagent was added and the positive results indicated if color changed), Indole (by using indole reagent and examine the change of color) and sugar fermentation tests were performed on suspected cultures according to Eyre (2009). Gelatin liquefaction test was done to detect the ability of the organism to produce gelatinase which hydrolysis the gelatin, according to Macfaddin (2000).

Nagler test

The Nagler test was done on Egg yolk agar plates according to Forbes et al. (2007) for the identification of lecithinase activity of alpha toxin of *C. perfringens*.

Dermonecrotic reaction

One side of shaved back of albino Guinea pig was injected by the prepared toxin while the other side injected by toxin after neutralization with standard antitoxin. The lesion of the reaction was interpretive according to Sterne and Batty (1975).

Toxicity test of the isolates

Toxin production medium were inoculated by pure colonies of isolates according to Chou (1971) and incubated at 37°Cand pH 7.4 for 4 hours. Sample was collected and centrifuged at 6000rpm/20min. The supernatant was taken and assayed for determination of lethality of the toxin as described by Fu et al. (2004).

DNA extraction

DNA extraction from samples was performed using the QIA amp DNA Mini kit (Qiagen, Germany, GmbH) with modifications based on the recommendations of manufacturer. Briefly, 200 µl sample from suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56° C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

PCR amplification

Oligonucleotide primers that used in current study were supplied from Metabion (Germany) and they have been listed in Table 1. The multiplex PCR for toxins was performed according to Meer and Songer (1997). The primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp® Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 11 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied bio system 2720 Thermal Cycler. The Uniplex PCR for Net B or CPE was performed. The primers were used in a 25- µl

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reaction containing 12.5 µl of Emerald Amp® Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an Applied bio system 2720 Thermal Cycler.

Analysis of the PCR products

Electrophoresis (1.5% agarose gel, AppliChem, Germany, GmbH) was performed to separate the products of in 1× TBE buffer at room temperature by using gradients of 5V/cm. In each gel slot, 30 µl of the products was loaded. The fragment sizes were determined by using Gelpilot100 bp DNA Ladder (Qiagen, Germany, GmbH). The gel was photographed by a gel documentation system (Alpha Innotech, Bio metra) and the data was analysed through computer software (Automatic Image Capture, USA).

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions that were used for PCR amplification of DNA extracts of *Clostridium perfringens* isolates

Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	References
			Primary denaturation	Secondary denaturation	Annealing	Extension		
Alpha	GTTGATAGCGCAGGACATGTTAAG	402	94˚C 5 min.	94˚C 45 sec.	50˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	YOO et al. (1997)
	CATGTAGTCATCTGTTCCAGCATC							
Beta	ACTATACAGACAGATCATTCAACC	236						
	TTAGGAGCAGTTAGAACTACAGAC							
Epsilon	ACTGCAACTACTACTCATACTGTG	541						
	CTGGTGCCTTAATAGAAAGACTCC							
Iota	GCGATGAAAAGCCTACACCACTAC	317						
	GGTATATCCTCCACGCATATAGTC							
Net B	GCTGGTGCTGGAATAAATGC	560	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Datta et al. (2014)
	TCGCCATTGAGTAGTTTCCC							
Enterotoxin (CPE gene)	ACATCTGCAGATAGCTTAGGAAAT	247	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 10 min.	Kaneko et al. (2011)
	CCAGTAGCTGTAATTGTTAAGTGT							

RESULTS

Bacterial identification of isolates

Morphological characterization

Figure 1. Colonies streaked on blood agar plate have shown a double zone of hemolysis (inner zone shown complete zone of hemolysis, and outer zone for incomplete hemolysis)

Figure 2. Gram staining of smear from suspected colonies that have shown Gram-positive bacilli

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Biochemical characteristics

The number of 26 *C. perfringens* isolates were collected from 135 intestinal samples as presented in Table2.Those colonies that presented double zone of hemolysis when inoculated in blood agar and revealed as Gram-positive bacilli were further examined for their biochemical reactions and toxicity test, as they were catalase, oxidase, and Indole tests negative and liquefies gelatin. Sugar fermentation (glucose, sucrose, maltose and lactose) of isolates was positive and also their Nagler's reaction was positive. Figure 3 indicated clear opalescence zone in the antitoxin free side (right) while it was inhibited in the left side where the antitoxin was added (no clear opalescence) due to toxin-antitoxin neutralization.

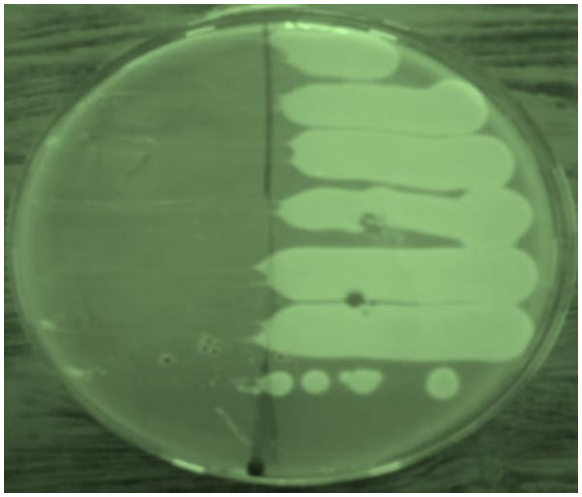

Figure 3. Nagler`s reaction on egg yolk agar for the isolates

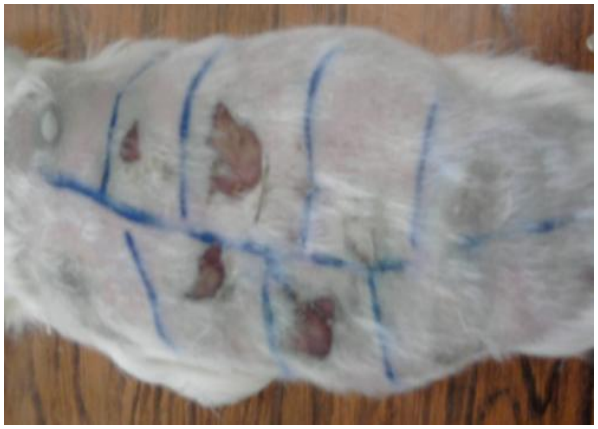

Figure 4. Dermonecrotic reaction performed on Guinea pig, the right side was inoculated by toxin and antitoxin, while the left side has indicated lesion on skin due to incubation only by toxin.

Toxicity test

All 26 isolates that have been inoculated in three mice for each isolate caused that inoculated mice died within 24 hours.

Table 2. Genotyping of *Clostridium perfringens* isolates from different animal species

Animal specie	No. of samples collected	*C. perfringens* positive %	Genotypes of *C. perfringens* isolates positive %					
			CPA	CPB	ETX	CPIi	Net B	CPE
Cattle	20	(4/20) 20	(4/4) 100	0	0	0	(4/4) 100	(4/4) 100
Sheep	20	(6/20) 30	(6/6) 100	0	0	0	0	(6/6) 100
Rabbit	40	(10/40) 25	(10/10) 100	0	0	0	0	(10/10) 100
Chicken	30	(2/30) 6.66	(2/2) 100	0	0	0	(2/2) 100	(2/2) 100
Turkey	20	(3/20) 15	(3/3) 100	0	0	0	0	(3/3) 100
Ostrich	5	(1/5) 20	(1/1) 100	0	0	0	0	(1/1) 100

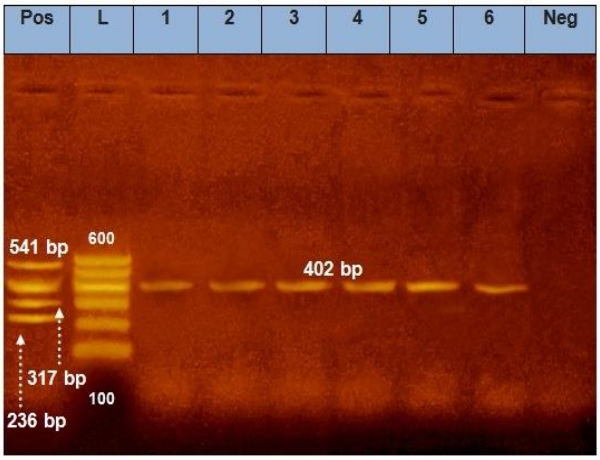

Figure 5. Agarose gel for Multiplex PCR of toxins**. Lane (L):** 100bp DNA Ladder**, Lane Positive:** *Clostridium perfringens* strains type B and E served as a positive control, **Lane Negative:** Negative**, Lanes (1-6):** samples isolated 1: Turkey, 2: Chicken, 3: Ostrich, 4: Rabbit, 5: Sheep, and 6: Cattle.

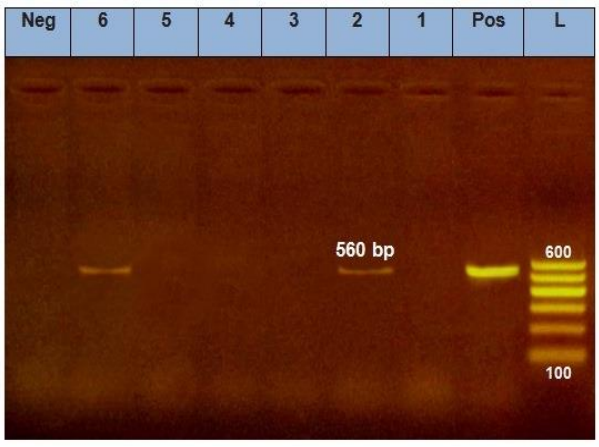

Figure 6. Agarose gel for Uniplex of Net B. Lane (L): 100bp DNA Ladder, Lane Positive: Positive control for Net B gene, Lane Negative: Negative, Lanes (1-6): Samples isolated. 1: Turkey, 2: Chicken, 3: Ostrich, 4: Rabbit, 5: Sheep, and 6: Cattle.

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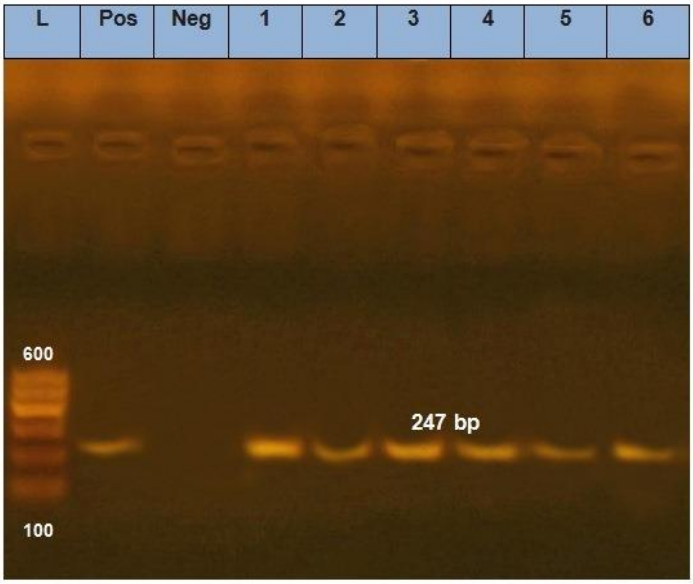

Figure 7. Agarose gel for uniplex of CPE. **Lane (L):** 100bp DNA Ladder**, Lane Positive:** Positive control for CPE gene, **Lane Negative:** Negative**, Lanes (1-6):** Samples isolated. 1: Turkey, 2: Chicken, 3: Ostrich, 4: Rabbit, 5: Sheep, and 6: Cattle.

Genotyping of the isolates

The 26 number of *C. perfringens* collected isolates (19.25%) from 135 intestinal samples were all CPA and CPE genes positive by multiplex PCR which included the number of 4 isolates (20%) from total 20 cattle samples, 6 isolates (30%) from total 20 sheep samples, 10 isolates (25%) from total 40 rabbit samples, 2 isolates (6.66%) from total 30 chicken samples, 3 isolates (15%) from total 20 turkey samples and 1 isolate (20%) from total 5 ostrich samples. The Net B gene was positive in six isolates (two isolates from chicken and four isolates from cattle).

DISCUSSION

C. perfringens can produce four major toxins (α, β, ε, and ι) and is accordingly divided into five serotypes, from A to E (Zhang et al., 2020). All five serotypes of *C. perfringens* carry and express the alpha toxin structural gene, but most of type A strains produce alpha toxin, while beta toxin is a major lethal toxin produced by both types B and C strains of *C. perfringens*. Epsilon toxin is produced as a proto toxin and activated by the proteolytic enzymes that produced by the same organism (Popoff, 2014). Mainly iota toxin secreted by type E of *C. perfringens* and consists of two separate proteins that are immunologically and chemically distinct (Awad et al., 2001).

Several virulence factors of *C. perfringens* including enterotoxins and Net B toxin have been studied (Silva et al., 2013). *C. perfringens* enterotoxin (CPE) is a 35kDa polypeptide consisting of three domains. The CPE forms an active pore that enhances calcium influx, consequently leads to cell death and intestinal damage (Freedman et al., 2016). Results from current study revealed that all isolates characterized by presence of CPE gene, and it comes in accordance with Li et al. (2010) who mentioned that the most CPE gene positive strains of *C. Perfringens was* classified as type A, moreover, type C and D strains are fairly common to produce the enterotoxin

In this study, 135 intestinal samples from different animal and poultry species were taken and the causative organism was isolated on cooked meat medium then streaked on blood agar as illustrated in figure 1double zone of hemolysis of the colonies (inner shown complete zone of hemolysis, and outer incomplete zone of hemolysis) have been detected in 26 samples from 135 intestinal samples, then the colonies of each blood agar plate were smeared and Gram stained separately, where Gram-positive bacilli seen in 26 out of 135 of the smears (Figure 2).

Biochemical tests for the isolates revealed that they were oxidase, catalase and indole tests negative, the isolates positively ferment glucose, sucrose, maltose and lactose. Figure 3 indicated that the isolates showed clear opalescence zone in the antitoxin free side, while in the side where the antitoxin was added, it was inhibited due to toxin-antitoxin neutralization. These results primarily concluded that 26 isolates from the 135 collected samples were *C. perfringens* as mentioned by Willis (1977).

Genotyping of the isolates was done by multiplex PCR using primers sequences, for the four major toxins genes as indicated in table1. The PCR results revealed that alpha toxin gene (402bp) was only detected among the major toxin in the obtained isolates (Figure 5). These results came in contact with the fact that alpha toxin is the virulence factor which is produced by all types of *C. perfringens* (Titball et al., 2000).

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Uniplex PCR for Net B and CPE genes by using their specific primer sequences 546 and 247 bp respectively, was performed separately as it has been presented in table 1. Figure 6 based on the Agarose gel of uniplex PCR using Net B primer revealed that six isolates(two from chicken and four from cattle)which previously had been isolated, were the only ones that contained Net B gene as a virulence factor beside the major alpha toxin, these results were in complete agreement with Anthony et al. (2006) as they stated alpha toxin is not the only essential causative agent of necrotic enteritis in chickens and this provides the basis for further studies to identify virulence factors. Moreover, Anthony et al. (2010) identified Net B in *C. perfringens* type A strains isolates from chickens suffering necrotic enteritis. Also, Thomas and Joan (2009) detected Net B gene in isolates of American strains of *C. perfringens* from chickens and cattle.

The results in Table 2 indicated that the *C. perfringens* type A isolates had Net B gene obtained from infected chickens which recently died and had sever inflammation in caecum and enteritis. This finding proved by Keyburn et al. (2010) who found that the majority of strains isolated from necrotic enteritis affected birds were Net B gene positive and there was correlation between Net B and in vitro expression of Net B protein and provide the evidence that Net B is important in pathogenesis as virulence factor for *C. perfringens*.

On the other side, the results from the Uniplex PCR by using CPE primer which has been illustrated inFigure7, revealed that *C. perfringens* enterotoxin secreted by all of the 26 isolates. Freedman et al. (2016) previously concluded that most CPE positive strains classify as *C. perfringens* type A and also added that during the disease when *C. perfringens* sporulates in the intestines, CPE produces, a process that in volves several sporulation specific alternative sigma factors.

CONCLUSION

Based on results from current study, it could be concluded that among 26 *Clostridium perfringens* isolates from different species, six isolates that have been isolated from chicken and cattle (two and four respectively) contained CPA, Net B and CPE genes as virulence factors and hence can be used in preparation of enterotoxaemia and necrotic enteritis vaccines from *Clostridium perfringens* type A as they are the more virulent strains.The authors of present study also recommend further studies on the virulence factors of *Clostridium perfringens*.

DECLARATIONS

Authors' contribution

El-Helw H.A. and Taha M.M. isolated *Clostridium perfringens* from the samples, El-Sergany E.F. identified the isolates by using morphological and biochemical tests. Ebtesam E.Z. Kotb and Hussein A.S. provided samples from farms while El-Helw H.A. and Abdalla Y.A. performed genotyping of isolates.

REFERENCES

Ahsani MR, Mohammadabadi MR, and Shamsaddini MB (2010). *Clostridium perfringens* isolates typing by multiplex PCR. Journal of Venomous Animals and Toxins including Tropical Diseases, 16: 573-578. DOI: https://www.doi.org/10.1590/S1678-91992010000400006

Anthony K, Sheedy S, Mark F, Williamson M, Milena A, Rood J, and Moore R (2006). Alpha toxin of *Clostridium perfringens* is not an essential virulence factor in necrotic enteritis in chickens. Infection and Immunity, 74(11): 6469-6500. DOI: https://www.doi.org/10.1128/IAI.00806-06

Anthony LK, Trudi LB, Robert JM, and Julian I (2010). Net B, a Pore-Forming Toxin from Necrotic Enteritis Strains of *Clostridium perfringens*. Toxins, 2: 1913-1927. DOI: https://www.doi.org/10.3390%2Ftoxins2071913

Awad MM, Ellemor DM, Boyd RI, Emmins JJ, and Rood JI (2001). Synergestic effects of alpha toxin and perfringolysin O in *Clostridium perfringens* mediated gas gangrene. Infection and Immunity Journal, 69: 7904-7910. DOI: https://www.doi.org/10.1128/IAI.69.12.7904-7910.2001

Boujon P, Henzi M, Penseyres JH, and Belloy L (2005). Enter otoxemia involving beta2-toxigenic *Clostridium perfringens* in a white stork (*Ciconiaciconia*). The Veterinary Research, 156: 746-747. DOI: https://www.doi.org/10.1136/vr.156.23.746

Chou G (1971). Medium for toxin production by *Clostridium perfringens* in continuous culture. Applied Microbiology, 21(5): 794-798. PMCID: 377283

Datta S, Rakha NK, Narang G, Arora D, and Mahajan NK (2014). Prevalence of α, ß, and Net B toxin producing strains of *Clostridium perfringens* in broiler chickens in Harayana. Haryana Veterinarian, 53(1): 39-42. Available at: https://www.luvas.edu.in/haryana-veterinarian/download/harvet2014/9.pdf

Eyre JWH (2009). The Project Gutenberg eBook of The Elements of Bacteriological Technique, A Laboratory Guide for Medical, Dental, and Technical Students. Second Edition Rewritten and Enlarged. eBook #27713 ISO-8859-8861. Available at: http://www.gutenberg.org/files/27713/27713-h/27713-h.htm

Ferreira MR, Gustavo MS, Carlos E, Marcelo M, Felipe M, Ângela N, and Fabricio RC (2016). Recombinant Alpha, Beta, and Epsilon Toxins of *Clostridium perfringens*: Production Strategies and Applications as Veterinary Vaccines Toxins, 8: 340-362. DOI: http://www.doi.org/10.3390/toxins8110340

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Fisher DJ, Miyamoto K, Harrison B, Akimoto S, Sarker Mr, and McClane BA (2005). Association of beta2 toxin production with *Clostridium perfringens* type A human gastrointestinal disease isolates carrying a plasmid enterotoxin gene. Molecular Microbiology, 56: 747-762. DOI: https://www.doi.org/10.1111/j.1365-2958.2005.04573.x

Forbes B, Daniel S, and Alice W (2007). Bailey and Scott's Diagnostic Microbiology, C.V. Mosby Company, St. Louis, MO. 12th ed. Pp. 182-191. Available at: https://www.worldcat.org/title/bailey-scotts-diagnostic-microbiology/oclc/77256475

Freedman JC, Shrestha A, and McClane BA (2016). *Clostridium perfringens* Enterotoxin: Action, Genetics, and Translational Applications. Toxins, (8): 73-81. DOI: https://www.doi.org/10.3390/toxins8030073

Fu SW, Xue J, Zhang YL, and Zhou DY (2004). Simplified purification method for *Clostridium difficile* toxin A. World Journal of Gastroenterology, 10(18): 2756- 2758. DOI: https://www.doi.org/10.3748/wjg.v10.i18.2756

Garmory HS, Chanter N, and French NP (2000). Occurrence of *Clostridium perfringens* beta2-toxin amongst animals, determined using genotyping and subtyping PCR assays. Epidemiology Infection, 124: 61-67. DOI: https://www.doi.org/10.1017/s0950268899003295

Gibert M, Jolivet-Reynaud C, and Popoff MR (1997). Beta2 toxin, a novel toxin produced by *Clostridium perfringens.* Gene, 203: 65–73. DOI: https://www.doi.org/10.1016/s0378-1119(97)00493-9

Jihong L, Daniel P, Mahfuzur RS, and Clane BAM (2016). *Clostridium perfringens* Sporulation and Sporulation-Associated Toxin Production. Microbiology Spectrum Journal, 4(3): 1-26. DOI: http://www.doi.org/10.1128/microbiolspec.TBS-0022-2015

Kaneko I, Miyamoto K, Mimura K, Yumine N, Utsunomiya H, Akimoto S, and McClane BA (2011). Detection of Enter otoxigenic *Clostridium perfringens* in Meat Samples by Using Molecular Methods. Applied and Envir. Microbiol, pp. 7526-7532. DOI: https://www.doi.org/10.1128%2FAEM.06216-11

Keyburn AL, Scott AS, Mark EF,Mark MW,Milena MA, Julian IR, and Robert JM (2006). Alpha-Toxin of *Clostridium perfringens* Is Not an Essential Virulence Factor in Necrotic Enteritis in Chickens. Infection and Immunity, 74(11): 6496-6500. DOI: https://www.doi.org/10.1128/IAI.00806-06

Keyburn AL, Yan X,Bannam TL, Van Immerseel FV, Rood JI, and Moore RJ (2010). Association between avian necrotic enteritis and *Clostridium perfringens* expression Net B toxin. Veterinary Research, 14: 21. DOI: https://www.doi.org/10.1051/vetres/2009069

Kitadokoro K, Nishimura K, Kamitani S, Fukui-Miyazaki A, Toshima H, Abe H, Kamata Y, Sugita-Konishi Y, Yamamoto S, and Karatani H (2011). Crystal structure of *Clostridium perfringens* enterotoxin displays features of beta-pore-forming toxins. Journal of Biological Chemistry, 286: 19549-19555. DOI: https://www.doi.org/10.1074/jbc.M111.228478

Li J, Miyamoto K, Sayeed S, and McClane BA (2010). Organization of the *cpe* locus in CPE-positive *Clostridum perfringens* type C and D isolates. PLoS One, 5: e10932. DOI: https://www.doi.org/10.1371/journal.pone.0010932

MacFaddin JF (2000). Biochemical tests for identification of medical bacteria, 3rd Edition. Baltimore (Md.): Williams and Wilkins, p.928. Available at: https://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55))/reference/ReferencesPapers.aspx?ReferenceID=1661468

Mariele S, Rubén P, Schocken I, Lívia B, Andressa P, and Karin W (2020). Necrotic Enteritis Caused by *Clostridium perfringens* in Blue and Gold Macaws (*Araararauna*). Journal of Avian Medicine and Surgery, 34(1): 65-69. DOI: https://www.doi.org/10.1647/1082-6742-34.1.65

Meer RR, and Songer JG (1997). Multiplex polymerase chain reaction assay for genotyping *Clostridium perfringens.* American Journal of Veterinary Research, 58(7): 702-705. Available at: https://pubmed.ncbi.nlm.nih.gov/9215442/

Popoff MR (2014). Clostridial pore-forming toxins: powerful virulence factors. Anaerobe, 30: 220-238. DOI: https://www.doi.org/10.1016/j.anaerobe.2014.05.014

Silva R, Ribeiro M, Palhares S, Borges AS, Maranhão RPA, and Silva MX (2013). Detection of A/B toxin and isolation of *Clostridium difficile* and *Clostridium perfringens* from foals. Equine Veterinary Journal, 45: 671-675. DOI: https://www.doi.org/10.1111/evj.12046

Sterne M, and Batty I (1975). Criteria for diagnosing Clostridial infection. Pathogenic clostridia, Butterworths, London, United Kingdom, pp. 79-122.

Thomas GM, and Joan AS (2009). Prevalence of *net B* among some clinical isolates of *Clostridium perfringens* from animals in the United States. Veterinary Microbiology, 136: 202-205. DOI: https://www.doi.org/10.1016/j.vetmic.2008.10.026

Titball RW, Naylor CE, Miller J, Moss DS, and Basak AK (2000). Opening of the active site of *Clostridium perfringens* α-toxin may be triggered by membrane binding. International Journal of Medical Microbiology, 290: 357-361. DOI: https://www.doi.org/10.1016/S1438-4221(00)80040-5

Uzal FA, Freedman JC, Shrestha A, Theoret JR, Garcia J, Awad MM, Adams V, Moore RJ, Rood JI, and McClane BA (2014). Towards an understanding of the role of *Clostridium perfringens* toxins in human and animal diseases. Future Microbiol, 9(3): 361-377. DOI: https://www.doi.org/10.2217%2Ffmb.13.168

Vaikosen ES, and Muller W (2001). Evaluating biochemical tests for isolation / identification of *C. perfringens in* faecal samples of small ruminants in Nigeria. Bulletin of Animal Health and Production in Africa, 49(4): 244-248.

Willis AT (1977). Anaerobic Bacteriology, Clinical and Laboratory Practice. 3rd Ed., Butter Worth, London, Boston, pp. 131-133. Available at: https://www.elsevier.com/books/anaerobic-bacteriology/willis/978-0-407-00081-0

Wilson GS, and Miles AA (1975). Principles of Bacteriology, Virology and Immunology. 6th Ed., vol. 1 and vol. 11, p. 1249 and p. 2706. Available at: https://www.cabdirect.org/cabdirect/abstract/19762704307

Yang W, Chou C, and Chinling W (2018). Characterization of toxin genes and quantitative analysis of netB in necrotic enteritis (NE)-producing and non-NE-producing *Clostridium perfringens* isolated from chickens. Anaerobe, 54: 115-120. DOI: https://doi.org/10.1016/j.anaerobe.2018.08.010

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Yoo HS, Lee SU, Park KY, and Park YH (1997). Molecular Typing and Epidemiological Survey of Prevalence of *Clostridium perfringens* Types by Multiplex PCR. Journal of Clinical Microbiology, 35(1): 228-232. PMCID: 229544

Zhang J, Shui L, Lining X, Zhongmei W, Naiyu H, Tingting W, Xuming D, Jiakang H, and Wang J (2020). Verbascoside Protects Mice From Clostridial Gas Gangrene by Inhibiting the Activity of Alpha Toxin and Perfringolysin O. Frontiers in Microbiology, 11: 1504-1510. DOI: https://www.doi.org/10.3389/fmicb.2020.01504

To cite this paper: El-Helw HA, Taha MM, EF El-Sergany, EEZ Kotb, Hussein AS and Abdalla YA (2020). Identifying the Virulent Factors of *Clostridium perfringens* Locally Isolated from Different Species. *World Vet. J.*, 10 (4): 617-624. DOI: https://dx.doi.org/10.54203/scil.2020.wvj74

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 625-633, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj75

Green Synthesis of Silver Nanoparticles Using Lactic Acid Bacteria: Assessment of Antimicrobial Activity

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ORIGINAL ARTICLE
pii: S232456820000075-10
Received: 26 Oct 2020
Accepted: 13 Dec 2020

ABSTRACT

The biosynthesis of silver nanoparticles (Ag-NPs) is a new methodology in nanotechnology with a hopeful implementation in medicine, food control, and pharmacy. The objective of the present research was to conduct a green synthesis of Ag-NPs using the cell-free supernatant of *Lactobacillus plantarum* and *Lactobacillus brevis* and evaluate their antibacterial and antifungal activities. The production of Ag-NPs was confirmed by the color alteration from yellow to brown. Using the UV-visible spectrophotometer, the biosynthesized Ag-NPs indicated an absorption peak at 410 nm. The transmission electron microscope was used for the determination of the size and morphology of the nanoparticles. Nanoparticles appeared in spherical or polyhedral form, poly-dispersed and their diameter ranged from 5 to 40 nm. The X-ray diffraction analysis exhibited the crystalline nature of the particles with a face-centered cubic (FCC) structure. The biosynthesized Ag-NPs were evaluated for their antimicrobial efficiency using the agar well diffusion method. The antibacterial activity of Ag-NPs was more potent against Gram-negative bacteria than Gram-positive bacteria. Ag-NPs synthesized from *Lactobacillus plantarum* recorded the maximum activity against *Escherichia coli* (ATCC® 10536™) and *Pseudomonas* (ATCC® 27853™) bacteria, while those synthesized from *Lactobacillus brevis* recorded the maximum activity against *Escherichia coli* (ATCC® 35218™). Ag-NPs synthesized from *Lactobacillus plantarum* and *Lactobacillus brevis* showed antifungal activity against *Candida albicans* (ATCC® 10231™). The effect of these nanoparticles on *Escherichia coli* (ATCC® 10536™) was examined and imaged by a transmission electron microscope that indicated damage to the plasma membrane and cell wall. In conclusion, the biosynthesized Ag-NPs have applications as antimicrobial agents in the medicine and food industry.

Keywords: Antimicrobial activity, *Lactobacillus brevis*, *Lactobacillus plantarum*, Silver nanoparticles, Transmission electron microscope

INTRODUCTION

At the present, bacterial infections are one of the most common dangers fronting medical treatment due to the emergence of drug-resistant bacteria resulted from the spread of resistance mechanisms (Holmes et al., 2016). The overuse of antibiotics for the treatment of infectious diseases creates complicated mediation problems. Moreover, these drugs cause various side effects and problems, including allergy, hypersensitivity, and immune-suppression. Therefore, there is a need to develop new antimicrobial drugs for the treatment of pathogenic diseases (Jain et al., 2009).

Nanotechnology is a novel field of preparation and use of nanomaterials less than 100 nm in size. Nanoparticles exhibit distinctive properties and a high surface-to-volume ratio (Khan et al., 2014). Nanotechnology enhances the production of different metal nanoparticles, especially silver nanoparticles (Ag-NPs), which have high bactericidal potential. Ag-NPs can be used as an alternative for antibiotic drugs due to their strong effect on multidrug-resistant bacteria (Song and Kim, 2009). It has been demonstrated that Ag-NPs have antibacterial potential against some antibiotic-resistant microorganisms (Birla et al., 2009; Inoue et al., 2010). The antimicrobial properties of Ag-NPs depend on silver ions (Ag^+) which intensely hinder microbial growth through the inhibition of electron transport components, respiratory enzymes, and interference with DNA functions (Li et al., 2006). Silver at a nanoscale (smaller than 100 nm) has a potent toxic effect on a broad group of microorganisms (Elechiguerra et al., 2005). Morones et al. (2005) determined the antimicrobial effect of Ag-NPs on four species of Gram-negative bacteria (*Pseudomonas aeruginosa*, *Salmonella* Typhi, *Escherichia coli (E. coli),* and *Vibrio cholera)* and suggested that Ag-NPs stick to the cell membrane surface and interrupt its function by permeation the bacterial cell wall and freeing of Ag^+ ions.

Chemical and physical methods used for the production of Ag-NPs are costly and include toxic substances that can harm public health and the surrounding environment. The latest studies indicated great attention for the preparation of Ag-NPs from eco-friendly and cost-efficient biological sources using various plant, microbial (bacterial and fungal), and algae extracts (Ratan et al., 2020).

To cite this paper: Syame SM, Mansour AS, Khalaf DD, Ibrahim ES and Gaber ES (2020). Green Synthesis of Silver Nanoparticles Using Lactic Acid Bacteria: Assessment of Antimicrobial Activity. *World Vet. J.,* 10 (4): 625-633. DOI: https://dx.doi.org/10.54203/scil.2020.wvj75

The purpose of the current research was the biosynthesis of Ag-NPs by extracellular reduction of silver nitrate ($AgNO_3$) using lactic acid bacteria isolated from fermented milk product samples. In addition, the antibacterial and antifungal effects of the Ag-NPs were examined against Gram-positive, Gram-negative bacterial, and fungal species.

MATERIALS AND METHODS

Isolation and identification of *Lactobacillus plantarum* and *Lactobacillus brevis*

Samples of fermented dairy products, including Karish cheese, fermented goat's milk, and Laban Rayeb (concentrated sour milk) were collected from Cairo and Giza governorates in Egypt. All samples were collected in sterile bags, transferred to the laboratory under aseptic cooled conditions, and stored at 3 ± 1 ºC for up to a maximum of 24 hours before the analysis. For the preparation of samples, 10 g of each sample were homogenized in a stomacher lab-blender with 90 ml of sterile sodium citrate solution (2% w/v) for cheese samples and 90 ml of sterile physiological saline (0.85% NaCl w/v) for fermented milk samples for 30 seconds. A loopful from each sample was cultured on de Man, Rogosa and Sharpe (MRS, Oxoid, England) Petri-dishes. All plates were incubated at 37 °C for 48-72 hours under anaerobic conditions. Single colonies were picked from MRS agar plates, purified, and identified using morphological and biochemical characterization (Mathara et al., 2004).

Preparation of cell-free supernatant of *Lactobacillus* strains

Single colonies of *Lactobacillus* isolates were inoculated in nutrient broth (Oxoid, England) and incubated at 37°C for 24 hours, then the culture was centrifuged at 4500 rpm for 10 minutes to prepare the cell-free supernatant. After centrifugation, the precipitated cells at the bottom of the tube were discarded and the cell-free supernatant was collected and used for the synthesis of Ag-NPs (Chaudhari et al., 2012).

Biosynthesis of silver nanoparticles using cell-free supernatant of *Lactobacillus* strains

$AgNO_3$ was a precursor for the biosynthesis of Ag-NPs by *Lactobacillus*. AgNO3 (Sigma-Aldrich) with a concentration of 2 mM was added to *Lactobacillus* cell-free supernatant in a ratio of 1:1 and was mixed well. This step was prepared in a dark condition to avoid oxidation of AgNO3. The pH of the mixture was adjusted to 8.3. The resultant solution was incubated in a shaking incubator at 150 rpm at 37 °C for 24 hours (Dakhil, 2017). After incubation, the color change was observed and the reaction mixture was centrifuged at 10000 rpm for 10 minutes. The supernatant was discarded and replaced with deionized distilled water and re-centrifuged three times at the same speed and time to remove the remaining supernatant. The pellet deposited at the bottom of the tube represented Ag-NPs and then dried in an oven at 40°C for 18-24 hours. The dried powder was collected carefully and stored in sample vials for further analysis (Chaudhari et al., 2012; Sarvamangala et al., 2013).

Characterization of silver nanoparticles

Ultraviolet-visible spectrophotometry

The ultraviolet-visible (UV-Vis) spectrum of the Ag-NPs was measured using a UV-Vis spectrophotometer (Shimadzu, 1600) in the central laboratory of the National Research Center, Egypt. The absorbance was measured in the range of 400-800 nm. Deionized water was used as the blank.

Transmission electron microscopy

The size and shape of the Ag-NPs were determined by transmission electron microscopy (TEM). The sample was prepared by placing a drop of nanoparticle solution on a carbon-coated copper grid and water was allowed to evaporate. The TEM measurements were performed on a JEM – JEOL model 2100 instrument, which was operated at an accelerating voltage of 120 kV (Dong et al., 2019).

X-ray powder diffraction

Pattern of dry nanoparticle powder was obtained with Cu Kα radiation (1.5406 Å; 45 kV, 30 mA). The X-ray powder diffraction (XRD) pattern was analyzed to determine position, peak intensity, and width. The nanoparticle size was calculated using the Scherrer formula as follows:

$D = 0.94\lambda / \beta \cos \theta$

Where, D is the mean diameter of the nanoparticles, β denotes the angular full width at half maximum (FWHM) of the XRD peak at the diffraction angle θ, and λ refers to the wavelength of the X-ray radiation source (Shehzad et al., 2018).

Antibacterial activity of silver nanoparticles

Antibacterial activity of biogenic Ag-NPs was carried using the agar well diffusion method against different types of pathogenic Gram-positive and Gram-negative bacteria as listed in Table 1. Standardized suspension of each tested bacterium (1.5×10^8 CFU/mL) was prepared to a 0.5 McFarland standard by measuring the absorbance at wavelength

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625 nm, and swabbed separately onto sterile Muller-Hinton Agar (MHA, Oxoid, England) plates using sterile cotton swabs. Agar was punched with a sterilized cork borer (6 mm) and 50 µl of biogenic Ag-NPs was added into each well. One petri-dish was cultured for each pathogenic bacterium and used as control. All plates were incubated at 37 °C for 24 hours and the inhibition zones were measured (Charannya et al., 2018).

Antifungal activity of silver nanoparticles

Antifungal activity of biogenic Ag-NPs was assayed against pathogenic fungus, *Candida albicans* (Table 1) using the agar well diffusion method. The concentration of fungal suspension was adjusted to 0.5 McFarland standard in normal saline (at wavelength 530 nm) to achieve concentration of 1-5 × 10^6 CFU/mL. This suspension was streaked on Sabouraud dextrose agar (Oxoid, England) plate by a cotton swab. Wells were made in the agar plates with a sterilized cork borer (6 mm) and 50 µl of biogenic Ag-NPs were added into wells. One Petri-dish was cultured as control. After incubation for 3 days at 28 °C, the inhibition zones were measured (Buszewski et al., 2018).

Table 1. Bacterial and fungal strains used in this study for antimicrobial activity test

Bacterial strains
Gram-positive bacteria
Staphylococcus aureus (ATCC® 25923™)
Enterococcus faecalis (ATCC® 29212™)
Staphylococcus epidermidis (ATCC® 12228™)
Staphylococcus aureus (ATCC® 29213™)
Clostridium perfringens (ATCC® 13124™)
Gram-negative bacteria
Escherichia coli (ATCC® 10536™)
Klebsiella pneumonia (ATCC® 700603™)
Pseudomonas aeruginosa (ATCC® 27853™)
Escherichia coli (ATCC® 35218™)
Neisseria gonorrhoeae (ATCC® 19424™)
Fungal strains
Candida albicans (ATCC® 10231™)

Assessment of effect of silver nanoparticle on *Escherichia coli* by transmission electron microscopy

In order to observe the impact of Ag-NPs on the morphology and structure of *E. coli* (ATCC® 10536™), isolates of *E. coli* were adopted, and each was proliferated to 1×10^8 CFU/ml. After being exposed to 11.25 µg/mL Ag-NPs for 12 hours, the culture was precipitated by centrifugation and washed once with phosphate buffer saline (PBS) and then centrifuged; the precipitates were fixed in 2.5% glutaraldehyde overnight, and rinsed three times with 0.1 M phosphoric acid; the bacteria were dehydrated, paraffin-embedded, and sliced, then double-stained with 3% uranium acetate and lead nitrate before being observed by TEM. The bacteria without Ag-NPs treatment were considered as control (Liao et al., 2019).

RESULTS

Biosynthesis of silver nanoparticles

Culture filtrates of *Lactobacillus plantarum* (*L. plantarum*) and *Lactobacillus brevis* (*L. brevis*) were added to the $AgNO_3$ solution, after 24 hours, the color of the mixture changed to yellowish-brown or brown by optical observation.

Characterization of silver nanoparticles

UV-visible spectroscopy

UV-Vis spectroscopy analysis showed that the formed Ag-NPs had an absorption peak at 410 nm (Figure 1).

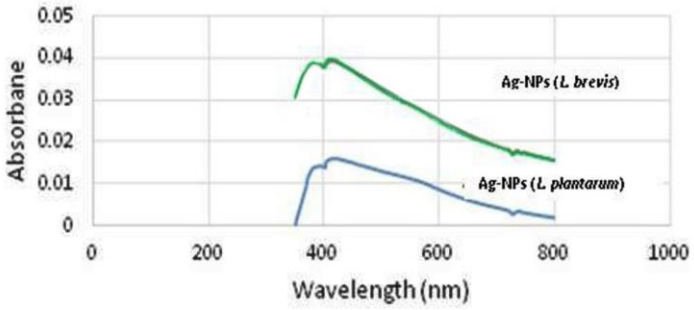

Figure 1. UV-visible absorption spectra of Ag-NPs synthesized by cell-free supernatant of *Lactobacillus brevis* and *Lactobacillus plantarurm*. The absorption spectra of Ag-NPs exhibit a strong broad peak at 410 nm.

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Transmission electron microscopy characterization

TEM results showed that most of the biogenic Ag-NPs were in spherical and polyhedral form, and poly dispersed nanoparticles (Figures 2 and 3). The diameter of_Ag-NPs was within the range of 5-40 nm. Selected area electron diffraction (SAED) patterns of the Ag-NPs presented in concentric circles and the Ag-NPs appeared in the form of crystals as can be seen in Figure 4.

X-ray diffraction pattern

The XRD patterns of the formed Ag-NPs were displayed in Figure 5. Ag-NPs exhibited unique diffraction peaks of metallic face-centered cubic (FCC) silver phase at 42.66° (111), 48.68° (200), 69.32° (220), and 82.12° (311) in 2θ.

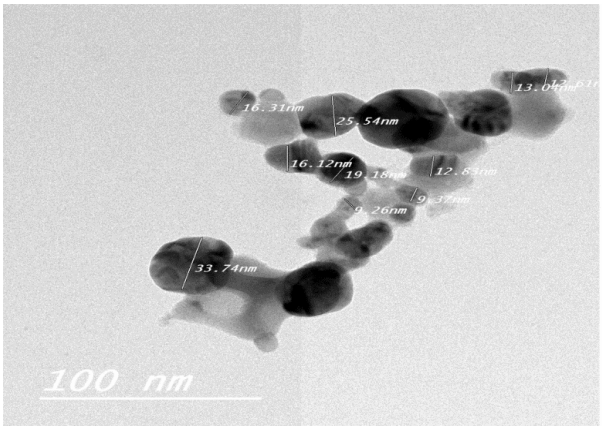

Figure 2. Transmission electron microscope micrograph of silver nanoparticles biosynthesized from *Lactobacillus plantarum*

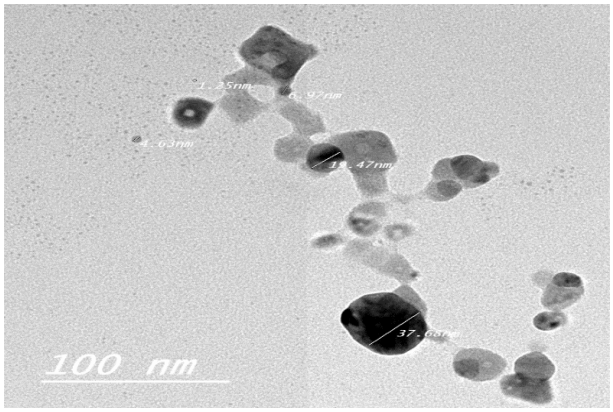

Figure 3. Transmission electron microscope micrograph of silver nanoparticles biosynthesized from *Lactobacillus brevis*

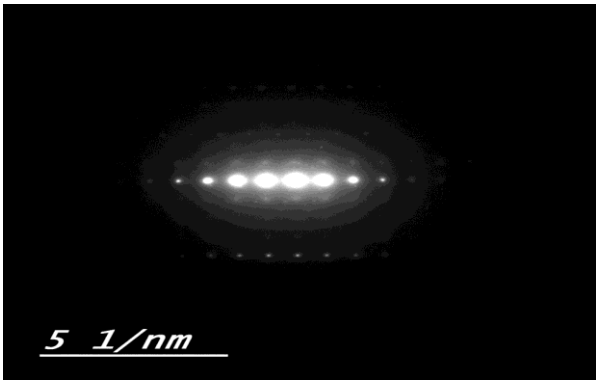

Figure 4. Selected area electron diffraction (SAED) patterns of the silver nanoparticles biosynthesized by *Lactobacillus plantarum*

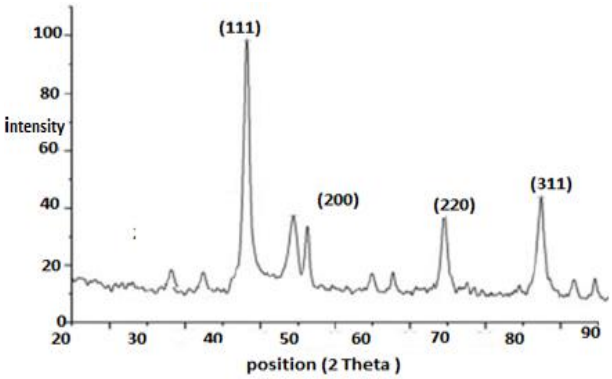

Figure 5. X-ray diffraction (XRD) pattern of silver nanoparticles biosynthesized by cell-free supernatant of *Lactobacillus brevis* and *Lactobacillus plantarum*

Antibacterial and antifungal activity of silver nanoparticles

Biosynthesized Ag-NPs prevented the growth of Gram-positive bacteria as well as Gram-negative bacteria (Table 2). The inhibition zones of Gram-negative bacteria were larger than those of Gram-positive bacteria for Ag-NPs synthesized from the culture filtrate of *L. plantarum*. The zone of inhibition against Gram-negative bacteria ranged 15-22 mm, the largest zone was against *E. coli* (ATCC® 35218™) while the smallest zone was against *Klebsiella pneumoniae* and *Neisseria gonorrhoeae*. The inhibition zone of Ag-NPs synthesized from the culture filtrate of *L. plantarum* against Gram-positive bacteria ranged 16-18 mm, the largest zone was against *Staphylococcus aureus* (ATCC® 25923™) whereas the smallest zone was against *S. epidermidis* and coagulase-negative *S. aureus* (ATCC® 29213™). The inhibition zone of Gram-positive bacteria was larger than that of Gram-negative bacteria for Ag-NPs synthesized from the culture filtrate of *L. brevis*. The diameter of inhibition zones against Gram-positive bacteria was within the range of 14-21 mm, the largest zone was against *Enterococcus faecalis* while the smallest zone was against *S. epidermidis* and coagulase-negative *S. aureus.* On the other hand, the inhibition zone diameter against Gram-negative bacteria ranged 13-22 mm, the greatest and the lowest zones were against *E. coli* and *Pseudomonas aeruginosa*, respectively. Antifungal activity of Ag-NPs was tested against *C. albicans* (Table 2) using the agar well diffusion method. The inhibition zones of Ag-NPs biosynthesized from culture filtrates of *L. plantarum* and *L. brevis* were 17 mm and 18 mm, respectively.

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Table 2. Antifungal and antibacterial activities of silver nanoparticles (Ag-NPs) biosynthesized from *Lactobacillus* species

Strain \ Inhibition zone diameter* (mm)	Ag-NPs biosynthesized by *Lactobacillus plantarum*	Ag-NPs biosynthesized *by* *Lactobacillus brevis*
Gram-positive bacteria		
Staphylococcus aureus (ATCC® 25923™)	18	16
Enterococcus faecalis (ATCC® 29212™)	17	21
Staphylococcus epidermidis (ATCC® 12228™)	16	14
Staphylococcus aureus (ATCC® 29213™)	16	14
Clostridium perfringens (ATCC® 13124™)	17	17
Gram-negative bacteria		
Escherichia coli (ATCC® 10536™)	22	16
Klebsiella pneumoniae (ATCC® 700603™)	15	15
Pseudomonas aeruginosa (ATCC® 27853™)	18	13
Escherichia coli (ATCC® 35218™)	16	22
Neisseria gonorrhoeae (ATCC® 19424™)	15	16
Fungus		
Candida albicans (ATCC® 10231™)	17	18

* Diameter of the zone of inhibition was determined using the agar well diffusion method.

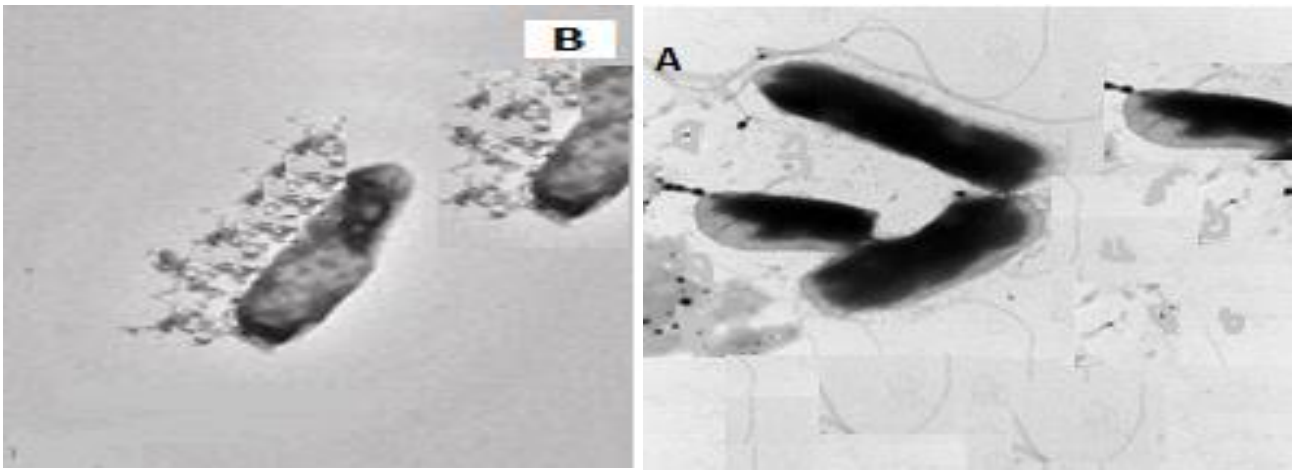

Figure 6. Transmission electron microscopy of *Escherichia coli* (ATCC® 10536™) before **(A)** and after treatment **(B)** with biogenic Ag-NPs.

Morphology and structure of *Escherichia coli* (ATCC® 10536™) after treatment with Ag-NPs by transmission electron microscopy

After treatment with Ag-NPs for 12 hours, the bacteria of *E. coli* (ATCC® 10536™) were collected for structural and morphological examinations by TEM. Ag-NPs-treated bacteria showed that the cell wall became significantly thin or even disappeared, crumpled, and released the cell contents (Figure 6B), in contrast to the untreated bacteria, which indicated the intact morphology of rod-shaped bacteria (Figure 6A).

DISCUSSION

Synthesis of metallic NPs has been established by microorganisms which are considered as potential bio-factories (Mukherjee et al., 2002). Cell-free culture of *Lactobacillus* is the easiest and simplest technique used for the synthesis of size-controlled Ag-NPs (Kalimuthu et al., 2008). Most organisms cannot synthesize Ag-NPs, only the bacteria which contain the "silver resistance machinery" can create Ag-NPs. The mechanism of production of Ag-NPs by culture filtrate of bacteria is not entirely understood. However, it is hypothesized that the enzymes produced by microorganism as reductase and nitrate reductase have a great role in the bio-reduction of silver ions to Ag-NPs (Duran et al., 2005). Nicotinamide adenine dinucleotide (NADH) and NADH-dependent enzymes play an important role in the biogenic synthesis of nanomaterials. The reduction is induced by electron transport from NADH by NADH-dependent reductase as an electron carrier (Thakkar et al., 2010). *Lactobacillus* can produce nitrate reductase at pH above 6 which causes biological reduction of Ag+ to Ag and creation of Ag-NPs (Ranganath et al., 2012). *Lactobacillus* species have the

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ability to secrete the aldehydic group through additional polysaccharides which are responsible for the reduction of Ag+ to Ag (Durán et al., 2011).

Addition of $AgNO_3$ to the supernatant of *Lactobacillus* bacteria produced Ag-NPs (at pH 8.3) and a reddish-brown coloration was obtained as a result of stimulation of surface plasmon resonance (SPR) of Ag-NPs (Sarvamangala et al., 2013; Natarajan et al., 2014; Sreedevi et al., 2015). The synthesis of Ag-NPs was confirmed by UV-Vis spectroscopy with collective excitation of conduction electrons in the metal determining the absorption band of the formed Ag-NPs which was at 410 nm (Figure 1). This absorption peak was also reported in Ag-NPs biosynthesized by *Neurospora crassa* (Longoria et al., 2011), *B. licheniformis* (Kalimuthu et al., 2008), *Aspergillus flavus* (Vigneshwaran et al., 2007), *Aeromonas* sp. SH10 (Saifuddin et al., 2009), *Bacillus subtilis* (Mouxing et al., 2006), and marine *Lactobacillus* species (Matei et al., 2015). The results of TEM indicated that the diameter of Ag-NPs biosynthesized from culture filtrates of both *Lactobacillus* species was approximately 5-40 nm that is in concordance with previous studies (Kalimuthu et al., 2008; Sintubin et al., 2009). The examination confirmed the crystalline nature of SAED patterns with clear lattice borders (Figure 4) as reported before (Gannimani et al., 2014).

Rajesh et al. (2014) reported that the average crystal size of Ag-NPs synthesized by *Lactobacillus acidophilus* culture filtrate, determined using the Scherrer equation, was 33 nm. They also found that Ag-NPs had a unique diffraction peak of metallic FCC silver phase at 38.31°, 44.41°, 64.58°, and 77.52° in 2θ.

Ag-NPs biosynthesis using bacteria is influenced by different factors, such as strain type, physical circumstances (including pH), temperature, time, concentration, and the type of metallic salt (Srivastava and Constanti, 2012). Many studies have been implemented to test the antibacterial activity of silver ions and zero-valent Ag-NPs and to apply them in various products, including bio-medical products for the cure of burns and bacterial diseases. Ag-NPs have a greater affinity to react with the cell surface than the silver ions. Moreover, the large surface area of nanoparticles permits their functionalization with different targets, such as antibodies and particular cell types, and prevents the growth of affected cells without upsetting the normal cells. The toxicity of Ag-NPs was studied in numerous studies and found that the production of reactive oxygen species (ROS) were the main causes of toxicity (Agnihotri et al., 2014). Ag-NPs destroy the bacterial cell membrane by endocytosis, penetrate the cells, and interact with glutathione or proteins to generate hydroxyl radicals or ROS. The generated ROS destruct the DNA and prevent the growth of the bacterial cell.

Commonly, the antibacterial effect of Ag-NPs depends on different factors, including their diameter, shape, and surface chemistry (Kora and Sashidhar, 2015; Wang et al., 2015). Bao et al. (2011) investigated the antibacterial activity of Ag-NPs depending on their size ranging from 10 to 100 nm and reported that nanoparticles of 10 nm presented a potent effect. They also mentioned that *E. coli* MTCC 443 and *S. aureus* NCIM 5201 were resistant to Ag-NPs regardless of their size. Moreover, the surface charge of nanoparticles affects efficiently the antibacterial activity of Ag-NPs. Kora and Sashidhar (2015) reported the antibacterial activity of Ag-NPs, synthesized using two plants (gum olibanum and gum ghatti), against different bacteria and found that the antibacterial activity of the Ag-NPs synthesized with gum olibanum was less than that of nanoparticles synthesized with gum ghatti. A bulk of studies have indicated that Ag-NPs are hopeful applicants for the treatment of bacterial diseases although Ag-NPs have an adverse effect on human health when used in high amounts due to high cytotoxicity (Agnihotri et al., 2014).

In the current study, there was a variation in the antibacterial efficiency of Ag-NPs between Gram-positive and Gram-negative bacteria resulting from the variance in the composition of their cell walls (Table 2). Inhibition zones of Ag-NPs biosynthesized from the culture filtrate of *L. plantarum* were 16-18 mm while those of Ag-NPs biosynthesized from the culture filtrate of *L. brevis* were 16-21 mm against Gram-positive bacteria. At the same time, the inhibition zones of Ag-NPs biosynthesized from the culture filtrate of *L. plantarum* were 16-22 mm while those of Ag-NPs biosynthesized from culture filtrate of *L. brevis* were 13-22 mm against Gram-negative bacteria.

Gram-positive bacteria are more resistant to the antibacterial action of Ag-NPs due to the presence of a denser cell wall than Gram-negative bacteria (Yin et al., 2015). Moreover, the lipopolysaccharides of the cell wall of Gram-negative bacteria have a greater negative charge than Gram-positive bacteria, which stimulates the adhesion of Ag-NPs, causing the sensitivity of Gram-negative bacteria to Ag-NPs (Bonnet et al., 2015). Electrostatic interaction between positively charged NPs and negatively charged bacterial cells is significant for the efficiency of nanoparticles as antibacterial materials (Kim et al., 2009).

Ag-NPs biosynthesized using culture filtrates of *Lactobacillus* spp. showed the inhibition zones of 17-18 mm against *C. albicans* due to the ability of Ag-NPs to adhere and saturate the fungal hyphae (Kim et al., 2009). It was also suggested that Ag-NPs cause damage to the cell membrane and subsequently prevent the ordinary budding route (Kim et al., 2009; Chadek et al., 2011; Elgorban et al., 2016). Bocate et al. (2019) studied the antifungal activity of Ag-NPs synthesized from fungi (*Fusarium oxysporum*) against three toxigenic species belonging to the genera *Aspergillus* (*Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus parasiticus*) and found that these nanoparticles can destruct the cell membrane and cause the cell death. Matei et al. (2015) reported that Ag-NPs biosynthesized from the cell-free supernatant of *L. plantarum* showed antifungal potency against the spoilage strains of fungi belonging to *Aspergillus*, *Fusarium*, and *Penicillium* genera and visual microscope investigation indicated hyphal changes of *Fusarium*.

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The effect of Ag-NPs on *E. coli* (ATCC® 10536™) was studied and morphological changes were investigated using TEM. The untreated bacteria indicated the intact morphology of rod-shaped bacteria. TEM images demonstrated that Ag-NPs were distributed and located at the surface of the plasma membrane of the bacterial cells. This could be due to the destruction of the plasma membrane and cell wall as a result of protein peroxidation and inactivation of membrane lipids, which causes membrane permeability resulting in potassium outflow, which was supported by the findings of a study conducted by Gopinath et al. (2017). The binding force between the bacteria and metal particles is dependent on the surface area of interaction. Sondi and Salopek-Sondi (2004) reported the action mode of the penetration of Ag-NPs nanoparticles into *E. coli* and studied their effects on the bacterial plasma membrane structure could increase the membrane permeability and the entrance of Ag-NPs inside the bacterial cell. That resulted in DNA damages and alteration of the bacterial membrane, which may affect bacterial metabolism, including bacterial respiration, resulting in cell death as a consequence of the consumption of ATP (El-Naggar et al., 2017; Sur et al., 2018; Dakshayani et al., 2019; Hamouda et al., 2019). Li et al. (2008) investigated the antibacterial effect of Ag-NPs on *E. coli* by TEM and found great damage to the bacterial cell membrane and inhibition of the function of some enzymes.

CONCLUSION

The current study established a modest method to prepare Ag-NPs from 1 mM AgNO3 using cell-free supernatant of lactic acid-producing bacteria. The diameter of biosynthesized Ag-NPs ranged from 5 to 40 nm and the form was spherical or polyhedral. The extracellular production of Ag-NPs using cell-free supernatant of *L. plantarum* and *L. brevis* is a safe, non-toxic, and cost-effective substitute to the toxic and expensive chemical and physical methods. These biosynthesized Ag-NPs from biological sources represent potential biomedical applications of promising antibacterial agents.

DECLARATIONS

Authors' contribution

Sohier M. Syame contributed to the planning of ideas, funding, and management of the research, laboratory work, results, and writing of the original draft. Asmaa S. Mansour participated in the laboratory work, writing, reviewing, and editing the final version of the manuscript. Doaa D. Khalaf, E. S. Ibrahim, and Gaber E. S. contributed to the practical part of the research.

Competing interests

The authors have not declared any conflict of interest.

REFERENCES

Agnihotri S, Mukherji S, and Mukherji S (2014). Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. Royal Society of Chemistry Advances, 4: 3974–3983. DOI: https://www.doi.org/10.1039/C3RA44507K

Bao Q, Zhang D, and Qi P (2011). Synthesis and characterization of silver nanoparticle and graphene oxide nanosheet composites as a bactericidal agent for water disinfection. Journal of Colloid and Interface Science, 360(2): 463–470. DOI: https://www.doi.org/10.1016/j.jcis.2011.05.009

Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP, and Rai MK (2009). Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Letters in Applied Microbiology, 48: 173–179. DOI: https://www.doi.org/10.1111/j.1472-765X.2008.02510.x

Bocate KP, Reisa GF, Souzaa PC, Juniora AGO, Duránb N, Nakazatoa G, Furlanetoa MC, Almeidaa RS, and Panagioa LA (2019). Antifungal activity of silver nanoparticles and simvastatin against toxigenic species of *Aspergillus*. International Journal of Food Microbiology, 291: 79-86. DOI: https://www.doi.org/10.1016/j.ijfoodmicro.2018.11.012

Bonnet M, Massard C, Veisseire P, Camares O, and Awitor KO (2015). Environmental toxicity and antimicrobial efficiency of titanium dioxide nanoparticles in suspension. Journal of Biomaterials and Nanobiotechnology, 6(3): 213–224. DOI: https://www.doi.org/10.4236/jbnb.2015.63020

Buszewski B, Railean-Plugaru V, Pomastowski P, Rafinska K, Szultka-Mlynska M, Golinska P, Wypij M, Laskowski D, and Dahm H (2018). Antimicrobial activity of biosilver nanoparticles produced by a novel *Streptacidiphilus durhamensis* strain. Journal of Microbiology, Immunology and Infection, 51(1): 45-54. DOI: https://www.doi.org/10.1016/j.jmii.2016.03.002

Chadek G, Mertas A, and Barszczewska-Rybarek I (2011). Antifungal activity of denture soft lining material modified by silver nanoparticles: a pilot study. International Journal of Molecular Sciences, 12(7): 4735–4744. DOI: https://www.doi.org/10.3390/ijms12074735

Charannya S, Duraivel D, Padminee K, Poorni S, Nishanthine C, and Srinivasan MR (2018). Comparative evaluation of antimicrobial efficacy of silver nanoparticles and 2% chlorhexidine gluconate when used alone and in combination assessed using agar diffusion method: An *in-vitro* study. Contemporary Clinical Dentistry, 9(S2): 204–209. DOI: https://www.doi.org/10.4103/ccd.ccd_869_17

Chaudhari PR, Masurkar SA, Shidore VB, and Kamble SP (2012). Antimicrobial activity of extracellularly synthesized silver nanoparticles using *Lactobacillus* species obtained from VIZYLAC capsule. Journal of Applied Pharmaceutical Science, 2(3): 25-29. DOI: https://www.doi.org/10.7324/JAPS.2012.2305

Dakhil AS (2017). Biosynthesis of silver nanoparticle (AgNPs) using *Lactobacillus* and their effects on oxidative stress biomarkers in rats. Journal of King Saud University – Science, 29: 462-467. DOI: https://www.doi.org/10.1016/j.jksus.2017.05.013

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Dakshayani SS, Marulasiddeshwara MB, Sharath Kumar MN, Golla R, Raghavendra Kumar P, Devaraja S, and Hosamani R (2019). Antimicrobial, anticoagulant and antiplatelet activities of green synthesized silver nanoparticles using Selaginella (Sanjeevini) plant extract. International Journal of Biological Macromolecules, 131: 787–797. DOI: https://www.doi.org/10.1016/j.ijbiomac.2019.01.222

Dong Y, Zhu H, Shen Y, Zhang W, and Zhang L (2019). Antibacterial activity of silver nanoparticles of different particle size against *Vibrio Natriegens*. PLoS One, 14(9): 1-12. DOI: https://www.doi.org/10.1371/journal.pone.0222322

Duran N, Marcato PD, De Souza GIH, Alve OL, and Esposito E (2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. Journal of Nanobiotechnology, 3: 1-7. DOI: https://www.doi.org/10.1186/1477-3155-3-8

Durán N, Marcato PD, Durán M, Yadav A, Gade A and Rai M (2011). Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi, and plants. Applied Microbiology and Biotechnology, 90: 1609-1624. DOI: DOI 10.1007s00253-011-3249-8.pdf

Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, and Lara HH (2005). Interaction of silver nanoparticles with HIV-1. Journal of Nanobiotechnology, 3(6): 1-10. DOI: https://www.doi.org/1477-3155-3-6.pdf

Elgorban AB, El-Samawaty AM, Yassin MA, Sayed SR, Adil SF, Elhindi KM, Bakri M, and Khan M (2016). Antifungal silver nanoparticles: synthesis, characterization and biological evaluation. Biotechnology & Biotechnological Equipment, 30(1): 56-62. DOI: https://www.doi.org/10.1080/13102818.2015.1106339

El-Naggar NE, Hussein MH, and El-Sawah AA (2017). Bio-fabrication of silver nanoparticles by phycocyanin, characterization, in vitro anticancer activity against breast cancer cell line and in vivo cytotxicity. Scientific Reports, 7: 10844-10863. DOI: https://www.doi.org/10.1038s41598-017-11121-3.pdf

Gannimani R, Perumal A, Krishna SB, Mathusamy SK, Mishra A, and Govender P (2014). Synthesis and antibacterial activity of silver and gold nanoparticles produced using aquous seed extract of *Protorhus longifolia* as a reducing agent. Digest Journal of Nanomaterials and Biostructures, 9(4): 1669. Available at: https://www.researchgate.net/publication/271367739

Gopinath V, Priyadarshini S, Oke MF, Arunkumar J, Marsili E, MubarakAli D, Velusamy P, and Vadivelu J (2017). Biogenic synthesis, characterization of antibacterial silver nanoparticles and its cell cytotoxicity. Arabian Journal of Chemistry, 10(8): 1107-1117. DOI: https://www.doi.org/10.1016/j.arabjc.2015.11.011

Hamouda RA, Hussein MH, Abo-elmagd RA, and Bawazir SS (2019). Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*. Scientific Reports, 9: 13071-13087. DOI: https://www.doi.org/10.1038/s41598-019-49444-y

Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, and Piddock LJV (2016). Understanding the mechanisms and drivers of antimicrobial resistance. Lancet, 38: 176–187. DOI: http://www.dx.doi.org/10.1016/S0140-6736(15)00473-0

Inoue Y, Uota M, Torikai T, Watari T, Noda I, and Hotokebuchi T (2010). Antibacterial properties of nanostructured silver titanate thin films formed on a titanium plate. Journal of Biomedical Material Research, 92A (3): 1171–1180. DOI: https://www.doi.org/10.1002/jbm.a.32456

Jain D, Daima HK, Kachhwaha S, and Kothari S (2009). Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. Digest Journal of Nanomaterials and Biostructures, 4: 557–563. Available at: https://www.researchgate.net/publication/215790280

Kalimuthu K, Babu RS, Venkataraman D, Mohd B, and Gurunathan S (2008). Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids and Surfaces B. Biointerfaces, 65(1): 150–153. DOI: https://www.doi.org/10.1016/j.colsurfb.2008.02.018

Khan AK, Rashid R, Murtaza G, and Zahra A (2014). Gold nanoparticles: Synthesis and applications in drug delivery. Tropical Journal of Pharmaceutical Research, 13(7): 1169-1177. DOI: https://www.doi.org/10.4314/tjpr.v13i7.23

Kim KJ, Sung WS, Suh BK, Moon SK, Choi JS, Kim JG, and Lee DG (2009). Antifungal activity and mode of action of silver nanoparticles on *Candida albicans*. Biometals, 22: 235–242. DOI: https://www.doi.org/10.1007/s10534-008-9159-2

Kora AJ, and Sashidhar RB (2015). Antibacterial activity of biogenic silver nanoparticles synthesized with *gum ghatti* and *gum olibanum*: a comparative study. Journal of Antibiotics, 68(2): 88–97. DOI: https://www.doi.org/10.1038/ja.2014.114

Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, and Alvarez PJJ (2008). Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. Water Research, 42: 4591–4602. DOI: https://www.doi.org/10.1016/j.watres.2008.08.015

Li Y, Leung P, Yao L, Song QW, and Newton E (2006). Antimicrobial effect of surgical masks coated with nanoparticles. Journal of Hospital Infection, 62: 58–63. DOI: https://www.doi.org/10.1016/j.jhin.2005.04.015

Liao S, Zhang Y, Pan X, Zhu F, Jiang C, Liu Q, Cheng Z, Dai G, Wu G, Wang L et al. (2019). Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant *Pseudomonas aeruginosa*. International Journal of Nanomedicine, 14: 1469-1487. DOI: https://www.doi.org/10.2147/IJN.S191340

Longoria EC, Nestor ARV, and Borja MA (2011). Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus *Neurospora crassa*. Colloids and Surfaces B. Biointerfaces, 83: 42-48. DOI: https://www.doi.org/10.1016/j.colsurfb.2010.10.035

Matei A, Cornea CP, Matei S, Matei GM, Cogălniceanu G, and Rodino S (2015). Biosynthesis of silver nanoparticles using culture filtrates of lactic acid bacteria and analysis of antifungal activity. Digest Journal of Nanomaterials and Biostructures, 10(4): 1201-1207. Available at: https://www.researchgate.net/publication/283317480

Mathara JM, Schillinger U, Kutima PM, and Holzapfel WH (2004). Isolation, identification and characterization of the dominant microorganisms of Kule naoto: The Maasai traditional fermented milk in Kenya. International Journal of Food Microbiology, 94(3): 269-278. DOI: https://www.doi.org/10.1016/j.ijfoodmicro.2004.01.008

Morones JR, Elechiguerra LJ, Camacho A, Holt K, Kouri BJ, Ramirez TJ, and Yocaman JM (2005). The bactericidal effect of silver nanoparticles. Nanotechnology, 16: 2346–2353. DOI: https://www.doi.org/10.1088/0957-4484/16/10/059

Mouxing FU, Qingbiao LI, and Doaha S (2006). Rapid preparation process of silver nanoparticles by bioreduction and their characterization. Chinese Journal of Chemical Engineering, 14: 114-117. DOI: https://www.doi.org/10.1016/S1004-9541(06)60046-3

Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, and Sastry M (2002). Extracellular synthesis of gold nanoparticles by the fungus *Fusarium oxysporum*. Combining Chemistry and Biology, 3(5): 461-463. DOI: https://www.doi.org/10.1002/1439-7633(20020503)3:5<461::AID-CBIC461>3.0.CO;2-X

Natarajan K, Selvaraj J, and Amachandra V (2014). Microbial production of silver nanoparticles. Digest Journal of Nanomaterials and Biostructures, 5(1): 135-140. Available at: http://admin.umt.edu.pk/Media/Site/STD/FileManager/OsamaArticle/August2015/10august/135_Natarajan.pdf

Rajesh S, Dharanishanthi V, and Vinoth Kanna A (2014). Antibacterial mechanism of biogenic silver nanoparticles of *Lactobacillus acidophilus*. Journal of Experimental Nanoscience, 10(15): 1143-1152. DOI: https://www.doi.org/10.1080/17458080.2014.985750

Ranganath E, Rathod V, and Banu A (2012). Screening of Lactobacillus spp. for mediating the biosynthesis of silver nanoparticles from silver nitrate. Journal of Pharmacy, 2(2): 237-241. Available at: https://www.researchgate.net/publication/267802632

To cite this paper: Syame SM, Mansour AS, Khalaf DD, Ibrahim ES and Gaber ES (2020). Green Synthesis of Silver Nanoparticles Using Lactic Acid Bacteria: Assessment of Antimicrobial Activity. *World Vet. J.*, 10 (4): 625-633. DOI: https://dx.doi.org/10.54203/scil.2020.wvj75

Ratan ZA, Haidere MF, Nurunnabi M, Shahriar SM, Ahammad AJS, Shim YY, Reaney MJT, and Cho JY (2020). Green chemistry synthesis of silver nanoparticles and their potential anticancer effects. Cancers, 12(4): 855-880. DOI: https://www.doi.org/10.3390/cancers12040855

Saifuddin N, Wong CW, and Yasumira AAN (2009). Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. E-Journal of Chemistry, 6: 61-70. DOI: https://www.doi.org/10.1155/2009/734264

Sarvamangala D, Kondala K, Murthy USN, NarasingaRao B, Sharma GVR, and Satyanarayana R (2013). Biogenic synthesis of AgNP's using Pomelo fruit—characterization and antimicrobial activity against Gram +ve and Gram −ve bacteria. International Journal of Pharmaceutical Sciences, 19(2): 30–35. Available at: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.403.6771&rep=rep1&type=pdf

Shehzad A, Qureshi M, Jabeen S, Ahmad R, Alabdalall AH, Aljafary MA, and Al-Suhaimi E (2018). Synthesis, characterization and antibacterial activity of silver nanoparticles using *Rhazya stricta*. PeerJ, 6: 1-15. DOI: https://www.doi.org/10.7717/peerj.6086

Sintubin L, De Windt W, Dick J, Mast J, van der Ha D, Verstraete W, and Boon N (2009). Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Applied Microbiology and Biotechnology, 84: 741–749. DOI: https://www.doi.org/10.1007/s00253-009-2032-6

Sondi I, and Salopek-Sondi B (2004). Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. Journal of Colloid and Interface Science, 275(1): 177–182. DOI: https://www.doi.org/10.1016/j.jcis.2004.02.012

Song JY, and Kim BS (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess and Biosystems Engineering, 32(1): 79-84. DOI: https://www.doi.org/10.1007/s00449-008-0224-6

Sreedevi TP, Thilagam M, Tamil Selvi A, and Chandrasekaran B (2015). Synthesis, Characterization and Antibacterial studies of silver nanoparticles using *Lactobacillus plantarum*; World Journal of Pharmaceutical Research, 4(8): 1757-1773. Available at: https://www.researchgate.net/publication/309321910

Srivastava SK, and Constanti M (2012). Room temperature biogenic synthesis of multiple nanoparticles (Ag, Pd, Fe, Rh, Ni, Ru, Pt, Co, and Li) by *Pseudomonas aeruginosa* SM1. Journal of Nanoparticle Research, 14(4): 831-840. DOI: https://www.doi.org/10.1007/s11051-012-0831-7

Sur UK, Ankamwar B, Karmakar S, Halder A, and Das P (2018). Green synthesis of silver nanoparticles using the plant extract of Shikakai and Reetha. Materials Today Proceedings, 5: 2321–2329. DOI: https://www.doi.org/10.1016/j.matpr.2017.09.236

Thakkar KN, Mhatre SS, and Parikh RY (2010). Biological synthesis of metallic nanoparticles. Nanomedicine Nanotechnology Biology and Medicine, 6(2): 257-262. DOI: https://www.doi.org/10.1016/j.nano.2009.07.002

Vigneshwaran N, Astaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, and Balasubramanya RH (2007). Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Materials Letters, 61: 1413-1418. DOI: https://www.doi.org/10.1016/j.matlet.2006.07.042

Wang Z, Xia T, and Liu S (2015). Mechanisms of nanosilver-induced toxicological effects: more attention should be paid to its sublethal effects. Nanoscale, 7(17): 7470-7481. DOI: https://www.doi.org/10.1039/c5nr01133g

Yin R, Agrawal T, Khan U, Gupta GK, Rai V, Huang YY, and Hamblin MR (2015). Antimicrobial photodynamic inactivation in nanomedicine: small light strides against bad bugs. Nanomedicine, 10(15): 2379-2404. DOI: https://www.doi.org/10.2217/nnm.15.67

To cite this paper: Syame SM, Mansour AS, Khalaf DD, Ibrahim ES and Gaber ES (2020). Green Synthesis of Silver Nanoparticles Using Lactic Acid Bacteria: Assessment of Antimicrobial Activity. *World Vet. J.*, 10 (4): 625-633. DOI: https://dx.doi.org/10.54203/scil.2020.wvj75

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 634-637, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wv76

Urinary Bladder Stone Removal Surgery in Sulcata tortoise (*Geochelon sulcata*) with Lateral Plastron Osteotomy Technique

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ORIGINAL ARTICLE
pii: S232245682000076-10
Received: 29 Oct 2020
Accepted: 20 Dec 2020

ABSTRACT

This paper aimed to examine the management of urinary calculi of a sulcata tortoise. A 5-year-old Sulcata tortoise (*Geochelon sulcata*) was presented with a history of dehydration, loss of appetite, and lameness. Clinical signs and radiographic examination indicated urinary calculi in the urinary bladder. Radiographic results revealed that there was a radiopaque urinary calculi mass. Plastron osteotomy and cystotomy techniques were used to remove urinary calculi. The appetite of the tortoise returned to normal in a week after the surgery. The lateral plastron is an appropriate osteotomy technique, especially for the immediate opening of the plastron with a stone-filled bladder. This was a safe area to open plastron since it was far from the heart. This method was not beneficial for the tortoise in their infancy since it would interrupt the development of plastron formation resulting in the postoperative asymmetrical plastron structure.

Keywords: Urinary calculi, Cystotomy, Plastron osteotomy, Sulcata tortoise

INTRODUCTION

Urinary calculi are solid calculi primarily found in the urinary bladder while regularly calcified, it forms non-calcific material. Tortoise urinary calculi are mainly composed of urates, a mixture of uric corrosive and metals, such as sodium, potassium, or calcium (Wright, 2008). Uroliths have been reported in amphibians, lizards, turtles, and snakes. These are mainly due to the accumulation of uric corrosive and no entry into water supplies leading to tortoise dehydration (Girolamo and Mans, 2016).

Cystotomy is a surgical incision into the bladder for the expulsion of urinary calculi, which is of utmost importance for a tortoise and might be huge. Depending on the organisms, surgical approaches to the coelom differ widely. Two methodologies of turtle coelom surgeries are plastron osteotomy and coeliotomy of the femoral fossa (Mitchell and Tully, 2008). The most suitable choice depends on the species and the exciting organ. Appropriate preparation of the reptile patient for coeliotomy is mandatory. Coeliotomy using the transplastron coeliotomy or plastron osteotomy approach allows the manipulation of the bladder through which the plastron is opened to approach the bladder in the caudoventral aspect of the coelomic cavity (Mitchell and Tully, 2008). Some of the techniques that have been used by (Girolamo and Mans, 2016) are plastron perforations in the central part. In some cases, it is important to consider the exposed plastron due to the surrounding veins and the heart b. When the plastron opens in the horizontal zone it reveals vesica filled with stones. Most of the innovative surgical techniques, as in another medial area, are described in papers, case series, or other empirical studies. This paper discusses the advantages and disadvantages of lateral plastron osteotomy techniques.

MATERIALS AND METHODS

A sulcata tortoise (Geochelon sulcata) was referred to the Out-Patient Clinics of DRD Veterinary in Surabaya, Indonesia, with a history of dehydration, loss of appetite, and lameness. Based on the clinical symptoms, an X-ray examination was performed (Figure 1) and a dorsoventral radiograph was taken. The results revealed an enhanced radioopacity at the middle-left of the coelomic cavity indicating urinary calculi. The administered general anesthetic drugs included a combination of tiletamine and zolazepam with a dose of 10 mg/kg body weight (Zoletil® from France). The size of the incision usually depends on the urinary calculi size observed in the radiography interpretation.

For a plastron osteotomy, the tortoise was positioned in dorsal recumbency (Figure 2A). The opening of the plastron was performed through the lateral abdominal shields using a circular mini grinding saw for home use. The entry

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point was rectangular with a 45° tendency. It was inundated with saline during the plastron osteotomy (Alworth et al., 2011). Firstly, the two lateral cuts were made, and a pincet was used to ensure the maximum thickness of the cuts, and the shell was pierced over the core. Using a forceps clamp, the cranial edge of the plastron was raised to reveal the muscle. To separate the plastron osteotomy segment from the underlying soft tissue, blunt dissection was used (Divers, 2012). The paired abdominal veins were closely connected with the plastron, and careful removal of the flap periosteum was required to maintain these vessels with the rest of the soft tissues (Figure 2B). The caudal soft tissue connections could be left unblemished as the plastron fold was reflected caudally and secured with ordinary saline. Stay suture was regularly utilized to forestall coelomic tainting by nonsterile urine, and liquid substance was suctioned before the entry (Figure 3).

The dorsal perspective was incised on the urinary bladder with sharp blade number 10. In the next step, urinary calculi were removed from the body of the tortoise. The bladder was flushed by sterile saline and closed with a clear continuous pattern using the absorbable suture. The coelomic membrane was closed in a continuous pattern using Safil®, Braun Surgical, Spain (Stephen and Scott, 2019). The recovery technique for the plastron legitimately relied on the quality of the repair. Precise reduction of the hard segment and plastron can increase the likelihood of recovery from the first or second target. Nonetheless, the plastron fold becomes a sequestrum that gives the fresh bone producing underneath a brief assurance (Girolamo and Mans, 2016).

The shell was thoroughly washed with an alcohol swab to remove any grease before using epoxy resin to seal the region where antibiotic powder and powder gips were added. In this regard, it was highly important to prevent infection and accelerate the healing process (Rodrigues et al., 2015). During 12-18 weeks, the recovery of plastron was observed. The coating material (i.e. epoxy resin) remained in place for 6-12 months before removal. The recuperate of the plastron bone fold could differ during the first two years.

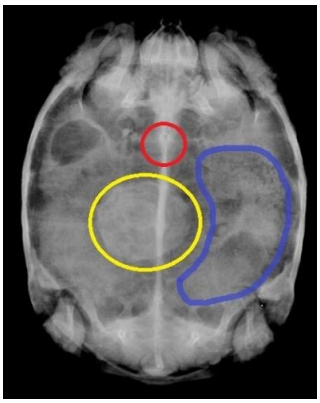

Figure 1. Dorsoventral radiographic image. Radiography interpretation of urinary calculi in vesica urinaria. An extensive mineral, opaque, rounded structure is seen in the mid-left lateral coelom (Yellow: urinary calculi, blue: large intestine, red: hearth).

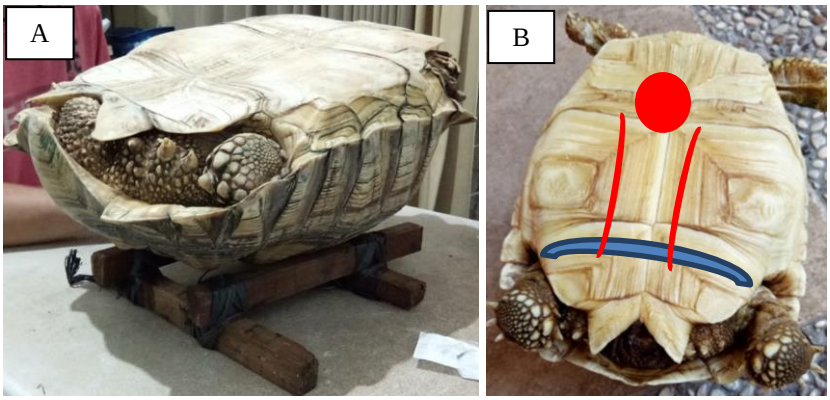

Figure 2. Tortoise is positioned in dorsal recumbency. **A:** Preparation before surgery. **B**: View of the centrum of a tortoise illustrating the position of the heart (red circle), paired ventral abdominal veins (red lines), caudal plastron hinge (blue line).

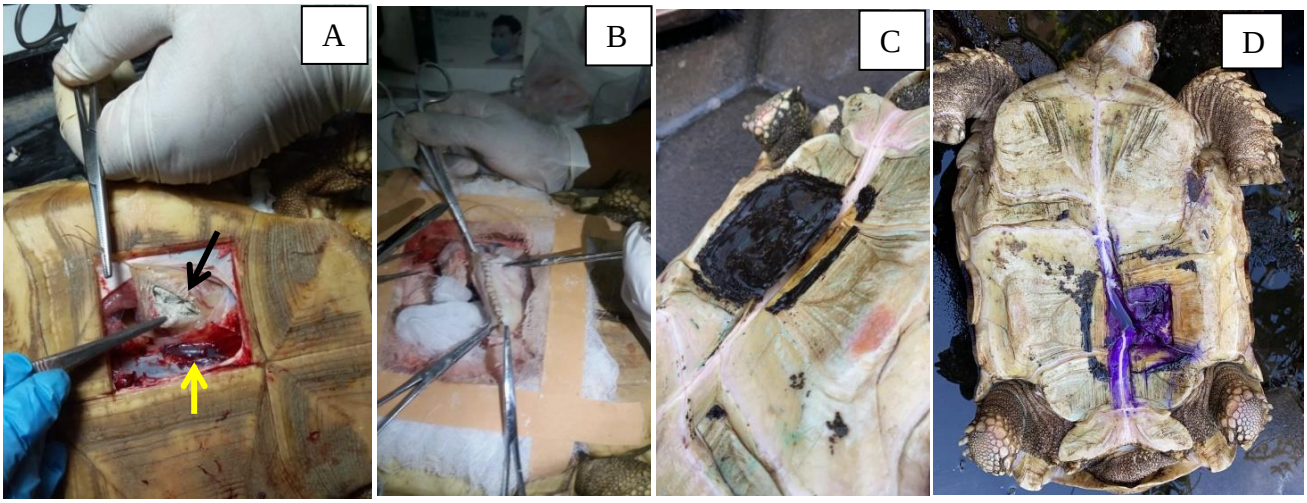

Figure 3. Plastron osteotomy in tortoises. **A:** The yellow arrow shows the abdominal vein, and the black arrow shows urinary calculi still inside of vesica urinaria. **B**: Closed vesica urinaria with simple continues the pattern. **C**: Final repair, repositioned, and held in place by epoxy plastron flap. **D**: An asymmetric plastron was observed six months after surgery.

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Ethical approval

This article is not an experimental investigation. This experiment was performed according to all ethics and animal welfare in DRD Veterinary Animals Clinic in Surabaya, Indonesia.

RESULTS AND DISCUSSION

A tortoise is a type of animal that may experience urinary calculi. In the form of uric acid, reptiles excrete their nitrogenous waste. Uric acid is water-insoluble and thus water cannot be used to dissolve it. The process of dissolving uric acid occurs to prevent the loss of water in reptiles, in particular desert tortoise. This mechanism is the key factor in the formation of calculi in reptiles. Calculi formation starts with the degradation of the protein into a nucleic acid. The nucleic acid in the diet is then degraded to nucleotides by nucleases. These nucleotides undergo additional enzymatic hydrolysis for the processing of free purines and pyrimidine bases. The amino acid is synthesized in the liver with additional purines and pyrimidine bases. If the body does not reuse these free bases, they are further depleted and eventually removed. The pyrimidines are catabolized to give the final products (CO2 and NH3). Purines in reptiles may undergo further breakdowns. Purine is degraded into guanine and adenine. Adenine is converted to hypoxanthine and converted by xanthine oxidase into xanthine and ultimately converted by xanthine oxidase into uric acid. Guanine is immediately transformed into xanthine and then converted into uric acid. In the bladder, uric acid is stored and combined with other cations, such as potassium, resulting in urate precipitation. In the end, the prolonged accumulation of urate salt contributes to calculi formation (Amat et al., 2012).

Calculi plans may be forestalled. The arrangement of calculi is significantly influenced by the tortoise's hydration status. Access to a clean and shallow container of water should be given to the tortoise (Amat et al., 2012) by supplying the tortoise with fresh water sources, such as ponds, or spraying water on their body. For drinking, urination, and even defecation, tortoises require water. In case of dehydration, the tortoise automatically holds their urine helping them to prevent further loss of water. Long accumulation of urine can ultimately contribute to the formation of calculi. Moreover, the tortoise diet should not include a high level of protein.

High protein diets contribute to further protein breakdown and formation of uric acid, which is the key component for the formation of calculi (Miller and Eric, 2015). The tortoise should be fed with vegetables and grass to have a balanced diet and enough nutrients. The diet should be rich in fiber and minerals, such as calcium, vitamin A, and vitamin D, in particular the calcium-phosphorus ratio, which is one of the components that contributes to the development of calculi with an average calcium-phosphorus ratio of 2:1 (McArthur et al., 2004).

To establish a correct diagnosis, it is important to have the history and x-ray examination of the tortoise. Radiography is necessary to confirm the diagnosis, especially in tortoise (Azlan et al., 2015). The dorsoventral view was taken on the radiograph. The radiograph is adequately clear in this case to diagnose that the urinary calculi were radiopaque. There are some methods to extract the urinary calculi, including cystotomy, but in tortoise, opening the plastron is necessary, which is plastron osteotomy (Mitchell and Tully, 2008). The most popular procedure is plastron osteotomy as it provides access to the bladder. Prolonged recovery and rehabilitation are frequently associated with this invasive procedure. The non-invasive technique is designed to extract urinary calculus under sedation or anesthesia through the vent with forceps. The drawback of this approach involves instances where the calculus is stuck within the pelvic region or where forceps will imagine the calculus and palpate it directly from the vent (Amat et al., 2012).

In the present case, the lateral plastron osteotomy technique is used. These strategies approached with negligible obtrusiveness as well as easy and straight forward endurance rates into the coelomic cavity. In the current study, plastron osteotomy was conducted, however, the surgeon's preference was to have more full access to the coelomic cavity (Keller and Weber, 2015). In comparison with medial plastron osteotomy technique, the solution to the lateral plastron osteotomy procedure can be even safer for the urinary bladder to conduct since there is less contact with the vein and the heart is not exposed. It is not advised to ligate both abdominal veins due to the possibility of serious circulatory disturbance (Stephen and Schot, 2019).

After six months of post-operation, the plastron structure was asymmetric due to the growth phase of tortoise plastron. The new growth of the plastron was not balanced between the right and left side since the incision was made in the lateral region. Although the shape of the plastron was not asymmetrical, behavior and appetite seemed to be good.

CONCLUSION

The proper surgical treatment for tortoise urinary calculi is a cystotomy performed using a lateral plastron osteotomy technique. This technique can only be conducted over the urinary calculi region so that the urinary bladder is much simpler to perform. There was a negligible contact of the vein in the present study and the heart was not exposed. This technique can cause asymmetrical plastron structure in the future. In a tortoise that reached to the limitation of growth, this technique can be used.

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DECLARATIONS

Authors' contribution

DAK.Sari is owner of DRD Veterinary Clinic. DAK.Sari and D.Apritya wrote the manuscript. D.Apritya checked and edited the final draft of the article. Both authors confirmed the final form of the manuscript for publication in the journal.

Competing interests

The authors have not declared any conflict of interest.

Acknowledgments

This research was funded by DRD Veterinary Clinic, Indonesia. The authors would like to acknowledge their gratitutde to Yayasan Wijaya Kusuma and Wijaya Kusuma Surabaya University.

REFERENCES

Alworth LC, Hernandez SM, and Divers SJ (2011). Laboratory Reptile Surgery: Principles and Techniques. Journal of the American Association for Laboratory Animal Science, 50(1): 1-16. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3035398/

Amat AC, Gabriel B, and Chee NW (2012). Cystic calculi removal in African spurred Tortoise (*Geochelone sulcata*) using transplastron coeliotomy. Veterinary World, 5(8): 489-492. DOI : https://doi.org/10.5455/vetworld.2012.489-492

Azlan CA, Saw MH, Noorshimah R, Cheah EHC, Lam JL, and Jefri NM (2015). Plastron Osteotomy in The Management Of Fishing Hook Ingestion In a Malayan Box Turtle (*Cuora ambonensis*). Malaysia Veterinary Journal, 27(1): 12-15. Available at: https://storage.unitedwebnetwork.com/files/478/f53a87f4bfa28724caf0a6b0af6efbb1.pdf

Divers SJ (2012). An Introduction to Reptile Surgery: A Chance to Cut is a Change to Cure. Proceedings:NAVC Conference Small Animal. Available at : https://www.vetfolio.com/learn/article/an-introduction-to-reptile-surgery-a-chance-to-cut-is-a-chance-to-cur

Girolamo ND, and Mans C (2016). Reptile Soft Tissue Surgery. Veterinary Clinics of North America Exotic Animal Practice Journal, 19: 97-131. DOI: http://dx.doi.org/10.1016/j.cvex.2015.08.010

Keller KA, and Weber EPS (2015). Diagnosis and Treatment of urolithiasis in client-owned Chelonians:40 cases (1987-2012). Journal of The American Veterinary Medical Association , 247(6): 650-658. DOI : http://www.dx.doi.org/10.2460/javma.247.6.650

McArthur S, Wilkinson R, and Meyer J (2004). Medicines and surgery of tortoise and turtles. Blackwell publishing ltd, pp. 31-407. available at : https://vetbooks.ir/medicine-and-surgery-of-tortoises-and-turtles/

Miller I, and Eric R (2015). Fowler's Zoo and Wild Animal Medicine, Cap. 4 Chelonians (Turtles, Tortoises) Saunders, an imprint of Elsevier Inc, available at: https://www.sciencedirect.com/science/article/pii/B9781455773978000049?via%3Dihub

Mitchell M, and Tully TN (2008). Manual of Exotic Pet Practice. Saunders Elsevier, pp. 243-245. Available at: https://books.google.co.id/books?id=JMTUKwzPEvwC&pg=PA245&lpg=PA245&dq=plastron+osteotomy&source=bl&ots=R1rpkyDoOS&sig=ACfU3U0GDia3TU_uG8yfOumAGcWSx23Uhw&hl=en&sa=X&ved=2ahUKEwihgonP3dPqAhUVH7cAHVU5B08Q6AEwBXoECAkQAQ#v=onepage&q=plastron%20osteotomy&f=false

Rodrigues MC, Lima WC, Quessada AM, Silva FAN, Silva LMC, de Souza AB, de Moura CRC, and Lima DASD (2015). Celiotomy by plastrotomy in a yellow-footed tortoise (Geochelone denticulata). Pesquisa Veterinaria Brasileira, 35 (2): 173-176. DOI: https://doi.org/10.1590/S0100-736X2015000200014

Stephen JD, and Scott JS (2019). Mader's Reptile and Amphibian Medicine and Surgery- E-Book. Elsevier. Available at: https://www.sciencedirect.com/book/9780323482530/maders-reptile-and-amphibian-medicine-and-surgery

Wright K (2008). Clinical Management of Bladder Stones in Tortoises. NAVC Conference. Available at: https://www.cabi.org/isc/FullTextPDF/2009/20093019001.pdf

To cite this paper: Kartika Sari DA and Apritya D (2020). Urinary Bladder Stone Removal Surgery in Sulcata tortoise (*Geochelon sulcata*) with Lateral Plastron Osteotomy Technique. *World Vet. J.*, 10 (4): 634-637. DOI: https://dx.doi.org/10.54203/scil.2020.wv76

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 638-652, December 25, 2020
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2020.wv77

Incidence of Appendicular Bone Fracture in Dogs and Cats: Retrospective Study at Veterinary Hospital of Cairo University and some Private Clinics in Egypt

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ORGINAL ARTICLE
pii: S232245682000077-10
Received: 31 Oct 2020
Accepted: 21 Dec 2020

ABSTRACT

Appendicular bone fractures in small animal practice constitute a major challenge facing veterinary orthopedic surgeons concerning affected limb and bone as well as the extent of tissue damage, site, and shape of the fracture line. Therefore, this retrospective study was designed to provide descriptive data at referral veterinary teaching hospital, faculty of veterinary medicine, Cairo University, and some private pet clinics in Cairo district, Egypt to identify and determine the prevalence of appendicular fractures arising from trauma in dogs and cats treated from January 2017 to January 2020, and emphasizing the information that characterized the population (breed, age, gender, and animal size). The investigated fractures were classified according to the specific limb (forelimbs / hind limbs), specific bone fractures (Humerus, radius and ulna, femur, tibia and fibula, and the other bones), extent of tissue damage (open or closed and incomplete or complete), site (proximal, diaphyseal or distal zones), number (single or comminuted), and the direction of the fracture line (transverse, oblique or spiral). From the obtained data, it could be concluded that there was a high incidence of the appendicular long bones concerning the different bone fractures with significantly higher records in dogs, compared to cats. The highest records of fracture were in mongrel dogs, and cats as rescued animals. Excluding mongrel dogs and cats, the highest incidence of fracture-cases in dogs was recorded in Miniature breeds and svelte breeds for cats. Male dogs and cats showed a higher incidence than females. The bone fracture mostly occurred in dogs younger than one-year-old, and cats aged one to three years. A fracture in the hindlimbs was more significant than forelimbs with the highest incidence in femoral bone among both dogs and cats. The percentage of open fractures were more common in cats than dogs. Incomplete fractures were recorded more frequently in dogs than cats. In dogs, the most common fractures in the femur, tibia/fibula, humerus, and radius/ulna were complete comminuted diaphyseal femoral, complete oblique diaphyseal tibial/fibular, complete transverse distal humoral, and complete transverse diaphyseal radial/ulnar fractures respectively. Moreover, cats were complete transverse distal femoral, complete oblique diaphyseal tibial/fibular, complete spiral diaphyseal humoral, and complete transverse distal radial/ulnar fractures. In conclusion, appendicular bone fracture among dogs and cats referred to the veterinary teaching hospital, Cairo University and some private clinics in Egypt showed high incidence (87% in dogs and 71.8% in cats) out of total fracture cases and this incidence correlated with some predisposing factors (including breeds, weight, age, and gender) and causative agents that resulted in different types of appendicular fractures.

Keywords: Cat, Dog, Femur, Fracture, Orthopedic

INTRODUCTION

Orthopedic cases constitute a major percentage of surgery caseload in most of veterinary clinics and referral centers in different parts of the world (Appari et al., 2013). Bone fractures, especially in long bones, constitute a major problem in small animal practice particularly in dogs (Gadallah et al., 2009). Violent trauma with a vehicular accident or minimal trauma with a pathological condition such as neoplasia usually are the most common causes of fractures (Beale, 2004; Fossum, 2013). The diagnosis of fractures is based on a history of trauma and clinical signs. Radiography, ultrasonography, and histology have been used as the tools for fracture-healing assessment (Rrisselada et al., 2005). When regular serial radiographs are available, they aid in monitoring the progress of bone healing, and facilitate the decision-making procedure for the removal of orthopedic implants (Hobbs, 2012).

It is extremely important to carry out retrospective and prospective studies to determine the prevalence of the most common diseases in a given geographic region (Chaves et al., 2014). The incidence of bone fractures in dogs and cats have been reported in different regions of the world (Thengchaisri et al., 2006; Shiju et al., 2010; Minar et al., 2013; Rhangani, 2014; Elzomor et al., 2014; Libardoni et al., 2016 and Lovrić et al., 2020).

A standardized description of a fracture is an important issue as it directs the orthopedic surgeons to classifying it very cautiously, thereby to choose the proper method of reduction, fixation and immobilization. Classification of appendicular fractures was extensively discussed (Harari, 2002; Lanz, 2002; Piermattei et al., 2006; Shales, 2008a and 2008b). Fractures could be classified according to different aspects of cause; open or closed, extent of bone damage,

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number and position of fracture lines, direction, location, forces acting on the fracture, stability, degree of soft tissue damage and age of the fracture. According to the cause of the fracture, fractures are classified into intrinsic (muscular, pathological and stress) and extrinsic (external trauma). Regarding the extent of bone damage, fractures are classified into incomplete (greenstick, fissure and depressed) and complete. According to the number and position of the fracture lines, they are classified into simple, segmental and comminuted. According to the direction of fracture lines, they are classified into transverse, oblique and spiral. According to direction of fracture location, fractures are classified into diaphyseal (proximal, mid or distal), metaphyseal, articular, condylar and physeal (classified according to the salter-harris system). Regarding the forces acting on the fracture site, fractures are classified into avulsion, impaction, compression and displacement. According to the stability, they are classified into stable (interlocking fragments may have inherent stability and be suitable for external coaptation or conservative management) and unstable (fractures are usually unstable, and they require stabilization in order to facilitate the weight-bearing and healing). Regarding the degree of age of fracture, fractures are classified into recent (having sharp fracture edges) and old (rounded edges form after 10 to 14 days or there is the callus formation) (Shales, 2008a).

Knowing the types and frequency of fractures in domestic animals, the professionals in the area of orthopedics and veterinary physiotherapy can direct their attention to the improvement of fixation techniques, correction and stabilization of fractures, thus increasing the efficiency in the treatment and repair (Vidane et al., 2014). Fossum (2013) suggested a fracture-assessment scoring system which includes three different factors; mechanical, biological, and clinical. Mechanical factors include a reducibility of the fracture, patient size and weight, and if an injury or a disease is present on other limbs. Evaluation of mechanical factors helps to evaluate how strong the fracture fixation should be for the individual patient. Biological factors take into account the age and general health of the patient, extent of the soft tissue damage around the fracture both due to high-velocity injury and the surgeon's skills during fracture repair. The biological factors play a role in estimating the healing time of the fracture. Clinical factors help to assess the fracture healing during the postoperative period. It includes the patients' and owner's compliance, the activity level of the patient, and the comfort during the fracture healing. The three factors should all be taken into account when the prognosis of a fracture and fracture surgery are assessed. Fractures with high scores generally heal with lower complication risks, whereas fractures assigned lower scores one can expect a greater risk for complications.

This retrospective study aimed to provide descriptive data at referral veterinary teaching hospital, faculty of veterinary medicine, Cairo University, and some private pet clinics in Cairo district, Egypt to identify and determine the prevalence of dogs and cats with appendicular fractures arising from a trauma treated from January 2017 to January 2020, and emphasizing the information that characterized the population (breed, age, gender, and size).

MATERIALS AND METHODS

Ethical approval

All procedures (including data collections, patients' information recording at the referral veterinary teaching hospital, Cairo University and some private clinics in Egypt and radiographs interpretations) of the current study were approved by the Institutional Animal Care and Use Committee, Cairo University (Vet CU 20022020127).

Data collection

Data were collected from patient's medical records at referral veterinary teaching hospital, faculty of veterinary medicine, Cairo University, and some private pet clinics in Cairo district, Egypt. Searches were made on all fractures during the period from January 2017 to January 2020. Dogs and cats with fractures were confirmed by the history, clinical, orthopedic, and radiographic examinations, then were submitted to osteosynthesis surgical treatment which was not included in this study.

Patients' information

Basic information about the patients included breeds, weight, age, sex, and causative agent were recorded. All dog breeds were listed according to Federation Cynologique Nationale (FCI), the World Canine Organization. Breeds not recognized by FCI were categorized as mixed-breed. Regarding dogs, 34 breeds were admitted to the hospital and clinics, while fracture was recorded in 18 dog breeds (English Bullmastiff, Saint Bernard, German Shepherd, Golden Retriever, German Rottweiler, Labrador Retriever, Siberian Husky, Alaskan Husky, American Pitbull, Mongrel (Mixed-breed), Doberman Pinscher, Grand Griffon Vendèen, Petit Basset Griffon Vendèen, English Cocker Spaniel, American Cocker Spaniel, Yorkshire Terrier, Pomeranian, and Chihuahua). Regarding cats, six breeds were admitted to the hospital and clinics, while the fracture was recorded in five cat breeds (Persian, Mongrel (Mixed-breed), Himalayan, Siamese, and Egyptian Mau).

The dog breeds were categorized according to body size or weight into Giant (more than 45 kg), Large (22-45 kg), Medium (12-22 kg), Small (5-12 kg), and Mini or toy breeds (less than 5 kg) (American Kennel Club, 2006; Fogle, 2009).

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Cat breeds were categorized according to body conformation types to three general groups: cobby, such as the Persian and Himalayan cat breeds; svelte, such as the Siamese, Egyptian Mau, and Sphynx cat breeds, and moderate such as the mongrel cat breed. The cobby's body design is short and compact, deep-chested, and broad across the shoulders and rump. The head is also large and round, and the tail is often shorter and blunt at the tip. In contrast, the svelte type is very slim and lithe with long tapering lines. The head is narrow and forms a wedge shape, and the tail is usually long, slender, and pointed at the tip. The moderate lies are between these two types, and is neither cobby nor svelte; several breeds are moderate in conformation (Helgren, 2013). Dogs' and cats' gender were recorded to study the relations between the incidence of fracture and gender of affected cases. Regarding the age, the animals were distributed into four age groups: Juvenile, puppies or kitten (less than 1 year), young adult (1-3 years), mature adult (3-10 years), and elder (over 10 years old) according to the methodology used in a case study (Shearer, 2011). Data about different causes of fracture were collected from the owners to evaluate the relations between the incidence of fracture and their causes.

Establishment of the types and frequency of bone fractures' occurrence

Fractures were classified according to Shales (2008b):

Classification of bone fracture based on the body parts, the incidence of specific limb and specific bone fractures which includes

a- Incidence of specific limb (forelimbs / hind limbs) fractures

b- Incidence of specific appendicular bone fractures. Forelimb (Humerus, radius and ulna), Hindlimb (Femur, tibia and fibula.), and the number of the other bone fractures were recorded.

Frequency of the different types of appendicular bone fracture based on the extent of tissue damage, site, and shape of the fracture line

a- The extent of tissue damage: 1) Open or closed fracture; 2) Incomplete or complete fracture.

b- Fracture location: Proximal, diaphyseal or distal zones

c- Fracture line: 1) The number of fracture line (single or comminuted); 2) The direction of fracture line (transverse, oblique or spiral)

Statistical analysis

Data that were collected about age, sex, and breeds were collected, and they were added to the Microsoft Excel 2010R spreadsheet, stored separately and exported to analytical software using the Chi-square test. Values of < 0.05 were considered as statistically significant.

RESULTS

Patients' information

Breeds, weight, age, gender, and causative agent

Out of 4625 cases of dogs (table 1) and 3712 cats (table 2) admitted to the hospital and clinics, a total of 324 (7.01%) records of dogs and 149 (4.01%) of cats were diagnosed with fractures which were selected from X-ray records at referral veterinary teaching hospital, faculty of veterinary medicine, Cairo University and some private pet clinics in Cairo district, Egypt; covering the period from January 2017 to January 2020.

Regarding dog breeds, the fracture was recorded in 18 breeds. The distribution of appendicular fractures among the different breeds of dogs in the present study is presented in table 3. The most affected breeds were Mongrel (Mixed-breed, 61.74%), Pomeranian (22.22%), Chihuahua (13.33%), Yorkshire Terrier (7.41%), Siberian Husky (5.56%), Golden Retriever (5.36%), German Shepherd (5.19%), American Cocker Spaniel (4.08%), German Rottweiler (3.90%), Alaskan Husky (3.26%) and Grand Griffon Vendèen (3.26%). Regarding cat breeds, the fracture was recorded in five breeds. The distribution of appendicular fractures among the different breeds of cats in the currents study is presented in table 4. The most affected breeds were Mongrel (Mixed-breed, 14.01%), Siamese (3.17%), Persian (1.99%), Himalayan (1.85%), and Egyptian Mau (1.47%). According to the body size or weight, the dog breeds were categorized into five groups; Giant, Large, Medium, Small, and Mini breeds (Table 5). The most affected breed according to the size was medium (26.84%), followed by mini (13.33%), large (4.83%), small (2.73%), and giant (1.89%) breeds. According to the body conformation, the cat breeds were categorized into three groups; Cobby, Moderate, and Svelte breeds (Table 6). The most affected breed according to the body conformation was moderate (14.01%), followed by svelte (2.58%) and cobby (1.98%) breeds. Regarding the gender (table 7), the incidence of fractures in male dogs was 65.43% out of total fracture cases; 7.61% out of total admitted male dogs and 4.58% out of total admitted (male and female) dogs, and the incidence of fractures in female dogs was 34.57% out of total fracture cases; 6.08% out of total admitted female dogs and 2.42% out of total admitted (male and female) dogs. Meanwhile, in cats, the incidence of fractures in male cats was

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66.44% out of total fracture cases; 6.81% out of total admitted male cats and 2.67% out of total admitted (male and female) cats, while the incidence of fractures in female cats was 33.56% out of total fracture cases; 2.21% out of total admitted female cats and 1.35% out of total admitted (male and female) cats.

Regarding the age (table 8), the incidence of the fracture in dogs were 54.94%, 29.63%, 12.96%, and 2.47% out of dog fracture cases; 10.72%, 6.93%, 3.09%, and 3.64% out of total admitted dogs of related age and 3.85%, 2.08%, 0.91%, and 0.17% out of total admitted dogs, distributing among ages (Juvenile (< 1 year), young adult (1-3 years), mature adult (3-10 years) and Elder (> 10 years)), respectively. Meanwhile, in cats, the incidence of bone fracture was 32.89%, 34.23%, 24.83%, and 8.05% out of cat fracture cases; 3.94%, 5.99%, 3.84%, and 1.83% out of total admitted cats of related age and 1.32%, 1.37%, 1.00%, and 0.32% out of total admitted cats, distributing among ages (Juvenile (< 1 year), young adult (1-3 years), mature adult (3-10 years) and Elder (> 10 years)), respectively.

The obtained results (table 9) showed that the causes of presented fracture cases in dogs and cats were trauma due to traffic accidents (141 dogs, 43.5% and 74 cats, 49.7%), falling from a height (63 dogs, 19.4% and 54 cats, 36.2%), indoor trauma (18 dogs, 5.6% and 8 cats, 5.4%), pathological conditions (12 dogs, 3.7% and 2 cats, 1.3%), animal bite (10 dogs, 3.1%) and human abuse (9 dogs, 2.8%), respectively. The cause which cannot be defined in 82 cases of fractures (71 dogs, 25.6%, and 11 cats, 8.7%) was because the owners had not witnessed the moment of the event.

Table 1. The total admitted dog cases to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020.

Dog breeds	Routine works (Vaccinations, deworming, external parasite control, etc.)	Medicinal cases	Surgical cases			Total
			Soft tissue surgical cases	Orthopedic surgical cases		
				Fracture cases	Joint affected cases	
1. German Shepherd	428	282	126	62	296	1194
2. Golden Retriever	206	141	116	36	173	672
3. German Rottweiler	123	104	80	18	136	461
4. American Pit bull	112	87	82	4	28	313
5. Mongrel (Mixed-breed)	16	13	66	163	6	264
6. Grand Griffon Vendèen	68	55	72	7	13	215
7. Labrador Retriever	52	46	18	5	48	169
8. Petit Basset Griffon Vendèen	62	48	27	2	8	147
9. French Bullmastiff	42	48	13	0	44	147
10. Siberian Husky	49	42	12	7	16	126
11. Maltese	55	18	29	0	7	109
12. English Cocker Spaniel	47	37	11	3	3	101
13. Alaskan Husky	42	28	7	3	12	92
14. English Bullmastiff	36	22	11	1	19	89
15. Saint Bernard	20	18	11	2	19	70
16. Neapolitan Mastiff	22	16	13	0	9	60
17. French Bulldog	27	19	2	0	3	51
18. American Cocker Spaniel	34	7	4	2	2	49
19. Doberman Pinscher	28	12	6	1	2	49
20. Cane Corso	31	9	2	0	1	43
21. Caucasian Shepherd	13	7	0	0	11	31
22. Yorkshire Terrier	16	2	3	2	4	27
23. German Boxer	11	7	6	0	0	24
24. Great Dane	10	6	0	0	7	23
25. Pekingese	12	2	6	0	1	21
26. Pomeranian	7	5	0	4	2	18
27. Chihuahua	8	2	0	2	3	15
28. Alabai (Central Asian Shepherd)	6	2	0	0	5	13
29. Chinese Pug	4	1	1	0	2	8
30. Rhodesian Ridgeback	6	0	0	0	1	7
31. Chow-Chow	5	0	0	0	2	7
32. Belgian Malinois	5	0	0	0	0	5
33. Basset Hound	1	0	0	0	2	3
34. Dalmatian	2	0	0	0	0	2
Total	1606	1086	724	324	885	4625
%	34.72%	23.48%	15.65%	7.01%	19.14%	100%

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Table 2. The total admitted cat cases to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020.

Cat breeds	Routine works (Vaccinations, deworming, external parasite control, etc.)	Medicinal cases	Surgical cases			Total
			Soft tissue surgical cases	Orthopedic surgical cases		
				Fracture cases	Joint affected cases	
35. Persian	638	1543	478	54	2	2715
36. Mongrel (Mixed-breed)	112	107	315	87	0	621
37. Himalayan	38	104	16	3	1	162
38. Siamese	26	87	7	4	2	126
39. Egyptian Mau	7	38	22	1	0	68
40. Sphynx	11	6	3	0	0	20
Total	832	1885	841	149	5	3712
%	22.41%	50.78%	22.66%	4.01%	0.14%	100%

Table 3. Number and percentage of fracture cases out of total cases admitted to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020 in relation to different dog breeds.

Dog breeds	Total admitted cases	Fracture cases	Percentage out of the total number of admitted breed cases
1. Mongrel (Mixed-breed)	264	163	61.74%
2. Pomeranian	18	4	22.22%
3. Chihuahua	15	2	13.33%
4. Yorkshire Terrier	27	2	7.41%
5. Siberian Husky	126	7	5.56%
6. Golden Retriever	672	36	5.36%
7. German Shepherd	1194	62	5.19%
8. American Cocker Spaniel	49	2	4.08%
9. German Rottweiler	461	18	3.90%
10. Alaskan Husky	92	3	3.26%
11. Grand Griffon Vendèen	215	7	3.26%
12. English Cocker Spaniel	101	3	2.97%
13. Labrador Retriever	169	5	2.96%
14. Saint Bernard	70	2	2.86%
15. Doberman Pinscher	49	1	2.04%
16. Petit Basset Griffon Vendèen	147	2	1.36%
17. American Pitbull	313	4	1.28%
18. English Bullmastiff	89	1	1.12%
Total	4071	324	7.96%

Table 4. Number and percentage of fracture cases out of total cases admitted to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020 in relation to different cat breeds.

Cat breeds	Total admitted cases	Fracture cases	Percentage out of the total number of admitted breed cases
19. Persian	2715	54	1.99%
20. Mongrel (Mixed-breed)	621	87	14.01%
21. Himalayan	162	3	1.85%
22. Siamese	126	4	3.17%
23. Egyptian Mau	68	1	1.47%
Total	3692	149	4.04%

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Table 5. Incidence of the fracture among affected dog breeds (Classified according to the body weight).

	Dog breeds	Fracture cases	The percentage from admitted breed
Giant breeds Over 45kg	English Bullmastiff	1	1.12%
	Saint Bernard	2	2.86%
	Total	3	1.89%
Large breeds 22-45 kg	German Shepherd	62	5.19%
	Golden Retriever	36	5.36%
	German Rottweiler	18	3.90%
	Labrador Retriever	5	2.96%
	Siberian Husky	7	5.56%
	Alaskan Husky	3	3.26%
	Total	131	4.83%
Medium breeds12-22kg	American Pitbull	4	1.28%
	Mongrel (Mixed-breed)	163	61.74%
	Doberman Pinscher	1	2.04%
	Total	168	26.84%
Small breeds 5-12kg	Grand Griffon Vendèen	7	3.26%
	Petit Basset Griffon Vendèen	2	1.36%
	English Cocker Spaniel	3	2.97%
	American Cocker Spaniel	2	4.08%
	Total	14	2.73%
Mini breeds less than 5kg	Yorkshire Terrier	2	7.41%
	Pomeranian	4	22.22%
	Chihuahua	2	13.33%
	Total	8	13.33%
Total		324	7.96%

Table 6. Incidence of the fracture among affected cat breeds (Classified according to the body conformation).

Cat breeds		Fracture cases	The percentage from admitted breed
Cobby	Persian	54	1.99%
	Himalayan	3	1.85%
	Total	57	1.98%
Moderate	Mongrel	87	14.01%
	Total	87	14.01%
Svelte	Siamese	4	3.17%
	Egyptian Mau	1	1.47%
	Total	5	2.58%
Total		149	4.04%

Table 7. Incidence of the fracture among affected dogs and cats (Classified according to the gender)

Species		Fracture cases			Total admitted cases		
		Male	**Female**	**Total**	**Male**	**Female**	**Total**
Dog	No.	212	112	324	2784	1841	4625
	% out of total fracture cases	65.43%	34.57%	100.00%			
	% out of total admitted related gender	7.61%	6.08%	7.01%			
	% out of total admitted cases	4.58%	2.42%	7.01%	60.19%	39.81%	100.00%
Cat	No.	99	50	149	1454	2258	3712
	% out of total fracture cases	66.44%	33.56%	100.00%			
	% out of total admitted related gender	6.81%	2.21%	4.01%			
	% out of total admitted cases	2.67%	1.35%	4.01%	39.17%	60.83%	100.00%
Total	No.	311	162	473	4238	4099	8337
	% out of total fracture cases	65.75%	34.25%	100.00%			
	% out of total admitted related gender	7.34%	3.95%	5.67%			
	% out of total admitted cases	3.73%	1.94%	5.67%	50.83%	49.17%	100.00%

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Table 8. The incidence of the fracture among the affected dogs and cats (Classified according to the age).

Species		*Fracture cases*					*Total admitted cases*				
		Juvenile (< 1 year)	Young adult (1-3 years)	Mature adult (3-10 years)	Elder (> 10 years)	Total	Juvenile (< 1 year)	Young adult (1-3 years)	Mature adult (3-10 years)	Elder (> 10 years)	Total
Dog	No.	178	96	42	8	324	1661	1386	1358	220	4625
	% out of total fracture cases	54.94%	29.63%	12.96%	2.47%	100%					
	% out of total admitted related age	10.72%	6.93%	3.09%	3.64%	7.01%					
	% out of total admitted cases	3.85%	2.08%	0.91%	0.17%	7.01%	35.91%	29.97%	29.36%	4.76%	100%
Cat	No.	49	51	37	12	149	1244	851	963	654	3712
	% out of total fracture cases	32.89%	34.23%	24.83%	8.05%	100%					
	% out of total admitted related age	3.94%	5.99%	3.84%	1.83%	4.01%					
	% out of total admitted cases	1.32%	1.37%	1.00%	0.32%	4.01%	33.51%	22.93%	25.94%	17.62%	100%
Total	No.	227	147	79	20	473	2905	2237	2321	874	8337
	% out of total fracture cases	47.99%	31.08%	16.70%	4.23%	100%					
	% out of total admitted related age	7.81%	6.57%	3.40%	2.29%	5.67%					
	% out of total admitted cases	2.72%	1.76%	0.95%	0.24%	5.67%	34.84%	26.83%	27.84%	10.48%	100%

Table 9. The causes of fracture among the affected dogs and cats cases admitted to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020.

Species	Cause of fracture							
	Road traffic accident	Fall from height	Unknown cause	Indoor trauma	Pathological conditions	Animal bite	Human abuse	Total
Dog	141	63	71	18	12	10	9	324
	43.5 %	19.4 %	21.9%	5.6 %	3.7%	3.1 %	2.8 %	100%
Cat	74	54	11	8	2	0	0	149
	49.7 %	36.2 %	7.4 %	5.4 %	1.3%	0%	0%	100%
Total	215	117	82	26	14	10	9	473
	45.5 %	24.7 %	17.3 %	5.5 %	3%	2.1 %	1.9 %	100%

Table 10. The incidence of fracture among the affected dogs and cats cases admitted to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020 (Classified according to the affected skeletal part of the body).

Species	The affected skeletal part						Total fracture cases
	Axial skeleton			Appendicular skeleton			
	Skull / Mandible	Vertebrae / ribs	Total	Forelimb	Hindlimb	Total	
Dog	13	29	42	88	194	282	324
	4 %	9 %	13%	27 %	60 %	87%	100%
Cat	31	11	42	30	77	107	149
	20.8 %	7.4 %	28.2%	20.1 %	51.7 %	71.8%	100%
Total	44	40	84	118	271	389	473
	9.3 %	8.5 %	17.8%	25 %	57.2 %	82.2%	100%

Table 11. Distribution of fractures in different appendicular bones in dogs' and cats' cases admitted to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020.

Species	Appendicular bone					
	Femur	Tibia & Fibula	Humerus	Radius & Ulna	Other bones	Total
Dog	108	63	14	52	45	282
	38.3%	22.3%	5%	18.4%	16%	100%
Cat	46	20	14	6	21	107
	43%	18.7%	13.1%	5.6%	19.6%	100%
Total	154	83	28	58	66	389
	39.6%	21.3	7.2%	14.9%	17%	100%

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Establishment of the types and frequency of the bone fractures' occurrence

Classification of bone fractures based on the body parts, the incidence of specific limb and specific bone fractures

According to the affected skeletal part (table 10), the incidence of fracture among the affected dogs and cats were recorded as axial fractures (42 dogs, 13% and 42 cats, 28.2%) and appendicular fractures (282 dogs, 87% and 107 cats, 71.8%).

Incidence of specific limb (forelimbs / hind limbs) fractures.

Incidence of the fractures was found predominantly in hind limbs in dogs and cats, as 60% (n: 194) in dogs and 51.7% (n: 77) in cats. In turn, 27% (n: 88) of the fractures occurred in the forelimbs in dogs and 20.1% (n: 30) in cats (Table 10).

Incidence of specific appendicular bone fractures.

Concerning both forelimbs and hind limbs, in dogs, femur was the most affected bone (38.3%, n: 108), followed by the tibia/fibula (22.3%, n: 63), radius/ulna (18.4%, n: 52) and humerus (5%, n: 14). Meanwhile in cats, femur was the most affected bone (43%, n: 46), followed by the tibia/fibula (18.7%, n: 20), humerus (13.1%, n: 14) and radius/ulna (5.6%, n: 6) (Table 11).

Frequency of the different types of appendicular bone fracture based on the extent of tissue damage, site, and shape of the fracture line

The extent of tissue damage

Open or closed fracture. In the present study, closed fractures were more frequently recorded in dogs (89%, n: 282) and cats (76.6% n: 82) than open fractures (11%, n: 31) in dogs and (23.4% n: 25) in cats (Table 12). Regarding the forelimb (118 cases; 88 dogs and 30 cats), closed fractures (96 cases; 69 dogs and 27 cats) were recorded in 14, 40, and 15 dogs and 14, 3, and 10 cats in the humerus, radius & ulna and other bones representing 100%, 76.9% and 68.2% in dogs and 100%, 50% and 100% in cats out of the related bone fracture cases respectively, while open fractures (22 cases; 19 dogs and 3 cats) were recorded in 0, 12, and seven dogs and 0, three, and 0 cats in the humerus, radius & ulna and other bones representing 0%, 23.1% and 31.8% in dogs and 0%, 50% and 0% in cats out of the related bone fracture cases respectively. Regarding hindlimb (271 cases; 194 dogs and 77 cats), closed fractures (237 cases; 182 dogs and 55 cats) were recorded in 102, 57, and 23 dogs, and 30, 14, and 11 cats in the femur, tibia & fibula and other bones representing 94.4%, 90.5% and 100% in dogs and 65.2%, 70% and 100% in cats out of the related bone fracture cases respectively, while open fractures (34 cases; 12 dogs and 22 cats) were recorded in the femur, tibia & fibula and other bones representing 5.6%, 9.5% and 0% in dogs and 34.8%, 30% and 0% in cats out of the related bone (femur, tibia & fibula and other bones) fracture cases respectively.

Incomplete or complete fracture. The obtained results revealed that incomplete fractures were recorded in 42dogs and three representing 17.7% and 3.5% out of total fore and hind limbs long bone fractures (femur, tibia NS fibula, humerus and radius and ulna), while complete fractures were recorded in 195 dogs and 83 cats representing 82.3% and 96.5% out of total fore and hind limbs long bone fractures (femur, tibia and fibula, humerus and radius and ulna) (Tables 13 and 14).

Fractures' location

Proximal, diaphyseal or distal zones. Regarding the location of the fracture among the affected long bones, proximal, diaphyseal, and distal fractures were recorded in 27, 156 and 54 cases in dogs and 13, 37, and 36 cases in cats representing 11.4%, 65.8%, and 22.8%, and 15.1%, 43%, and 41.9% out of total fore and hind limbs long bone fractures (femur, tibia and fibula, humerus, and radius and ulna) in dogs and cats respectively (Tables 13 and 14).

Fractures' line

The number of fractures' line (single or comminuted). Regarding the number of the fractures' line among the affected long bones, single and comminuted fractures were recorded in 151 and 44 dogs, and 76 and 7 cats representing 63.7% and 18.6%, and 88.4% and 8.1% out of total fore and hind limbs long bone fractures (femur, tibia & fibula, humerus, and radius & ulna) in dogs and cats respectively (Tables 13 and 14).

The direction of fractures' line (transverse, oblique or spiral). Regarding the location of the fracture among the affected long bones, transverse, oblique, or spiral fractures were recorded in 75, 56, and 20 dogs, and 41, 24, and 11 cats representing 31.7%, 23.6%, and 8.4%, and 47.7%, 27.9%, and 12.8% out of total fore and hind limbs long bone fractures (femur, tibia & fibula, humerus, and radius & ulna) in dogs and cats respectively (Tables 13 and 14).

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Table 12. Incidinces of the different types of appendicular bone fracture in dogs' and cats' cases admitted to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020 based on the extent of tissue damage.

Species	Fracture	Forelimb				Hindlimb				Total
		Humerus	Radius & Ulna	Other bones	Total	Femur	Tibia & Fibula	Other bones	Total	
Dog	Open	0 0% (0%)	12 38.7% (23.1%)	7 22.6% (31.8%)	19 61.3% (21.6%)	6 19.4% (5.6%)	6 19.4% (9.5%)	0 0% (0%)	12 38.7% (6.2%)	31 100% (11%)
	Closed	14 5.6% (100%)	40 15.9% (76.9%)	15 6% (68.2%)	69 27.5% (78.4%)	102 40.6% (94.4%)	57 22.7% (90.5%)	23 9.2% (100%)	182 72.5% (93.8%)	251 100% (89%)
	Total	14 5% (100%)	52 18.4% (100%)	22 7.8% (100%)	88 31.2% (100%)	108 38.3% (100%)	63 22.3% (100%)	23 8.2% (100%)	194 68.8% (100%)	282 100% (100%)
Cat	Open	0 0% (0%)	3 12% (50%)	0 0% (0%)	3 12% (10%)	16 64% (34.8%)	6 24% (30%)	0 0% (0%)	22 88% (28.6%)	25 100% (23.4%)
	Closed	14 17.1% (100%)	3 3.7% (50%)	10 12.2% (100%)	27 32.9% (90%)	30 36.6% (65.2%)	14 17.1% (70%)	11 13.4% (100%)	55 67.1% (71.4%)	82 100% (76.6%)
	Total	14 13.1% (100%)	6 5.6% (100%)	10 9.3% (100%)	30 28% (100%)	46 43% (100%)	20 18.7% (100%)	11 10.3% (100%)	77 72% (100%)	107 100% (100%)
Total	Open	0 0% (0%)	15 26.8% (25.9%)	7 12.5% (21.9%)	22 39.3% (18.6%)	22 39.3% (14.3%)	12 21.4% (14.5%)	0 0% (0%)	34 60.7% (12.5%)	56 100% (14.4%)
	Closed	28 8.4% (100%)	43 12.9% (74.1%)	25 7.5% (78.1%)	96 28.8% (81.4%)	132 42.6% (85.7%)	71 21.3% (85.5%)	34 10.2% (100%)	237 71.1% (87.5%)	333 100% (85.6%)
	Total	28 7.2% (100%)	58 14.9% (100%)	32 8.2% (100%)	118 30.3% (100%)	154 39.6% (100%)	83 21.3% (100%)	34 8.7% (100%)	271 69.6% (100%)	389 100% (100%)

Table 13. Incidince of the different types of appendicular bone fracture in dogs' cases admitted to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020 based on the extent, site, and shape of the fracture line.

Fracture type		Incomplete	Complete				Total
			Single			Comminuted	
Site	Bone		Transverse	Oblique	Spiral		
Proximal	Femur	7	3	-	-	-	10
		70.0%	30.0%	-	-	-	100%
		(43.8%)	(37.5%)	-	-	-	(37.0%)
		[16.7%]	[4.0%]	-	-	-	[4.2%]
	Tibia & fibula	9	3	3	-	-	15
		60.0%	20.0%	20.0%	-	-	100%
		(56.3%)	(37.5%)	(100%)	-	-	(55.6%)
		[21.4%]	[4.0%]	[5.4%]	-	-	[6.3%]
	Humerus	-	-	-	-	-	-
		-	-	-	-	-	-
		-	-	-	-	-	-
		-	-	-	-	-	-
	Radius & ulna	-	2	-	-	-	2
		-	100%	-	-	-	100%
		-	(25.0%)	-	-	-	(7.4%)
		-	[2.7%]	-	-	-	[0.8%]
	Total	16	8	3	-	-	27
		59.3%	29.6%	11.1%	-	-	100%
		(100%)	(100%)	(100%)	-	-	(100%)
		[38.1%]	[10.7%]	[5.4%]	-	-	[11.4%]
Diaphyseal	Femur	7	3	14	20	26	70
		10.0%	4.3%	20.0%	28.6%	37.1%	100%
		(29.2%)	(8.6%)	(35.0%)	(100.0%)	(70.3%)	(44.9%)
		[16.7%]	[4.0%]	[25.0%]	[100.0%]	[59.1%]	[29.5%]
	Tibia & fibula	3	13	18	-	7	41
		7.3%	31.7%	43.9%	-	17.1%	100%
		(12.5%)	(37.1%)	(45.0%)	-	(18.9%)	(26.3%)
		[7.1%]	[17.3%]	[32.1%]	-	[15.9%]	[17.3%]
	Humerus	2	4	-	-	1	7
		28.6%	57.1%	-	-	14.3%	100%
		(8.3%)	(11.4%)	-	-	(2.7%)	(4.5%)
		[4.8%]	[5.3%]	-	-	[2.3%]	[3.0%]
	Radius & ulna	12	15	8	-	3	38
		31.6%	39.5%	21.1%	-	7.9%	100%
		(50.0%)	(42.9%)	(20.0%)	-	(8.1%)	(24.4%)
		[28.6%]	[20.0%]	[14.3%]	-	[6.8%]	[16.0%]
	Total	24	35	40	20	37	156

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		15.4%	22.4%	25.6%	12.8%	23.7%	100%
		(100%)	(100%)	(100%)	(100%)	(100%)	(100%)
		[57.1%]	[46.7%]	[71.4%]	[100.0%]	[84.1%]	[65.8%]
	Femur	-	19	4	-	5	28
		-	67.9%	14.3%	-	17.9%	100%
		-	(59.4%)	(30.8%)	-	(71.4%)	(51.9%)
		-	[25.3%]	[7.1%]	-	[11.4%]	[11.8%]
	Tibia & fibula	-	3	2	-	2	7
		-	42.9%	28.6%	-	28.6%	100%
		-	(9.4%)	(15.4%)	-	(28.6%)	(13.0%)
		-	[4.0%]	[3.6%]	-	[4.5%]	[3.0%]
Distal	**Humerus**	-	5	2	-	-	7
		-	71.4%	28.6%	-	-	100%
		-	(15.6%)	(15.4%)	-	-	(13.0%)
		-	[6.7%]	[3.6%]	-	-	[3.0%]
	Radius & ulna	2	5	5	-	-	12
		16.7%	41.7%	41.7%	-	-	100%
		(100.0%)	(15.6%)	(38.5%)	-	-	(22.2%)
		[4.8%]	[6.7%]	[8.9%]	-	-	[5.1%]
	Total	2	32	13	-	7	54
		3.7%	59.3%	24.1%	-	13.0%	100%
		(100%)	(100%)	(100%)	-	(100%)	(100%)
		[4.8%]	[42.7%]	[23.2%]	-	[15.9%]	[22.8%]
Total		42	75	56	20	44	237
		17.7%	31.7%	23.6%	8.4%	18.6%	100%
		[100%]	[100%]	[100%]	[100%]	[100%]	[100%]

Table 14. Incidince of the different types of appendicular bone fracture in cats' cases admitted to the Referral Veterinary Teaching Hospital, Cairo University and some Private Clinics in Egypt from January 2017 to January 2020 based on the site, extend and shape of the fracture line.

Fracture type		**Incomplete**	**Complete**				**Total**
			Single			**Comminuted**	
Site	**Bone**		**Transverse**	**Oblique**	**Spiral**		
	Femur	1	5	6	-	-	12
		8.3%	41.7%	50%	-	-	100%
		(100%)	(83.3%)	(100%)	-	-	(92.3%)
		[33.3%]	[12.2%]	[25%]	-	-	[14%]
	Tibia & fibula	-	-	-	-	-	-
		-	-	-	-	-	-
		-	-	-	-	-	-
		-	-	-	-	-	-
Proximal	**Humerus**	-	-	-	-	-	-
		-	-	-	-	-	-
		-	-	-	-	-	-
		-	-	-	-	-	-
	Radius & ulna	-	1	-	-	-	1
		-	100%	-	-	-	100%
		-	(16.7%)	-	-	-	(7.7%)
		-	[2.4%]	-	-	-	[1.2%]
	Total	1	6	6	-	-	13
		7.7%	46.2%	46.2%	-	-	100%
		(100%)	(100%)	(100%)	-	-	(100%)
		[33.3%]	[14.6%]	[25%]	-	-	[15.1%]
	Femur	-	1	3	6	2	12
		-	8.3%	25%	50%	16.7%	100%
		-	(20%)	(21.4%)	(54.5%)	(33.3%)	(32.4%)
		-	[2.4%]	[12.5%]	[54.5%]	[28.6%]	[14%]
	Tibia & fibula	1	1	8	-	4	14
		7.1%	7.1%	57.1%	-	28.6%	100%
		(100%)	(20%)	(57.1%)	-	(66.7%)	(37.8%)
		[33.3%]	[2.4%]	[33.3%]	-	[57.1%]	[16.3%]
Diaphyseal	**Humerus**	-	3	2	5	-	10
		-	30%	20%	50%	-	100%
		-	(60%)	(14.3%)	(54.5%)	-	(27%)
		-	[7.3%]	[8.3%]	[54.5%]	-	[11.6%]
	Radius & ulna	-	-	1	-	-	1
		-	-	100%	-	-	100%
		-	-	(7.1%)	-	-	(2.7%)
		-	-	[4.2%]	-	-	[1.2%]
	Total	1	5	14	11	6	37
		2.7%	13.5%	37.8%	29.7%	12.2%	100%
		(100%)	(100%)	(100%)	(100%)	(100%)	(100%)
		[33.3%]	[12.2%]	[58.3%]	[100%]	[85.7%]	[43%]
	Femur	-	21	-	-	1	22
Distal		-	95.5%	-	-	4.5%	100%
		-	(70%)	-	-	(100%)	(61.1%)
		-	[51.2%]	-	-	[14.3%]	[25.6%]

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	Tibia & fibula	1	5	-	-	-	6
		13.0%	83.3%	-	-	-	100%
		(100%)	(16.7%)	-	-	-	(16.7%)
		[33.3%]	[12.2%]	-	-	-	[7%]
	Humerus	-	-	4	-	-	4
		-	-	100%	-	-	100%
		-	-	(100%)	-	-	(11.1%)
		-	-	[16.7%]	-	-	[4.7%]
	Radius & ulna	-	4	-	-	-	4
		-	100%	-	-	-	100%
		-	(13.3%)	-	-	-	(11.1%)
		-	[9.8%]	-	-	-	[4.7%]
	Total	1	30	4	-	1	36
		3.7%	83.3%	11.1%	-	2.8%	100%
		(100%)	(100%)	(100%)	-	(100%)	(100%)
		[33.3%]	[73.2%]	[16.7%]	-	[14.3%]	[41.9%]
Total		3	41	24	11	7	86
		3.5%	47.7%	27.9%	12.8%	8.1%	100%
		[100%]	[100%]	[100%]	[100%]	[100%]	[100%]

DISCUSSION

The present retrospective study provided novel descriptive data of the prevalence of dogs and cats with appendicular fractures in Egypt through the recorded cases in the referral veterinary teaching hospital, faculty of veterinary medicine, Cairo University and some private pet clinics in Cairo district, Egypt admitted from January 2017 to January 2020, and it emphasized the information that characterized the population (breed, age, gender and animal size).

The total number of the admitted cases to the hospital and the clinics during the current study period was 8337 pets (4625 dogs and 3712 cats) with a ratio of 55.5% to 44.5% respectively, which indicated that dog breeding is relatively more popular in the Egyptian society than raising cats. This may be due to the expansion of new communities with increasing numbers of compounds, resorts and villas in the regions with high social level which requires breeding guard dogs. This was clearly shown by the current findings that the most common admitted breeds were guard or outdoor dogs' breeds representing 68.8% of total admitted dogs. The results of the most common dog breeds in Egypt were in agreement with previous studies (Nouh et al., 2014; Rakha et al., 2015). More than the quarter of the admitted dogs was German Shepherd (25.8%). On the other hand, mongrel or stray dogs (representing 5.7% of total admitted dogs) were frequently admitted to the referral veterinary teaching hospital, faculty of veterinary medicine, Cairo university, through animal welfare societies and stray animal rescue persons or associations, and most of these cases were surgical injuries (representing 89% of total admitted mongrel dogs) with fracture incidence of 61.7%.

Regarding the cat breeds, relatively, there were few different breeds raised within the Egyptian society. The results were nearly similar to previously recorded ones (Farghali et al., 2020). Only six cat breeds were admitted to the hospital and the clinics during the study period with the highest admission of Persian cat breed (representing 73.1% of total admitted cats). On the other hand, mongrel cats (representing 16.7% of total admitted cats) were frequently admitted to the referral veterinary teaching hospital, faculty of veterinary medicine, Cairo university. Some of them were raised indoor, and the others were presented through animal welfare societies and stray animal rescue associations or persons, and most of these cases were surgical injuries (representing 64.7% of total admitted mongrel cats) with soft tissue surgical affections and fracture incidences (50.7% and 14% respectively).

In the present study, the incidence of fracture was higher in dogs (7%) than in cats (4%). Meanwhile, fractures observed in cats showed a similarity to those in dogs as in regard to the site of fracture. However, cats do have several advantages as orthopedic patients when compared to dogs including their light weight, straight bones, and anatomical configuration (Harasen, 2009; Singh et al., 2015). The highest incidences of the fracture cases were recorded in mongrel dogs and cats which may be due to the frequent exposure of stray animals to road traffic accidents. This result was similar to the previous study of Harasen 2003a (2004), Senn et al. (2004) and Uwagie-Ero et al. (2018).

With excluding of mongrel dogs (as anomalous fractures' incidence), the highest incidence of canine fracture cases was recorded in miniature breeds (less than 5kg, 13.33%), followed by large breeds (22-45 kg, 4.83%), small breeds (5-12kg, 2.73%), giant breeds (over 45kg, 1.89%) and medium breeds (12-22kg, 1.4%). These results were nearly similar to those recorded in previous studies where the most commonly affected breed was Yorkshire terrier (12%), followed by Poodle (12%) and Maltese (9%) (Minar et al., 2013). On the other side, some other previous studies have reported that German shepherds were the most affected breed (Ali, 2013; Rhangani, 2014; Libardni et al., 2016). Obviously, the variation of incidence of bone fractures in different breeds of dogs may be related to the regions/countries that the owners lived which may be different in behavior/life styles in the different countries (Minar et al., 2013). Other author mentioned that the size of the dog does not mean a condition of predisposition to fractures (Johnson, 2013). Many

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articles supported the theory that fractures were more often in smaller dogs (Brianza et al., 2006; Yu et al., 2010; De Arburn Parent et al., 2017). This may be due to the low muscle coverage in the limbs (Milovancev and Ralphs, 2004).

In cats, the highest incidence of bone fractures was recorded in mongrel breeds (14%). This was similar to previous study by Borges et al. (2016). Other factor contributing in the high incidence of fractures in mongrel cat breed rather than the road traffic accidents of stray cats, was the fact that the majority of cats recorded in the clinic were mixed breed. Regarding cat's body conformation, the highest incidence was recorded in moderate (14.01%) (Due to mongrel cats), followed by svelte (2.58%) and cobby (1.98%) breeds. It may be due to the normal playful behavior of svelte and moderate breeds with long limbs (Siamese and Egyptian Mau) resulting in frequent trauma and falling from a height more than the lazy cobby breeds with short limbs (Persian and Himalayan) (Helgren, 2013).

From the obtained data, male dogs (60.19%) were more frequently admitted than female ones (39.81%), while the opposite was recorded in cats (39.17% male and 60.83% female). These findings may be screening the fact of preferring of dog owners to rear male dogs more than females. The result which barrels to other previous researches which added that male dogs are more sociable, dominant, territorial, playful, active, and independent than females (Hart and Hart, 2016; Scandurra et al., 2018). On the other hand, most of cat owners prefer to raise female cats more than males (as it is obvious in this work where queens were admitted about one and half times more than tomcats), may be due to the disliked behavior of much more territorial male cats during the mating season, such as spraying which is not desired by many of the owners (Farghali et al., 2020).

In the current study, gender was among the predisposing factors where remarkable higher incidence was recorded in male dogs (65.4%) and cats (66.4%), than females (34.6% and 33.6%), respectively which is nearly the same in both species. This finding was similar to the reports elsewhere (Dvorak et al., 2000; Senn et al., 2004; Rhangani, 2014). As males are known to be more aggressive, and tend to roam for longer distances, it exposes them to external etiological agents (Simpson, 2004; Kumar et al., 2007; Ben Ali, 2013; Elzomor et al., 2014). Other factors, which may be incriminated, were escaping of uncastrated tomcat from homes during the mating season and exposure to falls from windows and road accidents (Farghali et al., 2020).

In relation to the age, bone fracture was mostly occurred in dogs of less than one-year-old. This finding was similar to many previous studies (Kushwaha et al., 2011; Ali, 2013; Minar et al., 2013; Uwagie-Ero et al., 2018). This higher incidence of fracture in young dogs up to 1 year of age might be due to being playful, active nature of the young ones compared to the adult dogs, and being inexperienced to escape from the hazards. Previous study mentioned that puppies were most affected by femoral fractures due to low bone density in their development (osteogenesis) phase (Libardoni et al., 2018). This was contrary to what was reported in Kenya revealing that the incidence of appendicular fractures was higher in adults (79%) as compared to the young dogs (21%) (Rhangani, 2014).

Regarding cats, the bone fracture was mostly occurred in cats of one to three years old. It may be resulted from reduced knowledge of neutralization importance of tomcats and queens in Egyptian society resulting in bad habits such as escaping of cats from homes during the mating season, cat fighting, exposure to falls from heights and traffic accidents (Farghali et al., 2020). The findings of the present study revealed that the extrinsic factors which include motor vehicle accidents, falling from height, and dog bites were the major etiological agents of bone fractures in dogs. These findings were consistent with those in other studies elsewhere (Simpson, 2004; Kumar et al., 2007; Ben Ali, 2013; Elzomor et al., 2014; Rhangani, 2014; Vidane et al., 2014). A high incidence of car accidents was due to the high number of animals with access to public roads, and the owners who suppress the containment and protection measures in their homes and during outings (Libardoni et al., 2016). Meanwhile, in cats, traffic accidents followed by falling from heights and cat bites were also the most common causes (Denny and Butterworth, 2000; Senna et al., 2004; Piermattei et al., 2006; Lovrić et al., 2020).

In the current work, there was a high incidence of the appendicular long bones concerning the different bone fractures. The same was reported in previous studies (Harasen, 2003a; Thengchaisri et al., 2006; Ali, 2013; Bennour et al., 2014; Rhangani, 2014; Libardoni et al., 2016).

On the other hand, the distribution of common orthopedic conditions in canine and feline species concerning the admitted cases revealed that the prevalence of appendicular fractures was significantly higher in dogs (87%) than in cats (71.8%). Nearly the same result was mentioned by Bennour et al. (2014) and Singh et al. (2015). Such variation may be attributed to the owners' close observation and care directed to cats comparing with dogs, as cats are kept most of the time inside the houses (Singh et al., 2015). In the current study, fractures in the hindlimbs were higher than in forelimbs in both dogs and cats which the finding was similar to the studies reported elsewhere with a different relationship (Souza et al 2011; Ben Ali, 2013; Minar et al., 2013; Bennour et al., 2014; Rhangani, 2014; Roush, 2014; Eyarefe and Oyetayo, 2016; Uwagie-Ero et al., 2018). Most of the long bone fractures in dogs and cats occurring in hindlimbs were found in the femur, followed by tibia and fibula. These results were similar to other studies in Thailand (Thengchaisri et al., 2006), India (Shiju et al., 2010), Korea (Minar et al., 2013), Kenya (Rhangani, 2014), Egypt (50.6%) (Elzomor et al., 2014), Philippines (Libardoni et al., 2016) and Austria (Lovrić et al., 2020). However, in the dogs' forelimb, radius and

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ulna were the bones with the most fractures (Minar et al., 2013; Bennour et al., 2014; Libardoni et al., 2016). This may be due to the low local muscle coverage (Milovancev and Ralphs, 2004).

The fracture of the humerus in cats were the most common sites of forelimb fracture. Similar results have been reported (Chandler and Beale, 2002; Senn et al., 2004; Ben Ali, 2013). Concerning both forelimbs and hindlimbs, the femur was the most affected bone, followed by tibia or fibula, radius or ulna, and humerus in dogs. Other studies showed higher incidences of occurrence with radius, ulna, and femur (Harasen, 2003b; Beale, 2004; Elzomor et al., 2014).

In cats, the femur was also the most frequently fractured bone, but there were variations in the prevalence of fractures among tibia or fibula, humerus and radius or ulna. This was in agreement with previous results (Piermattei et al., 2006; Lovrić et al., 2020). Some authors considered the fractures of the radius/ulna to be infrequent accounting for humeral fractures (Chandler and Beale, 2002). In the present study, closed fractures were more frequent than open fractures. The percentage of the open fractures were more common in cats (23.4%) than in dogs (11%). Open fractures were observed more frequently in the bones below the elbow and stifle in dogs due to poor soft-tissue coverage (Voss and Montavon, 2009). While, in cats, open fractures were more frequently recorded in supracondylar femoral fracture.

Incomplete fractures were recorded more frequently in dogs than in cats representing 17.7% and 3.5% out of the total fore and hind limbs long bone fractures. Greenstick, fissures, and folding fractures were the most common form of incomplete fractures among dogs. The results which were in agreement with previous findings described that greenstick or incomplete fractures were more commonly seen in juveniles (Jain et al., 2016). Complete fractures were recorded more frequently in cats than in dogs representing 96.5% and 82.3% out of total fore and hind limbs long bone fractures.

Regarding the number and direction of the fracture line, the most common type of fracture encountered in both fore and hind limbs in dogs was complete single transverse fracture (31.7%) followed by oblique (23.6%), comminuted (18.6%), incomplete (17.7%) and spiral (8.4%) fractures. This was similar to what has been reported in previous studies (Shiju et al., 2010; Shiju et al., 2011; Ben Ali, 2013).

In cats, the most common type of fracture encountered in both fore and hind limbs was also complete single transverse (47.7%) followed by oblique (27.9%), spiral (12.8%), comminuted (8.1%) and incomplete (3.5%) fractures. Out of 237 cases of appendicular long bone fractures in dogs, the diaphyseal fracture was the most common site of fracture (156 cases, 65.8%), followed by distal fractures (54 cases, 22.8%) and proximal fractures (27 cases, 11.4%). Out of 86 fracture cases in cats, 43% (37 cases) in the diaphyseal, 41.9% (36 cases) was in the distal part and 15.1% (13 cases) in proximal one.

CONCLUSION

From the obtained data, it could be concluded that there was a high incidence of the appendicular long bones concerning the different bone fractures with significantly higher records in dogs than in cats. The highest records of fracture were in mongrel dogs, and cats as rescued animals. With excluding of mongrel dogs and cats, the highest incidence of canine fracture cases was recorded in Miniature breeds, and feline fracture in svelte breeds. Male dogs and cats showed a higher incidence than females. The bone fracture has mostly occurred in dogs of less than one-year-old, and in cats of one to three years old. The fracture in the hindlimbs was higher than forelimbs with the highest incidence among femur in both dogs and cats. The percentage of open fractures were more common in cats than in dogs. Incomplete fractures were recorded more frequently in dogs than in cats. In dogs, the most common fracture in the femur, tibia/fibula, humerus and radius/ulna were complete comminuted diaphyseal femoral, complete oblique diaphyseal tibial/fibular, complete transverse distal humoral, and complete transverse diaphyseal radial/ulnar fractures respectively, and in cats, were complete transverse distal femoral, complete oblique diaphyseal tibial/fibular, complete spiral diaphyseal humoral, and complete transverse distal radial/ulnar fractures respectively.

DECLARATIONS

Acknowledgments

This study was supported by the department of surgery, anesthesiology and radiology, Faculty of veterinary medicine, Cairo University, Egypt. The authors would also like to thank the team of Alpha vet pet clinic, Office Building at Al-rehab Cairo, Cairo Governorate, Egypt for their assistance in data collection.

Competing interests

The authors declare that there is no conflict of interest.

Author's contribution

Ahmed Elsayed Ahmed and Haithem Ali Mohamed Ahmed Farghali designed the study and wrote the manuscript. Abeer Ali Mahmoud Abo-Soliman and Haithem Ali Mohamed Ahmed Farghali participated in data collection and analysis, writing. All authors have read and approved the final manuscript.

To cite this paper: Abo-Soliman AAM, Ahmed AE and Farghali HAMA (2020). Incidence of Appendicular Bone Fracture in Dogs and Cats: Retrospective Study at Veterinary Hospital of Cairo University and some Private Clinics in Egypt. *World Vet. J.*, 10 (3): 638-652. DOI: https://dx.doi.org/10.54203/scil.2020.wvj77

REFERENCES

Ali LB (2013). Incidence, occurrence, classification and outcome of small animal fractures: A retrospective study (2005-2010). WASET, Journal of Animal Veterinary Sciences, 7: 191-196. DOI: https://doi.org/10.5281/zenodo.1082359

American Kennel Club (2006). The Complete Dog Book. 20th ed. New York: Ballantine Books, . Available at: https://www.amazon.com/Complete-Dog-Book-20th/dp/0345476263

Appari AM, Johnson E, and Anthony DL (2013). Meaningful use of electronic health record systems and process quality of care: evidence from a panel data analysis of U.S. acute-care hospitals. Health Services Research, 48(2): 354-375. DOI: https://doi.org/10.1111/j.1475-6773.2012.01448.x

Beale B (2004). Orthopedic clinical techniques femur fracture repair. Clinical Techniques in Small Animal Practice, 19(3): 134-150. DOI: https://doi.org/10.1053/j.ctsap.2004.09.006

Ben Ali LM (2013). Incidence, occurrence, classification and outcome of small animal fractures: A retrospective study (2005-2010) World Academy of Science, Engineering and Technology, 7(3): 516-521. DOI: https://doi.org/10.5281/zenodo.1082359

Bennour E, Abushhiwa M, Ben Ali L, Sawesi O, Marzok M, Abuargob O, Tmumen S, Abdelhadi J, Abushima M, and Benothman M (2014). A Retrospective study on appendicular fractures in dogs and cats in Tripoli-Libya. Journal of Veterinary Advanced, 4: 425-431. Available at: https://www.ejmanager.com/mnstemps/74/74-1391243660.pdf

Borges C, Rahal S, Agostinho F, Mamprim M, Santos R, Silva FE, Carolina MA, and Monteiro FO (2016). Long bone fracture in cat. A retrospective study. Veterinaria Zootecnia, 23: 504-509. Available at: https://www.researchgate.net/publication/307598723

Brianza SZ, Delise M, Maddalena Ferraris M, D'Amelio P, and Botti P (2006). Cross-sectional geometrical properties of distal radius and ulna in large, medium and toy breed dogs. Journal of Biomechanics, 39(2): 302-311. DOI: https://doi.org/10.1016/j.jbiomech.2004.11.018

Chandler JC, and Beale BS (2002). Feline orthopedics. Clinical Techniques in Small Animal Practice, 17: 190-203. DOI: https://doi.org/10.1053/svms.2002.36607

Chaves RO (2014). Neurological diseases in dogs examined at the Veterinary Teaching Hospital of the Federal University of Santa Maria, RS: 1.184 cases (2006-2013). Pesquisa Veterinária Brasileira,34(10): 996-1001. DOI: https://doi.org/10.1590/S0100-736X2014001000012

De Arburn Parent R, Benamou J, Gatineau M, Clerfond P, and Planté J (2017). Open reduction and cranial bone plate fixation of fractures involving the distal aspect of the radius and ulna in miniature- and toy-breed dogs: 102 cases (2008-2015). Journal of the American Veterinary Medical Association , 250(12): 1419-1426. DOI: https://doi.org/10.2460/javma.250.12.1419

Denny HR, and Butterworth SJ (2000). Classification of fractures, p.83-86. In: Ibid. (Eds). A guide to canine and feline orthopedic surgery. Wiley-Blackwell, Oxford, P. 644. Available at: https://onlinelibrary.wiley.com/doi/pdf/10.1002/9780470699027

Dvorak M, Necas A, and Zatloukal J (2000). Complications of long bone fracture healing in dogs: Functional and radiological criteria for their assessment. Acta Veterinaria Brno, 69: 107-114. DOI: https://doi.org/10.2754/avb200069020107

Elzomor ST, Sheta EME, Farghali HA, and Ashour AE (2014). Prevalence of femoral fractures in dogs and cats .The Journal of the Egyptian Medical Association, 74: 269-278. Available at: https://www.academia.edu/download/35180585/PREVALENCE_OF_FEMORAL_FRACTURES_IN_DOGS_AND_CATS.pdf

Eyarefe O, and Oyetayo SN (2016). Prevalence and pattern of small animal orthopaedic conditions at the Veterinary Teaching Hospital, University of Ibadan. Sokoto Journal of Veterinary Sciences, 14: 8. DOI: https://doi.org/10.4314/sokjvs.v14i2.2

Farghali HA, Senna NA, Khattab MS, and Shalaby RKI (2020). Prevalence of most common feline genital surgical affections in teaching veterinary hospital, Cairo university, Egypt and different pet clinics. Advances in Animal and Veterinary Sciences, 8(7): 709-719. DOI: https://doi.org/10.17582/journal.aavs/2020/8.7.709.719

Fogle B (2009). The Encyclopedia of the dog. New York: DK Publishing. Available at: https://www.amazon.com/Encyclopedia-Dog-Bruce-Fogle/dp/0756660041

Fossum TW (2013). Small Animal Surgery. 4th.ed. St. Louis Missouri: Mosby Elsevier, p. 1619. Available at: https://www.elsevier.com/books/small-animal-surgery/fossum/978-0-323-10079-3

Gadallah SM, Farghali H, and Magdy A (2009). Combined different fixation systems for reconstruction of comminuted diaphyseal femoral fractures in dogs. Journal of the Egyptian Veterinary Medical Association, 69(2): 29-44. Available at: https://www.academia.edu/5474710/

Harari J (2002). Treatment of feline long bone fractures. Veterinary Clinics North America Small Animal Practice, 32(4): 927-947. DOI: https://doi.org/10.1016/S0195-5616(02)00025-6

Harasen G (2003a). Common long bone fractures in small animal practice. Part 1: Canadian Veterinary Journal, 44: 333-334. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC372259/

Harasen G (2003b). Common long bone fractures in small animal practice. Part 2. Canadian Veterinary Journal, 44: 503-504. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC340183/

Harasen G (2004). Atraumatic proximal femoral physeal fractures in cats. Canadian Veterinary Journal, 45: 359-360. Available at: https://europepmc.org/article/med/15144117

Harasen G (2009). Feline orthopedics. Canadian Veterinary Journal, 50(6): 669-670. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2684059/

Hart B ,and Hart L (2016). Breed and gender differences in dog behavior. 10.1017/9781139161800.007. In book: The Domestic Dog. Edition: 2nd, Chapter: 7, Publisher: Cambridge University Press, Editors: James Serpell, Pp. 118-132. DOI: https://doi.org/10.1017/9781139161800.007

Helgren JA (2013). Barron's encyclopedia of cat breeds: a complete guide to the domestic cats of North America / J. Anne Helgren; with photographs by Bob Schwartz; illustrations by Michele Earle-Bridges, 2nd edition. Copyright, text and Illustrations © 2013, 1997 by Barron's Educational Series, Inc., P. 60. Available at: https://trove.nla.gov.au/work/23422748

Hobbs SL (2012). Biological and radiological assessment of fracture healing. In Practice, 25: 26-35. DOI: https://doi.org/10.1136/inpract.25.1.26

Jain R, Parihar AS, Kamble S, Parihar YS, and Ganguly S (2016). Multiple Fractures in Tibia Bone of Dog: A Case Study. International Journal of Contemporary Microbiology, January-June, 2(1): 82-83. DOI: https://doi.org/10.5958/2395-1796.2016.00019.3

Kumar K, Mogha HP, Kinjavdekarp, Amarpal, Singh GR, Pawde AM, Kushwaha, and Kushwaha RB (2007). Occurrence and pattern of long bone fractures in growing dogs with normal and osteopenic bones. Journal of the American Veterinary Medical Association, 54: 484-490. DOI: https://doi.org/10.1111/j.1439-0442.2007.00969.x

Kushwaha RB, Gupta AK, Bhadwal MS, Kumar S, and Tripathi AK (2011). Incidence of fractures and their management in animals: a clinical study of 77 cases. Indian Journal of Veterinary Surgery, 32(1): 54-56. Available at: https://www.researchgate.net/publication/333311447_Incidence_of_fracture_and_its_management_in_animals

Lanz OI (2002). Lumbosacral and pelvic injuries. The Veterinary clinics of North America. Small animal practice, 32(4): 949-962. DOI: https://doi.org/10.1016/S0195-5616(02)00029-3

To cite this paper: Abo-Soliman AAM, Ahmed AE and Farghali HAMA (2020). Incidence of Appendicular Bone Fracture in Dogs and Cats: Retrospective Study at Veterinary Hospital of Cairo University and some Private Clinics in Egypt. *World Vet. J.*, 10 (3): 638-652. DOI: https://dx.doi.org/10.54203/scil.2020.wvj77

Libardoni RDN, Serafini GMC, Oliveira CD, Schimites PI, Chaves RO, Feranti JPS, Costa CAS, Amaral ASD, Raiser AG, and Soares AV (2016). Appendicular fractures of traumatic etiology in dogs: 955 cases 2004-2013. Ciência Rural, 46: 542-546. DOI: https://doi.org/10.1590/0103-8478cr20150219

Libardoni RDN, Da Costa D, Menezes FB, Cavalli LG, Pedr otti LF and Kohlrausch PR (2018). Classification, fixation techniques, complications and outcomes of femur fractures in dogs and cats: 61 cases (2015-2016). Ciência Rural, 48(6): 1-6. DOI: https://doi.org/10.1590/0103-8478cr20170028

Lovrić L, Kreszinger M and Pećin M (2020). Surgical Treatment of Canine Femoral Fractures - a Review. World Veterinary Journal, 10 (2): 137-145. DOI: https://dx.doi.org/10.36380/scil.2020.wvj18

Johnson AL (2013). Management of specific fractures. In: Fossum, T.W. Small animal surgery. 4th.ed. St. Louis, Missouri: Mosby Elsevier, pp. 1106-1214. Available at: https://www.elsevier.com/books/small-animal-surgery/fossum/978-0-323-10079-3

Milovancev M, and Ralphs SC (2004). Radius/Ulna fracture repair. Clinical Techniques in Small Animal Practice, 19(3): 128-133. DOI: https://doi.org/10.1053/j.ctsap.2004.09.005

Minar M, Hwang Y, Park M, Kim S, Oh C, Choi S, and Kim G (2013). Retrospective study on fractures in dogs. Journal of Biomedical Research, 14: 140-144. DOI: https://doi.org/10.12729/jbr.2013.14.3.140

Nouh SR, Abo-Ahmad HM, Farghali HA, and Saleh MM (2014). A Retrospective Study on Canine Hip Dysplasia in Different Breeds in Egypt. Global Veterinaria, 13(4): 503-510. Available at: http://scholar.cu.edu.eg/?q=haithem_farghail/files/10.pdf

Piermattei DL, Flo G, and DeCamp C (2006). Brinker, Piermattei, and Flo's handbook of small animal orthopedics and fracture repair. 4th Edition., St. Louis, Missouri, Saunders, Elsevier, Pp. 549-553. Available at: https://www.elsevier.com/books/brinker-piermattei-and-flos-handbook-of-small-animal-orthopedics-and-fracture-repair/decamp/978-1-4377-2364-9?aaref=https%3A%2F%2Fwww.google.com%2F

Rakha GMH, Abdl-Haleem MM, Farghali HAM, and Abdel-Saeed H (2015). Prevalence of common canine digestive problems compared with other health problems at teaching. veterinary hospital, Faculty of Veterinary Medicine, Cairo University, Egypt. Veterinary World, 8(3): 403-411. DOI: https://doi.org/10.14202/vetworld.2015.403-411

Rhangani AT (2014). Incidence, classification and management of appendicular bone fractures in dogs in Nairobi country, Kenya. A retrospective study. Master thesis of veterinary surgery, university of Nairobi, Kenya. Available at: http://erepository.uonbi.ac.ke/bitstream/handle/11295/74296/Rhangani_Incidence,%20Classification%20And%20Management%20Of%20Appendicular%20Bone%20Fractures%20In%20Dogs%20In%20Nairobi%20County,%20Kenya.%20A%20Retrospective%20Study.pdf?sequence=5&isAllowed=y

Roush (2014). Pet Health by the Numbers: Prevalence of Bone Fractures in dogs & cats at 890 Banfield Pet Hospital USA. Journal of Today's Veterinary Practice, pp.1-17. Available at: https://todaysveterinarypractice.com/pet-health-by-the-numbers-prevalence-of-bone-fractures-in-dogs-cats/

Rrisselada M, Kramer M, and van Bree H (2005). Ultrasonographic and radiographic follow up of uncomplicated secondary fracture healing of long bones in dogs and cats. Veterinary Surgery, 34: 99-107. DOI: https://doi.org/10.1111/j.1532-950X.2005.00017.x

Scandurra A, Alterisio A, Di Cosmo A, and D'Aniello B (2018). Behavioral and Perceptual Differences between Sexes in Dogs: An Overview. Animals, 8(151): 1-26. DOI: https://doi.org/10.3390/ani8090151

Senna NA, Gadallah SM, and Zabady MK (2004). Studies on some bone disorders in cats: incidence, radiological assessment and surgical management. Journal of the Egyptian Veterinary Medical Association, 64(3): 113-137. Available at: https://scholar.google.com/scholar?cluster=10334186832027713353&hl=ar&as_sdt=2005&sciodt=0,5

Shales C (2008a). Fracture management in small animal practice: 1. Triage and stabilisation. In practice, 30(6): 314-320. DOI: https://doi.org/10.1136/inpract.30.6.314

Shales C (2008b). Fracture management in small animal practice: 2. Assessment and planning. In practice, 30(7): 374-384. DOI: https://doi.org/10.1136/inpract.30.7.374

Shearer (2011). Epidemiology of orthopedic disease. Veterinary Focus, 21(2): 24-25. DOI: https://doi.org/10.1055/s-0034-1381849

Shiju MS, Ganesh R, Ayyappan S, Rao GD, Kumar RS, Kundave VR and Das BC (2010). Incidence of pelvic limb fractures in dogs: a survey of 478 cases. Veterinary World Journal, 3(3): 120-121. Available at: http://www.veterinaryworld.org/Vol.3/March/Incidences%20of%20pelvic%20limb%20fractures%20in%20dogs.pdf

Shiju MS, Ganesh R, Ayyappan S, and Kumar RS (2011). Incidence of pectoral limb fractures in dogs: a survey of 331 cases. Tamilnadu Journal of Veterinary and Animal Sciences, 7 (2): 94-96. Available at: https://pdfs.semanticscholar.org/3cde/06a59f5912f6c7751b9276ce487ec55c0259.pdf

Simpson AM (2004). Fractures of the humerus. Clinical Techniques in Small Animal Practice, 19(3): 120-127. DOI: https://doi.org/10.1053/j.ctsap.2004.09.004

Singh R, Chandrapuria VP, Shahi A, Bhargava MK, Swamy M, and Shukla PC (2015). Fracture occurrence pattern in animals. Journal of Animal Research, 5(3): 611-616. DOI: https://doi.org/10.5958/2277-940X.2015.00103.5

Thengchaisri N, Chaiyakorn T, Pailin P, and Jadee T (2006). Classification of long bone fractures in dogs and cats, Proceeding of the 32th Veterinary Medicine and Livestock Development Annual Conference, Faculty of Veterinary Medicine Mahanakorn University of Technology, Bangkok, pp. 57-63. Available at:: http://www.vet.cmu.ac.th/cmvj/document/vol.17/number2/2019%2017-2%20%5B21%5D.pdf

Uwagie-Ero EA, Abiaezute CN, Okorie-Kanu OJ, Odigie EA, and Asemota OD (2018). Retrospective evaluation of canine fractures in southern Nigeria. Comparative Clinical Pathology, pp.1127-1132. DOI: https://doi.org/10.1007/s00580-018-2708-3

Vidane AS, Elias MZJ, Cardoso JMM, Come JAS., Harun M, and Ambrósio CE (2014). Incidence of fractures in the dogs and cats in Maputo (Mozambique) between 1998 and 2008. Brazilian Journal of Animal Science, 15: 490-494. DOI: https://doi.org/10.1590/1089-6891v15i424279

Voss K, and Montavon PM (2009). Fractures, p.129-151. In: Montavon P.M., Voss K. & Langley-Hobbs S.J. (Ed.), Feline orthopedic surgery and musculoskeletal disease. Mosby Elsevier, Edinburgh, P. 582. DOI: https://doi.org/10.1016/B978-0-7020-2986-8.00022-7

Yu B, Han K, Zhan C, Zhang C, Ma H, and Su J (2010). Fibular head osteotomy: a new approach for the treatment of lateral or posterolateral tibial plateau fractures. Knee, 17(5): 313-318. DOI: https://doi.org/10.1016/j.knee.2010.01.002

To cite this paper: Abo-Soliman AAM, Ahmed AE and Farghali HAMA (2020). Incidence of Appendicular Bone Fracture in Dogs and Cats: Retrospective Study at Veterinary Hospital of Cairo University and some Private Clinics in Egypt. *World Vet. J.*, 10 (3): 638-652. DOI: https://dx.doi.org/10.54203/scil.2020.wvj77

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 653-657, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj78

The Effects of Adding Lysin Essential Amino Acid to Commercial Feed on Fatty Acid Contents of Pangasius Fish

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ORGINAL ARTICLE
pii: S232245682000078-10
Received: 02 Oct 2020
Accepted: 22 Dec 2020

ABSTRACT

Pangasius is a medium to very large freshwater shark catfish primarily used for consumption with high economic value. The content of pangasius fatty acids is higher than in marine fish, since marine fish have a lower saturated fatty acid composition than freshwater fish. The present research aimed to determine the effects of adding lysine essential amino acid to commercial feeds on the saturated and unsaturated fatty acids contents of pangasius fish. In the present research, an experimental method with completely randomized design was used. The treatment was done by adding lysine with different doses including P0 (0%), P1 (1.2%), P2 (2.2%), and P3 (3.2%). Each treatment was repeated five times. The main parameters studied were the content of saturated and unsaturated fatty acids in pangasius fish meat. The observed parameter was water quality. The present results indicated the use of lysine in commercial feed caused significant differences in the content of saturated fatty acids, Monounsaturated Fatty Acids (MUFA) and Polyunsaturated Fatty Acids (PUFA) in pangasius meat; a decrease in the saturated fatty acids content was found in P3 with 3.2% (3.5882 mg/dl). In P2, an increase in the MUFA content of 2.2% (5.9630 mg/dl) was found. An increase in the PUFA content was found in P3 treatment with 3.2% Lysin (23.1082 mg/dl). P1, P2 and P3 indicated lower results than control treatments (P_0). The use of lysine in commercial feed indicated significant differences in the content of saturated fatty acids, MUFA and PUFA in pangasius.

Key words: Lysine essential amino acid, Saturated fatty acids, Unsaturated fatty acids.

INTRODUCTION

Pangasius is a type of freshwater consumption fish that has high economic value. However, the fatty acids content in fresh pangasius meat is high, which could reach up to 61.64% (Prananingtyas and Rahardja, 2019). The composition of fatty acids in pangasius in freshwater differed from the fish that live in marine waters. Marine fish had a lower composition of Saturated Fatty Acids (SFA), which are high in Polyunsaturated Fatty Acids (PUFA), which has been proven to be good for consumption as Unsaturated Fatty Acids (UFA) in marine fish are good for health (Weya et al., 2017).

Fatty acids are divided into SFA and UFA. Saturated fatty acids have a higher melting point than UFA and are the basis for determining the physical properties of fat (Bell et al., 2017). The excessively high-fat content led to an accumulation of fat in the body and disrupted the body's metabolic processes. Fatty acids that contain two or more double bonds are called PUFA (Sokoła-Wysoczańska et al., 2018).

One of the factors affecting pangasius fatty acids is their feed. The fat in their feed affected the composition of fatty acids in the body of pangasius. Fish feed was made of a material that can be eaten, digested, and absorbed either in whole or in part and would not cause poisoning or harm the health of the fish that consume it. The feed quality was considered to be poor if the content of essential amino acid was low (Van Doan et al., 2014; Bayati Zadeh et al., 2017; Mahmud et al., 2020).

Pangasius farmers were used commercial feed containing a variety of nutrients. However, the fatty acid content of pangasius still tends to be high. Additional feed supplement must be given to stimulate growth or increase productivity, reduce fat content and increase production efficiency. Feed supplement is a mixture of ingredients to improve the nutrients balance, which could be given without mixing with other foods to form a complete food. The use of feed supplements could increase the efficiency of food digestion so that fish farm production could be increased (Jana et al., 2014).

One of the feed supplements that could be added to the feed is lysine. Lysine is one of the essential amino acids for fish. Lysine plays a role in the formation of carnitine, which acts as a growth booster (Fitriani et al., 2019). The impact of the lysine, which is added to pangasius in commercial feed given to, is that it could stimulate the metabolic process of

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fatty acids in the body of pangasius (Liu et al., 2011). The current study aimed to determine the effects of adding lysine to commercial feed to reduce the content of SFA and increase the UFA contents of pangasius meat.

MATERIALS AND METHODS

Ethical approval

Present experiment was conducted on fish farm of the Universitas Airlangga of Indonesia**.** The research process included animals as a subject that was consistent with the ethical research principle based on the regulation of Research Ethic Committee. The present study implemented the basic principles of ethics of respect, benefit, non-deficiency, and justice.

Experimental design

The present study used a four-treatment experimental method to compare the effects of commercial feed without lysine with commercial feed with lysine. The addition of lysine was given a different dose for each treatment. Treatments P_0, P_1, P_2, and P_3 indicated the effects of different dosage of lysin on saturated and unsaturated fatty acid content of pangasius (Kim et al., 2018). The treatments used in the present study were the addition of lysine to commercial feeds at varying doses and were monitored and controlled. Treatment and repetition consisted of treatment P_0 (100%) and commercial feed. The experimental design used was Completely Randomized Design (CRD) with four treatments and five replications. The experiments were performed with a simple random sample (Laake et al., 2013).

Pangasius fish were hybrid pasupati pangasius larvae that were kept in a fiber tube measuring 57 cm × 36 cm × 29 cm for 42 days. The results indicated that the initial stocking density was 20 heads/L, 40 heads/L, 60 heads/L, 80 heads/L and 100 heads/L, there were differences in survival, in absolute length growth and in absolute weight growth. The best initial stocking density is 40 heads/L with a survival rate of 66.14%, an absolute increase in length of 5.03 cm and an absolute weight increase of 2.05 grams. The feed measure was five percent of biomass of pangasius by weighing the entire fish population. The feed was given twice every seven days, in the morning at 08:00 and in the evening at 16:00 (Jana et al., 2014). The frequency of feeding was determined by the species and size of the fish (Yoo and Lee, 2016), as well as by the factors that affect the appetite of fish. Basically, these three factors were closely related. The smaller the fish, the more often it is fed (Jana et al., 2014).

Freshwater with a volume of 22.5 liters per aquarium and 20 aquariums with a size of 30 centimeter (cm) × 30 cm ×40 cm, and a water height of 25 cm were used as Maintenance media. The fish used in the present study was pangasius with a size of seven to nine cm, an average weight of 6.48 ± 0.68 g/head and a stocking density of up to one fish /l. The feed used in the present research is commercial pellet feed, which is Hi-Pro-Vite 781. The first step was to grind the pellet so that it became smaller. The ground pellet feed was then sieved to obtain the desired particles. The next+++ step was to weight the ground pellet. The Analysis of Variance (ANOVA) was applied to the data analysis. If a difference was found, the analysis was followed by Duncan's Multiple Range Test at a real level of 5% to determine whether the addition of lysine in commercial feed to fish has an effect (Adi and Nugroh, 2020).

RESULTS AND DISCUSSION

Based on Duncan's multiple range test, it can be seen that P0 ($p < 0.05$) was not significantly different in P1, P2, and P3 treatments. P1 was not significantly different in P0 and P2 but was significantly different in P3. The P3 treatment was not significantly different compared to P0 but was significantly different from P1 and P2 treatments. In summary, the lowest SFA content in pangasius meat was found in P3 treatment (3.5882%), while the highest SFA content was 5.6868% as found in the P1 treatment with the addition of essential amino acids by 1.2% (Table 1).

Based on Duncan's Multiple Range Test, P0 ($p < 0.05$) was not significantly different from treatments P1, P2, and P3. The treatment of P1 was not significantly different from P2, and P3. The lowest SFA content in pangasius meat was 3.5882% and could be found in the P3 treatment with the addition of lysine of 3.2%, while the highest SFA content was 5.6868% in the P1 treatment with the addition of 1.2% essential amino acids (Table 2).

Duncan's multiple range test results indicated that P3 was significantly different ($p < 0.01$) between P0, P1, and P2 treatments, while the P0 treatment was not significantly different from the P1 and P2 treatments. The lowest content of PUFA treatment in pangasius meat was found in the P0 treatment (15.7392%) without essential lysine, while the PUFA content was 23.1082% in the P3 treatment with the addition of 3.2% essential amino acids (Table 3).

The observations on water quality revealed that the lowest average temperature was 27.5 to 28.7 o C in treatment P1, while the highest temperature was 27.6-28.6oC in the treatment P0. The P1 treatment had the highest dissolved oxygen (DO) (4.81-6.92 mg/l), while the P0 treatment had the lowest DO (4.47-6.98 mg/l). The pH (7) and ammonia were the same in all treatment. Water quality is related to the metabolism and physiological processes in the absorption of feed, which were reported in the present study.

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Table 1. Average saturated fatty acid content of Pangasius fish (42 day-old) in Airlangga fish farm.

Treatment	SFA (%) + SD	SFA ± SD Transformation
P0 (0%)	4.9668^{ab} ± 1.4542	2.3205 ± 0.3203
P1 (1.2%)	5.6868^{a} ± 1.2616	2.4763 ± 0.2618
P2 (2.2%)	5.4032^{a} ± 0.6675	2.4266 ± 0.1358
P3 (3.2%)	3.5882^{b} ± 0.9282	2.0110 ± 0.2371

Different superscripts in the same column indicate differences ($p < 0.05$). SFA: Saturated Fatty Acid content, SD: Standard Deviation

Table 2. Average content of monounsaturated fatty acids of Pangasius Fish (42 day-old) in Airlangga fish farm.

Treatment	MUFA (%) + SD	MUFA Transformation ± SD
P0 (0%)	7.2466^{a} ± 1.1659	2.7774 ± 0.2014
P1 (1.2%)	5.7120^{b} ± 0.3942	2.4914 ± 0.0780
P2 (2.2%)	5.9630^{b} ± 0.7992	2.5384 ± 0.1557
P3 (3.2%)	5.5686^{b} ± 0.8716	2.4581 ± 0.1818

*Different superscripts in the same column show differences of ($p<0.05$). SD: Standard Deviation, MUFA: Monounsaturated Fatty Acids

Table 3. Average content of monounsaturated fatty acid of Pangasius Fish (42 day-old) in Airlangga fish farm.

Treatment	MUFA (%) + SD	MUFA Transformation ± SD
P0 (0%)	15.7392^{b} ± 4.2449	4.0003 ± 0.5439
P1 (1.2%)	15.2388^{b} ± 1.5001	3.9634 ± 0.1937
P2 (2.2%)	15.8804^{b} ± 1.1152	4.0454 ± 0.1386
P3 (3.2%)	23.1082^{a} ± 3.4405	4.8483 ± 0.3570

*Different superscripts in the same column show differences ($p<0.01$). SD: Standard Deviation, MUFA: Monounsaturated Fatty Acids

Table 4. Average results of water quality observation in Airlangga fish farm for rearing of Pangasius Fish.

Treatment	Temperature (^{o} C)	Dissolved oxygen mg/L	pH	Ammonia mg/l
P0	27.6 - 28.6	4.47 - 6.98	7	0.003
P1	27.4 - 28.7	4.81 - 6.92	7	0.003
P2	27.5 - 28.6	4.67 - 7.02	7	0.003
P3	27.5 - 28.7	4.74 - 7.08	7	0.003

The current research indicated positive results in which the saturated fatty acids content was reduced when the commercial feed was mixed with lysine at 3.2% (P_3), with the amount of lysine added being 2.1% (P_2) and 1.2% (P_1), respectively, the treatments indicated higher results than the control treatment (P_0). It is believed that the metabolic process and feed absorption in *Pangasius* species causing the supplemented amino acids in the commercial feed to be effective in reducing the saturated fatty acid content of pangasius meat. Treatment P1 and P2 revealed no decrease because the dose of the addition of lysine was in low level, and the absorption process in the body of pangasius was not optimal, suggesting that the addition of lysine did not decrease the SFA content of pangasius meat (Prananingtyas and Rahardja, 2019).

The data in table 1 indicated that the SFA content was 24.70%. While in the present study, the total percentage of saturated fatty acids was 4.9112%. The measurement of SFA was done using Analysis of Variants (ANOVA) and was obtained from two SFAs found in pangasius meat. It can be seen that the SFAs of pangasius have decreased due to the addition of the lysine in commercial feed. The lowest SFA content in the pangasius meat was 3.5882% found in the P3 treatment, while the highest UFA content was 5.5686% found in the P1 treatment (Tables 1 and 2; Pamula, 2019).

The different results could be seen by good feed intake in each treatment significantly. The efficient use of the feed indicated the value of the percentage of feed that could be utilized by the body of the fish (Glencross et al., 2007). Saturated fatty acids have only a single bond on their hydrocarbon chains. In the meat of pangasius, the most SFAs were palmitic, stearic, and myristic acids. Palmitic acid is the highest saturated fatty acid (16.74%). Pentadecanoic acid was the highest SFA in foods, accounting for 15-50% of all the fatty acids present (Putri and Dewi, 2019).

Based on the statistical calculations in table 3, adding lysine to the commercial feed caused a significant difference in the SFA content in the present study ($p < 0.05$). The myristic fatty acid content was 4.9668% for P0, 5.6868% for P1, 5.4032% for P2, and 3.5882% for P3, whereas the pentadecanoic acid average value was 4.5576% for P0, 4.9804% for P1, 4.559% for P2, and 3.0978% for P3.

The main effect of SFA was an increase in total cholesterol and LDL levels (LDL cholesterol) (Prananingtyas and Rahardja, 2019). The average intake of unsaturated fat to lower LDL cholesterol levels was 10% of total energy.

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Excessive consumption of saturated fat could cause the liver to produce large amounts of LDL cholesterol and increase blood cholesterol levels, which could later lead to thrombosis (Sonawane et al., 2017).

The content of MUFA in pangasius in the current study was quite high, with a size of 6-8 cm and a weight of 7-10 grams/head with a total amount of 6.1226% MUFA. In present experiment, the addition of lysine was proposed for increasing the MUFA level.

The present research indicated an increase in the total UFA level with the addition of lysine (1.2% P_1, 2.2% P_2, and 3.2% P3) to the commercial feed, which indicated lower results than the control treatment (P_0). This is presumably due to the pangasius metabolism in treatment P_1, P2, and P3, which differed from the pangasius metabolism in P_0. Thus, the addition of the lysine indicated no increase in the MUFA content of pangasius meat. According to Pamula (2019), the content of fatty acids in the body depended on the ability of the fish break down essential fatty acids both naturally, both anabolically and catabolically (Pamula, 2019).

Monounsaturated fatty acid test results revealed that there are four types of fatty acids, such as palmitoleic acid, elaidic acid, and eicosenoic acid. Monounsaturated fatty acids have one carbon- carbon double bond in its carbon chain. These fatty acids are usually olefinic compounds with configuration cis and the double bonds are usually in certain positions. The most common type of MUFA group is ω-9 with the position of a double bond on the 9th carbon atom of the carboxyl group (Djoussé et al., 2012).

The results of the statistical analysis indicated a very significant difference ($p < 0.01$) with each treatment. The PUFA level in pangasius meat increased due to the addition of the amino acid lysine in commercial feed. The highest PUFA level was found with the P3 treatment (23.1082%), while the lowest was in the P0 treatment (15.7392%).

β-oxidation is a process of synthesizing UFAs. Unsaturated fatty acids are more prone to oxidation and are easily exposed to foreign substances. However, β-oxidation could be prevented, because the metabolic process of UFA with the double bonds will be released at the stage of dehydrogenase II process. The process of dehydrogenase II would continue until the double bond is broken down into the acetyl-coA molecule.

The results of observations revealed that the good water temperature for pangasius is between 27.4 ^{o}C and 28.7^{o}C (Table 4). Drastic changes in temperature could kill the fish due to the changes in their blood carrying capacity. Temperature also affected the appetite of fish. Pangasius' appetite increased relatively in the morning and evening when the water temperature was between 27°C and 28°C. The increase in temperature in the medium is influenced by the rate of metabolism (Fernandes et al., 2014). The temperature, as a physical quality of water, could affect fish activities, such as breathing, growth and reproduction. One of the water parameters that is very important for fish growth and survival was temperature. Temperature has a powerful impact on fish as fish are ectothermic, which means that fish could not produce body heat, thus their body temperature is dependent on the surrounding environment (Fitriani et al., 2019).

CONCLUSION

The use of amino acid lysine in commercial feed indicated significant differences in the saturated fatty acids, MUFA and PUFA levels in pangasius. The addition of lysine in commercial feed needs further investigation in the context of pangasius growth and reproduction cycle in order to increase pangasius productivity.

DECLERATION

Author's contribution

W. Nopita, M. Lamid, and Agustono had continuous and similar attempts in conduction and collection of samples and data, writing manuscript, and analysis of data. Authors approved the final version of the manuscript.

Competing interests

The author did not report any conflicts of interest in the current research.

REFERENCES

Adi P and Nugroh IMN (2020). Depiction of connection between library and information science in articles published by universitas airlangga's academics. International Journal of Innovation, Creativity and Change, 12: 963-976. Available at: https://www.ijicc.net/images/vol12/iss12/121292_Nugroho_2020_E_R.pdf

Bayati Zadeh J, Moradi kor Z, and Moradi kor N (2017). Synchronization of energy and protein on supply synthesis microbial protein. International Journal of Advanced Biological and Biomedical Research, 5(4): 174-177. Available at: http://www.ijabbr.com/article_33969.html

Bell RC, Zahradka P, Aliani M, Liang Y, McCargar LJ, Chan C, Ozga J, Proctor S, Wishart D, and Taylor C (2017). Dried beans lower cholesterol and glycated hemoglobin while peas lower blood pressure in adults with mild hypercholesterolemia. Federation of American Societies for Experimental Biology Journal, 31: 913-966. DOI: https://www.doi.org/10.1096/fasebj.31.1_supplement.966.13

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Djoussé L, Akinkuolie AO, Wu JHY, Ding EL, and Gaziano JM (2012). Fish consumption, omega-3 fatty acids and risk of heart failure: a meta-analysis. Clinical Nutrition, 31: 846-853. DOI: https://www.dx.doi.org/10.1016%2Fj.clnu.2012.05.010

Fernandes CE, da Silva Vasconcelos MA, De Almeida Ribeiro M, Sarubbo LA, Andrade SAC, and de Melo Filho AB (2014). Nutritional and lipid profiles in marine fish species from Brazil. Food Chemistry, 160: 67-71. DOI: https://www.doi.org/10.1016/j.foodchem.2014.03.055

Fitriani EN, Arief M, and Suprapto H (2019). Prevalence and intensity of ectoparasites in gabus fish (Channa striata) at cangkringan fishery cultivation technology development center, Sleman, Yogyakarta, in: IOP Conference Series: Earth and Environmental Science. IOP Publishing, p. 12095. Available at: https://www.iopscience.iop.org/article/10.1088/1755-1315/236/1/012095

Glencross B, Booth M, and Allan G (2007). A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition, 13: 17-34. DOI: https://www.doi.org/10.1111/j.1365-2095.2007.00450.x

Jana A, Saroch JD, and Borana K (2014). Effect of Spirulina as a feed supplement on survival and growth of Pangasius sutchi. International Journal of Fisheries and Aquatic Studies, 1: 77-79. Available at: http://www.fisheriesjournal.com/archives/2014/vol1issue5/PartB/98.pdf

Kim NK, Zealous Gietbong F, Andriyono S, Kim AR, and Kim HW (2018). The complete mitogenome of Bagrid catfish Chrysichthys nigrodigitatus (Siluriformes: Claroteidae). Mitochondrial DNA Part B, 3: 1239-1240. DOI: https://www.doi.org/10.1080/23802359.2018.1532341

Laake I, Carlsen MH, Pedersen JI, Weiderpass E, Selmer R, Kirkhus B, Thune I, and Veierød MB (2013). Intake of trans fatty acids from partially hydrogenated vegetable and fish oils and ruminant fat in relation to cancer risk. International Journal of Cancer, 132: 1389-1403. DOI: https://www.doi.org/10.1002/ijc.27737

Liu XY, Wang Y, and Ji WX (2011). Growth, feed utilization and body composition of Asian catfish (Pangasius hypophthalmus) fed at different dietary protein and lipid levels. Aquaculture Nutrition, 17: 578-584. DOI: https://www.doi.org/10.1111/j.1365-2095.2011.00859.x

Mahmud A, Girmatsion M, and Abraha B (2020). Fatty acid and amino acid profiles and digestible indispensable amino acid score of grass carp (Ctenopharyngodon idella) protein concentrate supplemented noodles. Journal of Food Measurement and Characterization, 14: 2370-2379. DOI: https://www.doi.org/10.1007/s11694-020-00484-3

Pamula OYT (2019). Optimization on survival and growth rate of African catfish (Clarias sp.) using water spinach (Ipomoea aquatica)-based aquaponics system. Aquarium, Conservation and Legislation, 12: 716-723. Available at: https://www.ec.europa.eu/research/participants/documents/downloadPublic/WjZnY0hKTTdFM3YyT1gyblRldlc1QVF0MzFYNGNGdHZRVERSL3BqL3BpV3o5bEVhak1FN2FnPT0=/attachment/VFEyQTQ4M3ptUWNDRDc2cnlEcVhQRjdlbWdyeEQ4MXI

Prananingtyas D, and Rahardja S (2019). Effect of different salinity level within water against growth rate, survival rate (FCR) of catfish (Clarias sp.), in: IOP Conference Series: Earth and Environmental Science. IOP Publishing, P. 12035. Available at: https://www.iopscience.iop.org/article/10.1088/1755-1315/236/1/012035/pdf

Putri FP, and Dewi NN (2019). Growth monitoring of koi fish (Cypri nus carpio) in natural hatchery techniques in Umbulan, Pasuruan, East Java, in: IOP Conference Series: Earth and Environmental Science. IOP Publishing, p. 12016. DOI: https://www.iopscience.iop.org/article/10.1088/1755-1315/236/1/012016/pdf

Sokoła-Wysoczańska E, Wysoczański T, Wagner J, Czyż K, Bodkowski R, Lochyński S, and Patkowska-Sokoła B (2018). Polyunsaturated Fatty Acids and Their Potential Therapeutic Role in Cardiovascular System Disorders-A Review. Nutrients, 10: 1561. DOI: https://www.doi.org/10.3390/nu10101561

Sonawane PD, Pollier J, Panda S, Szymanski J, Massalha Yona M, Unger T, Malitsky S, Arendt P, and Pauwels L (2017). Corrigendum: Plant cholesterol biosynthetic pathway overlaps with phytosterol metabolism. Nature Plants, 3: 17101. DOI: https://www.doi.org/10.1038/nplants.2017.101

Van Doan H, Doolgindachbaporn S, and Suksri A (2014). Effects of low molecular weight agar and Lactobacillus plantarum on growth performance, immunity, and disease resistance of basa fish (Pangasius bocourti, Sauvage 1880). Fish Shellfish Immunology, 41: 340-345. DOI: https://www.doi.org/10.1016/j.fsi.2014.09.015

Weya JM, Rumbiak NS, Hariyanto S, Irawan B, and Soegianto A (2017). Length-weight relationship and condition factor of crayfish from South Sorong and Jayawijaya, Papua, Indonesia. Croatian Journal of Fisheries, 75: 18-24. DOI: https://www.doi.org/10.1515/cjf-2017-0004

Yoo G, and Lee J (2016). The effect of feeding frequency, water temperature, and stocking density on the growth of river puffer Takifugu obscurus reared in a zero-exchange water system. Fisheries and Aquatic Sciences, 19: 23. DOI: https://www.doi.org/10.1186/s41240-016-0024-x

To cite this paper: Nopita W, Lamid M and Agustono (2020). The Effects of Adding Lysin Essential Amino Acid to Commercial Feed on Fatty Acid Contents of Pangasius Fish. *World Vet. J.*, 10 (4): 653-657. DOI: https://dx.doi.org/10.54203/scil.2020.wvj78

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 658-664, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.29252/scil.2020.wv79

Effect of Heat Stress on Developmental Competence of *In Vitro* Matured Oocytes of *Camelus* Dromedaries with Different Qualities

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ORIGINAL ARTICLE
pii: S232245682000079-10
Received: 11 Oct 2020
Accepted: 24 Nov 2020

ABSTRACT

The deleterious effect of heat stress on cumulus-oocytes complexes (COCs) competence is well recognized in different livestock species. Therefore, the present study aimed to investigate the effect of physiologically relevant heat stress on the developmental competence of camel COCs during *in vitro* maturation (IVM). A total of 1548 COCs were divided into six groups in this study. The groups were named K1 and K2 representing good and low-quality COCs incubated at 38.5°C for 30 hours. While K3 and k4 represent good and low-quality COCs exposed to 41°C for the first 6 hours of IVM. Finally, K5 and k6 represent the groups of good and low-quality COCs exposed to 42°C for the first 6 hours of IVM. After exposure of COCs to heat stress at 41°C and 42°C during the first 6 hours of *in vitro* maturation, the COCs were incubated at 38.5°C for 24 hours of IVM. The *in vitro* matured COCs were activated to cleave using ethanol followed by 4 mM 6-DMAP and developed embryos were cultured *in vitro* for 7 days post parthenogenetic activation. The results of this study indicated that heat stress at 42°C significantly decreased the Pb (polar body) extrusion rate in K4 and K6, compared to other groups. Additionally, the embryo cleavage rate was significantly lower for good and low-quality oocytes exposed to heat stress (K2, K3, K4, K5, and K6), compared to good quality COCs of the control group (K1). The cleavage rate was lower for low quality (K2; 63 ± 1.28) than good quality COCs (K1; 53 ± 1.85). The percentages of oocytes that developed to the blastocyst stage were lower for K2, K3, K4, K5, and K6 than K1. Moreover, the blastocyst rate was lower for K2 (9 ± 0.22) than K1 (15 ± 0.22). The results of this study indicated that exposure of camel oocytes to heat stress for 6 hours during *in vitro* maturation severely reduced extrusion of polar body, cleavage, and blastocyst rates. The low-quality camel COCs were reduced developmental capacity than good quality oocytes.

Keywords: Camel, Embryo development, Heat stress, Oocyte

INTRODUCTION

Nowadays, there has been concerted attention to global warming, especially in tropical and subtropical regions, due to the increased level of average air temperature during recent years (Rabie, 2020). Egypt is located in a subtropical climatic region, which encourages the adaptation to the extreme heat stress and drought of the desert. In this regard, the Arabian camel, known as Camelus dromedaries, has developed unique anatomical as well as physiological and biochemical characteristics (Hoter et al., 2019) to cope with such challenges. Camelus dromedarius is a source for meat, wool, and milk in desert areas heavily affected by the heat and shortage of feed and water (Yaqoob et al., 2017).

Camels' population in Egypt consists of 148060 animals contributing to approximately 36398 tons of meat (i.e., 1.7 of total meat production in Egypt, (FAO, 2015). In response to climatic change progress, there is a promising role for camels due to their high resistance to heat and arid conditions. The reproductive performance of Camelus dromedaries is considered one of the most important factors affecting camel productivity (Tibary and El Allali, 2020). Heat stress has negative effects on the reproductive efficiency of dairy cows. It has been reported that heat stress could increase ovarian inactivity, which is manifested by anestrous (Wolfenson et al., 2000; Oseni et al., 2003). The reduction in estradiol biosynthesis is the mechanism by which heat stress deteriorates ovarian follicle development and impaired expression of estrus (Badinga et al., 1993; Wolfenson et al., 1988). The ovarian follicles and their enclosed oocytes are highly sensitive to hyperthermia (Roth, 2017; Islam et al., 2018). (Camargo et al., 2019) stated that heat shock increases the proportion of apoptotic oocytes during *in vitro* maturation (Camargo et al., 2019). The findings of a recent study suggest that camel oocytes are less tolerant of the short acute heat shock, which is linked with reduced ooplasm diameter and maturation rate as well as increased incidence of chromosomal count abnormalities (Islam et al., 2018). In fact, the slow development of assisted reproductive technologies, such as Artificial Insemination (AI), embryo transfer, and IVEP, represents a major obstacle in the wide application of these techniques in Camelus dromedaries (Abdoon et al., 2014). P

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Parthenogenetic activation of the metaphase II oocytes in such species as camelids provides the chance to evaluate the development ability of oocytes without the need to an external factor (sperms). Therefore, the present study was carried out to examine the effect of heat stress during *in vitro* maturation on the developmental competence of camel oocytes with different morphological qualities up to the blastocyst stage after parthenogenetic activation.

MATERIALS AND METHODS

This study was carried out at the Central Laboratory for Research, MSA University, Egypt. All the employed reagents and media used in the current study were obtained from Sigma–Aldrich (St. Louis, MO, USA) otherwise, their incorporations were addressed.

Ethical approval

The current study does not require an ethical approval as the main materials are ovaries that collected from slaughtered animals in local abattoir according to Minister of Agriculture regulations.

Experimental design

The control groups K_1 (control for the good morphological quality group) and K_2 (control for the low morphological quality group) were subjected to an incubation temperature of 38.5°C. The treated groups of good quality oocytes K_3 and K_4 were exposed to 41°C and 42°C, respectively. The treated groups of low quality oocytes K_5 and K_6 were exposed to 41°C and 42°C, respectively. The four treated groups (about 260 oocyte/group) were exposed to the assigned temperature for the first 6 hours of *in vitro* maturation, thereafter, the incubation temperature for all treatments decreased to 38.5°C for 24 hours until the end of IVM duration. Maturation rates of the K_1, K_2, K_3, K_4, K_5, and K_6 oocytes were estimated based on the expansion of cumulus cells and the Pb extrusion ratio, then all groups were subjected to parthenogenetic activation to follow up early embryo developmental rate until blastocyst stage.

Procedures

Ovaries collection and oocytes recovery

Camel ovaries were collected from nearby abattoirs. The collected ovaries were transported to the laboratory within 3 hours in pre-warmed (30°C) physiological saline (0.9% NaCl) supplemented with (100 µg/ml streptomycin sulfate and 100 IU/ml penicillin). The collected ovaries were washed once in 70% ethanol and then three times in phosphate buffer saline supplemented with (100 µg/ml streptomycin sulfate and 100 IU/ml penicillin). Cumulus–oocyte complexes (COCs) were aspirated from follicles 2-8 mm in diameter using a 10 mL disposable syringe with an 18-gauge needle. The recovered COCs were washed three times in Hepes-buffered Medium199 (22340; Gibco, UK) supplemented with 100 µg/ml streptomycin sulfate, 100 IU/ml penicillin, and 10% inactivated fetal bovine serum (FBS). Cumulus-oocyte complexes were classified into good (grade A and B) and low quality (grade C) based on morphological assessment. The assessment of oocyte quality was based on the number of cumulus cell layers and the homogeneity of cytoplasm (Mesbah et al., 2016).

In vitro maturation of oocytes

The *in vitro* maturation medium is a basic maturation medium (TCM199) supplemented with 20 µg/mL follicle-stimulating hormone, 10% FBS, 10 ng/mL epidermal growth factor, 100 µg/ml streptomycin sulfate, and 100 IU/ml penicillin. The COCs were washed twice in IVM medium according to the experiment design. A total of 25-30 COCs/well were cultured in 400 µl of IVM medium overlaid with 400 µl mineral oil in 4 well culture plate (Nunc, Denmark) under 5% CO_2 and humidified air for 30 hours. Examination of matured oocytes was performed after 30 hours of incubation and based on cumulus expansion and extrusion of the first polar body using Optica stereomicroscope (SZM-LED1, Italy) and Leica inverted microscope.

Parthenogenetic activation and culture

The matured oocytes were denuded by gentle repetitive pipetting in a 0.1% hyaluronidase solution (Irivine Scientific, CA 92705 USA). Cumulus-free oocytes were then incubated in TCM-199 containing 10% FBS supplemented with 7% ethanol for 7 min (Wani, 2007) in a dark chamber. Oocytes were then washed in TCM-199 with 10% FBS, then transferred to 100-µL micro-drops of 4 mM 6-dimethylaminopurine (6-DMAP, D-2629), covered with mineral oil, and cultured in a humid atmosphere of 5% CO2 at 38.5°C for 4 hours. After activation, activated oocytes were washed twice in the *in vitro* culture medium (Continuous Single Culture -NX Complete, cat no. 90168, Irivine Scientific, CA 92705 USA) placed into 4-well culture Plates (400 µl culture media per well) under mineral oil in the humidified atmosphere of 5% CO2 at 38.5^{o}C. Cleavage rate (2-8 cells) was recorded 48 hours after culture. The number of embryos developed to the blastocyst stage was assessed 7 days after *in vitro* culture.

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Statistical analysis

Experimental Statistical analysis for maturation rate (expansion level and extrusion of the polar body) and embryonic development rates were performed using a randomized complete block design with six replications for each group using the web Agri Stat Package. The treatment means were compared by the least significant difference (LSD) test as reported by Snedecor and Cochran (1994) using ASSISTAT software, version 7.7 beta freeware. P-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effect of heat stress on maturation rate of camel oocytes

Climate change has raised tangible concerns about global warming representing a significant threat to the feasibility and sustainability of livestock farming worldwide, particularly in regions located in tropical and subtropical zones. Egypt is located in a subtropical area, where the intense heat stress of summer is accompanied by high ambient humidity. This issue worsens the situation and represents a constraint to farm animal welfare, productivity, and reproductive performance (Marai and Habeeb, 2010). In this regard, cumulus-oocyte complexes are highly affected when the female is exposed to environmental heat stress during follicular development, ovulation and *in vivo* events of oocyte maturation (Al-Katanani et al., 2002; Gendelman and Roth, 2012; Sadeesh et al., 2016).

There is a growing interest in establishing and improving assisted reproductive techniques, such as *in vitro* embryo production in camel. In addition, the *in vitro* maturation of oocyte is the key step for improving the success rate of *in vitro* embryo production in this species (Trasorras et al., 2013; Mesbah et al., 2016; Moulavi and Hosseini, 2018). Accordingly, cumulus expansion and extrusion of the first polar body are the main criteria used to evaluate the nuclear maturation of camel oocytes (Yaqoob et al., 2017; Saadeldin et al., 2019). The data of the current study indicated no change in the incidence of cumulus expansion in good quality COCs before (92.98 ± 0.95%) and after heat shock exposure at 41°C (93.61 ± 1.32%) and 42°C (93.96 ± 0.64%). However, the low quality COCs demonstrated no sign of cumulus expansion, which may be due to decreased number of cumulus layers in this quality category or low expression of TGFβ genes. In support to this idea, the lowest first polar body extrusion rate (11.2% ± 2.5%) was found in the group of camel oocytes treated with the inhibitor of the TGFβ pathway (SB-431542) which interferes with the activity of activin receptor-like kinases accompanied by no expansion of cumulus cells (Saadeldin et al., 2019).

The results of this study indicated that the heat stress has the same effect on both good and low-quality oocytes concerning nuclear maturation represented by Pb extrusion percentage and cytoplasmic maturation represented by cumulus expansion score. The cumulus expansion level was significantly higher in good quality oocyte groups K1 ($P < 0.01$, 92.98 ± 0.95%), K3 (93.61 ± 1.32%), and K4 (93.96 ± 0.64%) compared to low quality oocyte groups of K2 (0%), K5 (0%), and K6 (0%). Moreover, exposure of COCs to heat stress at 42^{o}C significantly decreased the Pb ratio in K4 ($P < 0.01$, 20.73 ± 0.49%) and K6 (20.30 ± 0.52%) compared to the control K1 (32.31 ± 0.54%), K2 (30.98 ± 1.36%), and heat-stressed groups at 41^{o}C K3 (31.44 ± 0.92%) and K5 (31.63 ± 0.47%). Indeed, buffalo cumulus-oocyte complexes that were collected during hot season had a high percentage of arrested oocytes in metaphase I stage after in vitro maturation (Abdoon et al., 2014). Interestingly, bovine oocytes that have been exposed to heat shock at 40.0°C and 41.0°C recorded lower rates of nuclear and cytoplasmic maturation (Maya-Soriano et al., 2013). Similarly, El-Sayed et al. (2018) have indicated that exposure of buffalo COCs to heat shock reduced the percentage of in vitro matured oocytes at temperature of 39.5°C and 40.5°C compared with that of non-treated control group (38.5°C).

Effect of heat stress on the development rate of in vitro produced camel embryos from oocytes with different quality

After parthenogenesis, development rate until 2-8 cells embryo was lower ($P < 0.01$) for good (K3= 50 ± 0.92 and K4= 55 ± 1.58) and low (K5= 45 ± 1.28 and K6= 27 ± 0.56) quality oocytes exposed to heat stress, compared with control K1 (63 ± 1.28) and K2 (53 ± 1.85). Moreover, development rate up to 2-8 cells embryo was lower ($P < 0.01$) for low quality oocytes exposed to heat stress K5 (45 ± 1.28) and K6 (27 ± 0.56) compared with good quality oocytes exposed to the same heat stress K3 (50 ± 0.92) and K4 (55 ± 1.58). The development rate was significantly lower for control low quality ($P < 0.01$, 53 ± 1.85) compared to the control good quality oocytes (63 ± 1.28). Bovine oocytes exposed to heat stress at 40.0°C and 41.0°C reduced in vitro embryo development rate (Maya-Soriano et al., 2013). Saadeldin et al. (2018) demonstrated that camel oocytes exposed to short acute heat shock at 45°C for 2, showed reduction in maturation rate, linked with decreased ooplasmic diameter and increased percentage of chromosomal abnormalities.

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The percentage of oocytes developed to the blastocyst stage was significantly lower for good quality oocytes exposed to heat stress K3 and K4 ($P < 0.01$), compared with control K1. Blastocyst rate was significantly lower for low quality oocytes exposed to heat stress 42°C K6 ($P < 0.01$), compared with low quality oocytes exposed to 41°C K5 and control low quality oocytes K2 (0, 3 ± 0.22, and 9 ± 0.22, respectively). Blastocyst rate was lower for control low quality oocytes K2 compared with control good quality oocytes K1 ($P < 0.01$, 9 ± 0.22, and 15 ± 0.22, respectively). There was no significant difference between good quality oocytes exposed to 42°C K4 and low-quality oocytes exposed to 41°C (2 ± 0.21 and 3 ± 0.22, respectively). In support to previous observation, experimental exposure of COCs to heat shock during the maturation has revealed reduced cleavage rate as well as blastocyst development (Edwards and Hansen, 1997). In addition, *in vivo* experiments have reported reduced pregnancy rate by 25% for each °C elevation in body temperature, which is due to the negative impact of heat stress on preimplantation development embryos (Nabenishi et al., 2012). Recently, heat shock at 40°C for 24 h during IVM of bovine COCs had no effect on Pb extrusion rate however; it reduced the rate of embryo cleavage and blastocyst development (Pöhland et al., 2020).

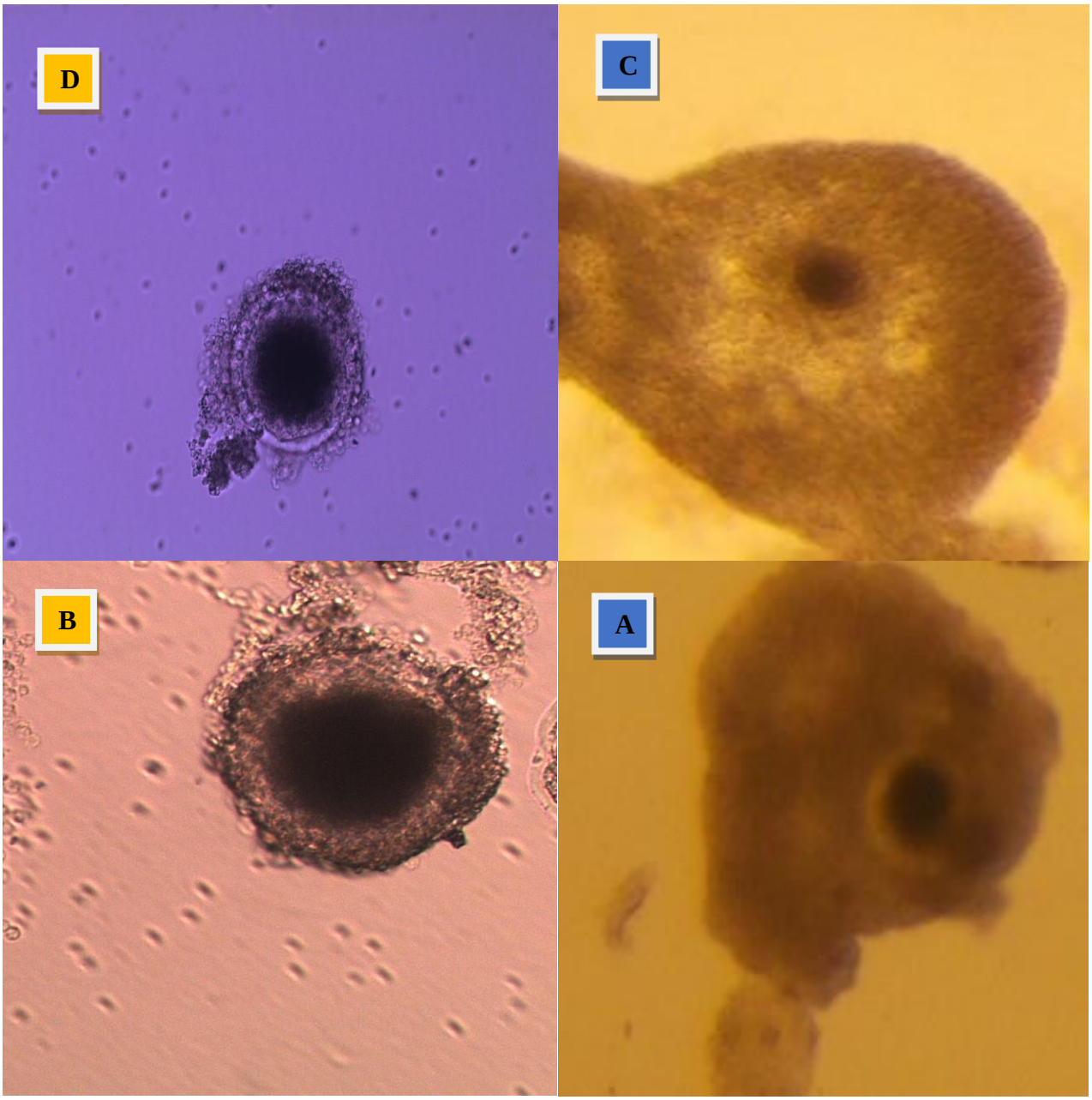

Figure 1. Immature and mature oocytes with different quality. **A**: Immature good quality oocyte. **B**: Immature low quality oocyte. **C**: Mature good quality oocyte. **D**: Mature low quality oocyte

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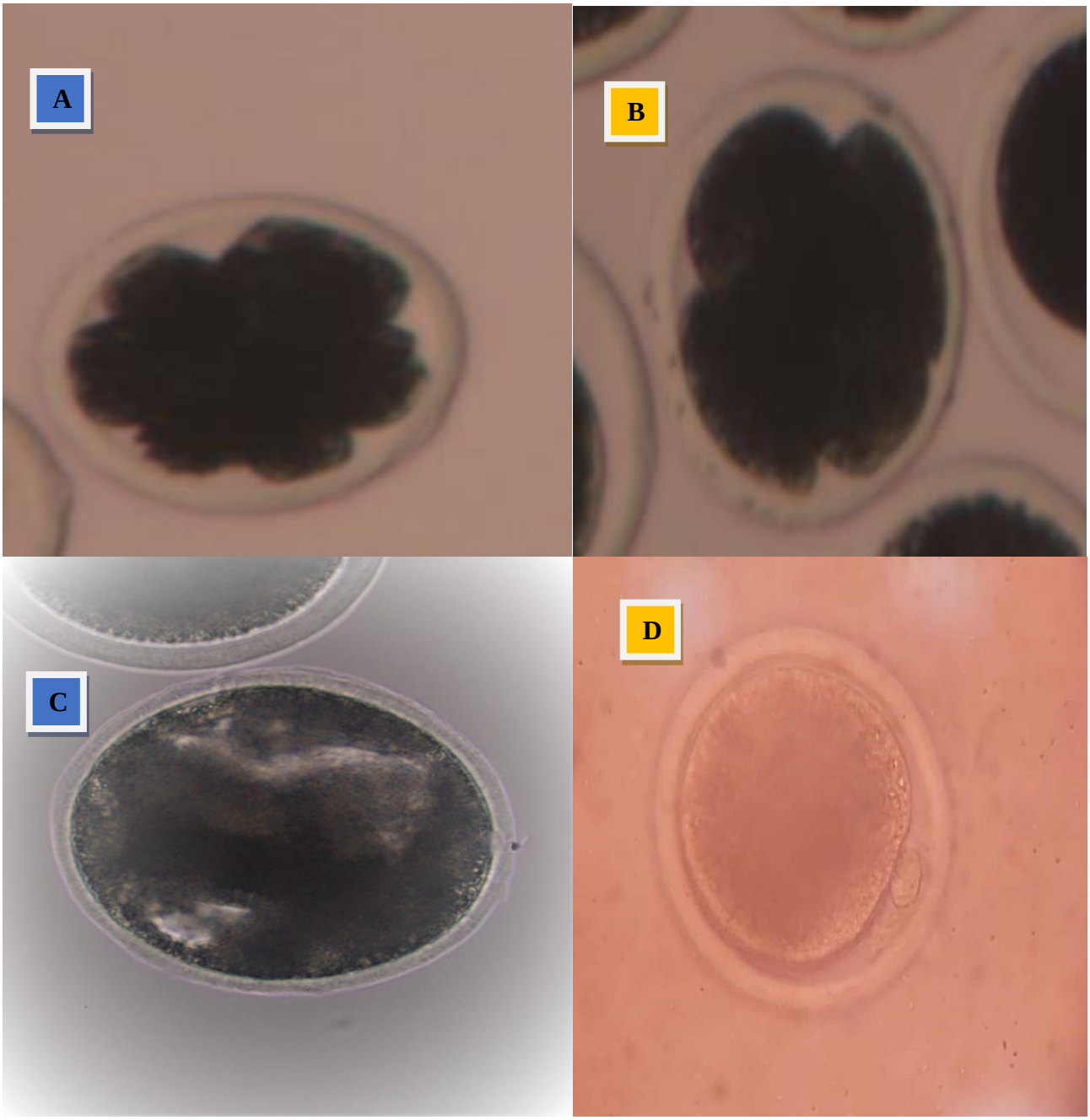

Figure 2. Polar body and different developmental stages of camel embryos. **A**: Blocked embryo. **B**: Four cell stage embryo. **C**: Blastocyst**. D**: Mature oocyte with polar body.

Table 1. Effect of heat stress on developmental rate of *in vitro* produced camel embryos from oocytes with different quality

group	Oocytes (n)	Cumulus expansion (%)*	Matured oocytes (n)**	Polar body extrusion (%)***	Polar body (n)
K1	266	92.98 ± 0.95^{a}	247 ± 6.34^{a}	32.31 ± 0.54^{a}	86 ± 2.25a
K2	273	0^{b}	0^{b}	30.98 ± 1.36^{a}	87 ± 2.77a
K3	230	93.61 ± 1.32^{a}	216 ± 4.03^{a}	31.44 ± 0.92^{a}	72 ± 1.21b
K4	275	93.96 ± 0.64^{a}	258 ± 7.01^{a}	20.73 ± 0.49^{b}	57 ± 1.59c
K5	282	0^{b}	0^{b}	31.63 ± 0.47^{a}	89 ± 2.47a
K6	222	0^{b}	0^{b}	20.30 ± 0.52^{b}	45 ± 0.85c

$^{a,b;}$ Means within a column with different superscripts differ significantly (P < 0.01). *Evaluated after 30 h. **Based on cumulus cells expansion. ***Based on polar body extrusion. K1: Good quality oocytes incubated at 38.5°C for 30 hours; K2: Low quality oocytes incubated at 38.5°C for 30 hours; K3: Good quality oocytes exposed to 41°C for the first 6 hours of maturation; K4: Good quality oocytes exposed to 42°C for the first 6 hours of maturation; K5: Low quality oocytes exposed to 41°C for the first 6 hours of maturation; K6: Low quality oocytes exposed to 42°C for the first 6 hours of maturation. n: Number

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Table 2. Effect of heat stress on development rate of *in vitro* produced camel embryos from oocytes with different quality

group	Oocytes (n)	Development 2-8 cells (n)*	Blastocyst (n)**	blocked embryos (n) ***
K1	266	63 ± 1.28^{a}	15 ± 0.22^{a}	48 ± 1.06^{e}
K2	273	53 ± 1.85^{c}	9 ± 0.22^{b}	44 ± 1.36^{d}
K3	230	50 ± 0.92^{b}	6 ± 0^{c}	$44 \pm .92^{c}$
K4	275	55 ± 1.58^{bc}	2 ± 0.21^{de}	$53 \pm 1.45a^{b}$
K5	282	45 ± 1.28^{d}	3 ± 0.22^{d}	$42 \pm 1,06^{b}$
K6	222	27 ± 0.56^{e}	0^{e}	27 ± 0.56^{a}

[a,b,c,d,e;] Means within a column with different superscripts differ significantly ($P < 0.01$). *Calculated as % of total oocytes. **Calculated as % of cleaved embryos. ***Calculated as % of cleaved embryos. K1: Good quality oocytes incubated at 38.5°C for 30 hours; K2: Low quality oocytes incubated at 38.5°C for 30 hours; K3: Good quality oocytes exposed to 41°C for the first 6 hours of maturation; K4: Good quality oocytes exposed to 42°C for the first 6 hours of maturation; K5: Low quality oocytes exposed to 41°C for the first 6 hours of maturation; K6: Low quality oocytes exposed to 42°C for the first 6 hours of maturation. n: Number

CONCLUSION

The results of this study indicated that the exposure of camel oocytes to heat stress during the first 6 hours of *in vitro* maturation could severely affect their developmental competence. The low morphologically quality COCs have no signs of cumulus expansion and had lower development competence than good quality after exposure to *in vitro* heat shock.

DECLARATIONS

Authors' contribution

All authors have contributed equally in laboratory works, the experimental design, writing, and revision of the manuscript.

Competing interests

All authors declare no competing interests that might interfere with the data provided in the current manuscript.

REFERENCES

Abdoon AS, Gabler C, Holder C, Kandil OM, and Einspanier R (2014). Seasonal variations in developmental competence and relative abundance of gene transcripts in buffalo (Bubalus bubalis) oocytes. Theriogenology, 82(8): 1055-1067. DOI: https://www.doi.org/10.1016/j.theriogenology.2014.07.008

Al-Katanani YM, Paula-Lopes FF, and Hansen PJ (2002). Effect of Season and Exposure to Heat Stress on Oocyte Competence in Holstein Cows. Journal of Dairy Science, 85(2): 390-396. DOI: https://www.doi.org/10.3168/jds.S0022-0302(02)74086-1

Badinga l, Thatcher WW, Diaz T, Drost M, and Wolfenson D (1993). Effect of environmental heat stress on follicular development and steroidgenesis in lactating Holstein cows. Theriogenology, 39: 797. DOI: https://www.doi.org/10.1016/0093-691X(93)90419-6

Camargo LSA, Costa FQ, Munk M, Sabine WV, Raquel VS, Bruno CC, Paulo HCJr, Alex CV, Luiz AGN, Joao HMV et al. (2019). Contrasting effects of heat shock during *in vitro* maturation on development of *in vitro*-fertilized and parthenogenetic bovine embryos. Reproduction Domestic Animals, pp. 1-9. DOI: https://www.doi.org/10.1111/rda.13544

Edwards JL, and Hansen PJ (1997). Differential responses of bovine oocytes and preimplantation embryos to heat shock. Molecular Reproduction and Development, 46(2): 138-145. DOI: https://www.doi.org/10.1002/(SICI)1098-2795(199702)46:2<138::AID-MRD4>3.0.CO;2-R

El-Sayed A, Nagy R, El-Asheeri A, and Eid L (2018). Developmental and molecular responses of buffalo (Bubalus bubalis) cumulus–oocyte complex matured *in vitro* under heat shock conditions. Zygote, 26(2): 177-190. DOI: https://www.doi.org/10.1017/S0967199418000072

Food Agriculture Organization (FAO) (2015). Climate change and food systems: global assessments and implications for food security and trade. Food Agriculture Organization of the United Nations, Available at: http://www.fao.org/3/a-i4332e.pdf

Gendelman M, and Roth Z (2012). Seasonal effect on germinal vesicle-stage bovine oocytes is further expressed by alterations in transcript levels in the developing embryos associated with reduced developmental competence. Biology of Reproduction, 86(1): 1-9. DOI: https://www.doi.org/10.1095/biolreprod.111.092882

Hoter A, Rizk S, and Naim HY (2019). Cellular and Molecular Adaptation of Arabian Camel to Heat Stress. Front. Genet, 10: 588. DOI: https://doi.org/10.3389/fgene.2019.00588.

Islam M Saadeldin, Ayman Abdel-Aziz Swelum, Mona Elsafadi, Amer Mahmood, Musaad Alfayez, and Abdullah N Alowaimer (2018). Differences between the tolerance of camel oocytes and cumulus cells to acute and chronic hyperthermia, Journal of Thermal Biology, 47: 47-54. DOI: https://www.doi.org/10.1016/j.jtherbio.2018.03.014

Marai IFM, and Habeeb AAM (2010). Buffalo's biological functions as affected by heat stress - A review. Livestock Science, 127(2): 89-109. DOI: https://www.doi.org/10.1016/j.livsci.2009.08.001

Maya-Soriano MJ, López-Gatius F, Andreu-Vázquez C, and López-Béjar M (2013). Bovine Oocytes Show a Higher Tolerance to Heat Shock in the Warm Compared with the Cold Season of the Year. Theriogenology, 79(2): 299-305. DOI: https://www.doi.org/10.1016/j.theriogenology.2012.08.020

Mesbah F, Kafi M, and Nili H (2016). Cumulus cell expansion and first polar body extrusion during *in vitro* oocyte maturation in relation to morphological and morphometric characteristics of the dromedary camel ovary. Reproduction Domestic Animals, 51(6): 916-923. DOI: https://www.doi.org/10.1111/rda.12758

Moulavi F, and Hosseini SM (2018). Diverse patterns of cumulus cell expansion during in *vitro* maturation reveal heterogeneous cellular and molecular features of oocyte competence in dromedary camel. Theriogenology, 119: 259-267. DOI: https://www.doi.org/10.1016/j.theriogenology.

Nabenishi H, Ohta H, Nishimoto T, Morita T, Ashizawa K, and Tsuzuki Y (2012). The effects of cysteine addition during *in vitro* maturation on the developmental competence, ROS, GSH and apoptosis level of bovine oocytes exposed to heat stress. Zygote, 20(3): 249-259. DOI: https://doi.org/www.10.1017/S0967199411000220

Oseni S, Misztal I, Tsuruta S, and Rekaya R (2003). Seasonality of days open in US Holsteins. Journal of Dairy Science, 86: 3718-3725. DOI: https://www.doi.org/10.3168/jds.S0022-0302(03)73977-0

Pöhland R, Souza-Cácares MB, Datta TK, Vanselow J, Martins MIM, da Silva WAL, Cardoso CJT, and Melo-Sterza FA (2020). Influence of long-term thermal stress on the in vitro maturation on embryo development and Heat Shock Protein abundance in zebu cattle. Animal Reproduction, 17(3): e20190085. DOI: https://www.doi.org/10.1590/1984-3143-ar2019-0085

Rabie TSKM (2020). Potential Climate Change Impacts on Livestock and Food Security Nexus in Egypt. In: Ewis Omran ES, Negm A. (eds) Climate Change Impacts on Agriculture and Food Security in Egypt. Springer Water. Springer, Cham, pp. 423-450. DOI: https://www.doi.org/10.1007/978-3-030-41629-4_17

Roth Z (2017). Effect of Heat Stress on Reproduction in Dairy Cows: Insights into the Cellular and Molecular Responses of the Oocyte. Annual Review of Animal Biosciences, 8(5): 151-170. DOI: https://www.doi.org/10.1146/annurev-animal-022516-022849

Saadeldin IM, Swelum AA, Elsafadi M, Mahmood A, Yaqoob SH, Alfayez M, and Alowaimer AN (2019). Effects of all-trans retinoic acid on the *in vitro* maturation of camel (Camelus dromedarius) cumulus-oocyte complexes. Journal of Reproduction and Development, 14(3): 215-221. DOI: https://www.doi.org/10.1262/jrd.2018-073

Saadeldin IM, Swelum AA-A, Elsafadi M, Mahmood A, Alfayez M, and Alowaimer AN (2018). Differences between the tolerance of camel oocytes and cumulus cells to acute and chronic hyperthermia. Journal of Thermal Biology, 74: 47-54. DOI: https://www.doi.org/10.1016/j.jtherbio.2018.03.014

Sadeesh EM, Sikka P, Balhara AK, and Balhara S (2016). Developmental Competence and Expression Profile of Genes in Buffalo (Bubalus Bubalis) Oocytes and Embryos Collected Under Different Environmental Stress. Cytotechnology, 68(6): 2271-2285. DOI: https://www.doi.org/10.1007/s10616-016-0022-y

Snedecor GW, and Cochran WG (1994). Statistical Methods. 9th Ed., Iowa State Univ. Press, Ames, Iowa, USA, Available at: https://journals.sagepub.com/doi/abs/10.3102/10769986019003304

Tibary A, and El Allali K (2020). Dromedary camel: A model of heat resistant livestock animal. Theriogenology, 154: 203-211. DOI: https://doi.org/10.1016/j.theriogenology.2020.05.046

Trasorras V, Giuliano S, and Miragaya M (2013). *In vitro* production of embryos in South American Camelids. Animal Reproduction Science, 136: 187-193. DOI: https://www.doi.org/10.1016/j.anireprosci.2012.10.009

Wani NA (2007). Chemical activation of in vitro matured dromedary camel (Camelus dromedarius) oocytes: Optimization of protocols. Theriogenology, 69: 591-602. DOI: https://www.doi.org/10.1016/j.theriogenology.2007.11.011

Wolfenson D, Flamenbaum I, and Berman A (1988). Dry period heat stress relief effects on prepartum progesterone, calf birth weight and milk production. Journal of Dairy Science, 71: 809-818. DOI: https://www.doi.org/10.3168/jds.S0022-0302(88)79621-6

Wolfenson, D, Roth Z, and Meidan R (2000). Impaired reproduction in heat-stressed cattle: Basic and applied aspects. Animal Reproduction Science, 60-61: 535-547. DOI: https://www.doi.org/10.1016/S0378-4320(00)00102-0

Yaqoob SH, Saadeldin IM, Swelum AA-A, and Alowaimer AN (2017). Optimizing camel (Camelus dromedarius) oocytes *in vitro* maturation and early embryo culture after parthenogenetic activation. Small Ruminants Research, 153: 81-86. DOI: https://www.doi.org/10.1016/j.smallrumres.2017.05.011

Yaqoob, Hilal S, Saadeldin, Islam M, Swelum, Ayman A, Al-Owaimer, and Abdullah N (2017). Optimizing camel (*Camelus dromedarius*) oocytes *in vitro* maturation and early embryo culture after parthenogenetic activation. Small Ruminant Research, DOI: https://www.dx.doi.org/10.1016/j.smallrumres.2017.05.011.

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2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 665-669, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj80

The Effect of Dietary Supplementation of Cod Liver Oil on Ratio of Saturated and Unsaturated Fatty Acids in Giant Prawn (*Macrobrachium rosenbergii*) Meat

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ORGINAL ARTICLE
pii: S232245682000080-10
Received: 09 Oct 2020
Accepted: 20 Dec 2020

ABSTRACT

The existence of feed plays an important role in aquaculture activities. This is due to the dominant influence on fish feed growth. Feeding with the right nutritional components can produce healthy and high-quality fish products. One of the nutrients needed by fish is fatty acids. In fact, the provision of fatty acids, one of which is not in the meat, has an important influence. So, this study aims to determine the effect of adding cod liver oil to commercial feed on the ratio of saturated and unsaturated fatty acids to the meat of giant prawn. This research was conducted experimentally with a completely randomized design. The treatment is given a dose of cod liver oil 0% (control), and treatments 1-4 use 3% dose addition to each treatment. On the other hand, in the data analysis stage, the researchers used ANOVA and continued with the Duncan's test. Based on the results, the study notes that the administration of cod liver oil in commercial feed does not affect the decreasing content of saturated fatty acids in giant prawn meat. On the other hand, the results also showed that the best ratio was found in treatment 4 at a dose of 12%. Therefore, it is concluded that the provision of nutrients for feed related to fatty acids in the pole has not reduced the content of saturated fatty acids. As well as, the best ratio of saturated fatty acids and unsaturated fatty acids was 1.21:1 with cholesterol content of 88.34 mg/dl and this ration resulted by the greatest growth rate in present study.

Key words: Cod liver oil, Feed, Giant prawn, Saturated fatty acids

INTRODUCTION

Prawn in Indonesia is produced from the results of capture and cultivation, and Giant prawn cultivation has developed quite rapidly in the country. In this regard, it can be seen that the production of giant prawns in 2013 reached 3.171 tons (Indonesia report, 2013).

In overall, feed plays an important role in aquaculture activities because it serves a dominant role on fish growth in term of economic (Melianawati and Suwirya, 2010). Feeding with the right nutritional components can produce healthy and high-quality fish products. One of the nutrients needed by fish is fatty acids (Pangkey, 2011). Fatty acids are collected into two types, based on the degree of saturation; namely saturated and unsaturated fatty acids (Bragagnolo and Rodriguez-Amaya, 2001; Venugopal and Gopakumar, 2017; Allen et al., 2019). Saturated fatty acids do not affect oxidation and free radical formation as do unsaturated fatty acids. The dominant effect of saturated fatty acids is an increase in total cholesterol and LDL (Low-Density Lipoprotein) cholesterol (Sartika, 2008). On the other hand, it is known that giant prawns have 35% saturated fatty acid levels and 139 mg/100g cholesterol levels which was higher than sea shrimp (Bragagnolo and Rodriguez-Amaya, 2001; Venugopal and Gopakumar, 2017; Allen et al., 2019). The lack of unsaturated fatty acids will cause disruption in fish health including reduced fecundity, ability to form embryos and abnormal growth (Pangkey, 2011; de Carvalho and Caramujo, 2018).

Fat does indeed play an important role in increasing growth rate in aquacultures (Stoneham et al., 2018). Meanwhile, the percentage of saturated fatty acids can be reduced by adding essential fatty acids (Stoneham et al., 2018). Essential fatty acids include unsaturated fatty acids that have a double-bond that cannot be synthesized in the body of animal, so it needs to be intake through feed (Meliandasari et al., 2016). In fact, cod liver oil can be supplemented to increase the content of unsaturated fatty acids in diet (Turchini et al., 2009). It is known that unsaturated fatty acids are divided into 2 namely monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). MUFA has one double carbon bond, which can occur in any position. PUFA has more than one double carbon bond, if the first double bond is found between the third and fourth carbon bonds it is called ω-3 fatty acids. The first double bond between the sixth and seventh carbon atoms is called ω-6 (Rustan and Drevon, 2005). Cod liver oil have a higher content of unsaturated fatty acids, and more than saturated-fatty acids (Das et al., 2007). So, present study was conducted with the aim to determine the effect of adding cod liver oil to commercial feed on the ratio of saturated and unsaturated fatty acids to giant prawn meat.

To cite this paper: Marzuki L, Agustono and Rahardja BS (2020). The Effect of Dietary Supplementation of Cod Liver Oil on Ratio of Saturated and Unsaturated Fatty Acids in Giant Prawn *(Macrobrachium rosenbergii)* Meat. *World Vet. J.*, 10 (4): 665-669. DOI: https://dx.doi.org/10.54203/scil.2020.wvj80

MATERIALS AND METHODS

This experiment was carried out at Prigi Brackish Aquaculture Plant, Trenggalek- East Java. Moreover, the research activities were carried out in June to July 2016.For the analysis of cod liver oil fatty acids and giant prawn meat, the activities were conducted on unit testing service at Faculty of Pharmacy, Universitas Airlangga, Surabaya.

The tools used include 20 pieces of aquarium measuring 35×20×25 cm^3, aerators, aerated hoses, *sipon* (suction device), aeration stones, large plastic tubs, measuring cups, digital scales, thermometers, pH meters, DO meters, ammoniates test kits. On the other hand, the main ingredient used is giant prawns (*Macrobrachium rosenbergii*) weighing 9 grams. Other ingredients include cod liver oil baby's Docosahexaenoic Aacid (DHA) brand, fish commercial feed, and chlorine (National Chlorine Industries Co. Ltd, Japan).

This research was carried out using an experimental method that is an experiment in the field or testing in a laboratory (Arifin, 1998). The design was based on Completely Randomized Design (CRD) with a diversity of cod liver oil (Kusriningrum, 2008). This study used five treatments with four replications in each treatment. Determination of the number of repetitions in each treatment, then, the following formula is as follows:

$t (n-1) \geq 15$

Notes: t = the treatments tested; n = number of tests or groups.

The dosage of cod liver oil in each feed was 0% (control), 3%, 6%, 9%, and 12%. Determination of the dose is based on the results of study (Faradilah, 2015) that has been done at a dose of 9% of the amount of feed which shows the best decrease in cholesterol in *vannamei* shrimp meat with the addition of cod liver oil.

Water quality

Water quality parameters measured during the study were temperature, pH, ammonia and Dissolved Oxygen (DO). The temperature was measured three times per day during the study, which was carried out at 06.00, 12.00, and 16.00 *WIB* (Indonesia's West Time Zone). Moreover, the measurement of pH and DO was done once a week while ammonia measurements are carried out twice during the study on the first day and the last day. This water quality measurement aims to monitor water quality conditions for giant prawns during maintenance. Temperature measurements were carried out three times a day, i.e. at 6:00 a.m. (Indonesia's West Time Zone), noon at 12.00 pm and evening at 4pm using a thermometer. Observation of temperature in the range 28-29 ° C. Measurement of the degree of acidity was done 3 times per day using a pH pen. From the observations, the acidity content is 6.5-7.5. This is in accordance with the statement where the optimal pH for giant prawn ranges from 6.0 to 8.5 (Erlangga, 2012). Measurement of marine oxygen (DO) was done once a week using a DO meter. DO observation results obtained 6-7 mg/l. This is appropriate where giant prawns can live in dissolved oxygen content in water that can support shrimp life which is between 4-8 mg/L (Fatagar, 2014). Ammonia is measured once a week using test-kit ammonia. In this study, ammonia content of 0.02-0.75 mg/l was obtained. This is not appropriate where ammonia for giant prawn ranges from 0-0.3 (Arthur, 2004). This difference is influenced by the rest of the feed, where changes in ammonia can be caused by temperature, pH and feed (Kamarudin et al., 1994).

RESULTS AND DISCUSSION

Saturated fatty acids

The results showed that the value of giant prawn saturated fatty acid content ranged from 54.87% - 59.13%. Data on the average content of giant prawn saturated fatty acids are in table 1. Calculation of Analysis of Variants (ANOVA) showed no significant difference between each treatment ($P> 0.05$). Duncan's test results showed that P_0 was not significantly different with P_1, P_2, P_3 and P_4 ($P>0.05$). From the *in vitro* analysis, the cod liver oil fatty acids obtained 8.9% content of dietary saturated fatty acids. When supplementing to commercial feed with a composition of P_0 (0%) obtained analysis of saturated fatty acids of 11.85%, P_1 (3%) has a saturated fatty acid content of 10.496%, P_2 (6%) of saturated fatty acid content of 10.496%, P_3 (9%) 8.99% and P_4 (12%) 8.97% data on saturated fatty acid content. A slight difference in the one factor; that supplement does not affect saturated fatty acids content (Table 1). This is in accordance with the feeding of fortified components of essential fatty acids which is an alternative method of regulating fat in livestock products (Legowo, 2004). The results presented in table 1 were not significantly different. It could be caused by the absorption of feed that it was not the same amount for each treatment.

Unsaturated fatty acids

The results showed the value of unsaturated fatty acid content of giant prawns ranged from 40.87% - 45.14%. Data on the average content of unsaturated fatty acids on giant prawn in table 2. The calculation of Analysis of Variants (ANOVA) showed that there is no significant difference ($P> 0.05$) between each treatment. Duncan's distance test results show that P_0 is not significantly different ($P> 0.05$) with P_1, P_2, P_3 and P_4. The calculation of Analysis of Variant

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(ANOVA) results showed that there is no significant difference (P> 0.05) for each treatment. This may be caused by the utilization of unsaturated fatty acids as energy and growth (possible mechanism). Tartrakoon et al. (2016) stated that the addition of unsaturated fatty acids to saturated fatty acids in feed will be accompanied by an increase in energy utilization. It was found out later that the process of utilizing unsaturated fatty acids as energy by means of fatty acids had undergone esterification that is forming esters with glycerol to triglycerides as energy reserves. Fatty acids are oxidized through beta oxidation and produce acetyl CoA which combines with acetyl CoA from the metabolism of carbohydrates and proteins, then, substitute to the citric acid cycle to produce energy (Lodish et al., 2000).

Table 1- Average saturated fatty acids contents of giant prawn

Treatments	Saturated Fatty Acids Contents (%) ± SD
P_0	59.13^a±10.2298
P_1	58.41^a ±5.4192
P_2	57.71^a±6.0569
P_3	55.81^a±5.0055
P_4	54.87^a±4.3496

Notes: P_0= commercial feed without the addition of cod liver oil, P_1= 100% commercial feed + 2 % tapioca flour + 3% cod liver oil, P_2= 100% commercial feed + 2 % tapioca flour + 6% cod liver oil, P_3= 100% commercial feed + 2% tapioca flour + 9% *cod liver oil*, P_4= 100% commercial feed + 2% tapioca flour + 12% *cod liver oil*. SD = Standard Deviation.

Table 2. Average unsaturated fatty acid contents

Treatments	Unsaturated Fatty Acids Contents (%) ± SD
P_0	40.87^a±10.2255
P_1	41.59^a ±5.4146
P_2	42.29^a±6.0543
P_3	44.18^a±4.9998
P_4	45.14^a±4.3820

Notes: P_0= commercial feed without the addition of cod liver oil, P_1= 100% commercial feed + 2 % tapioca flour + 3% cod liver oil, P_2= 100% commercial feed + 2 % tapioca flour + 6% cod liver oil, P_3= 100% commercial feed + 2% tapioca flour + 9% *cod liver oil*, P_4= 100% commercial feed + 2% tapioca flour + 12% *cod liver oil*. SD = Standard Deviation.

Ratio of saturated and unsaturated fatty acids

The data on the ratio of saturated and unsaturated fatty acids is obtained from the average content of saturated fatty acids divided by the average unsaturated fatty acid content of each treatment. The best ratio value is obtained from the lowest total cholesterol content in giant prawn meat and the best growth rate. Data on the ratio of saturated fatty acids and unsaturated fatty acids are presented in table 3. The best ratio of saturated fatty acids and unsaturated fatty acids can be seen from the value of cholesterol content in giant prawn meat and growth rate (Table 3). Cholesterol serves as a precursor of a number of compounds, such as sex hormones, adrenal cortex, bile acids and vitamin D. High cholesterol levels can cause heart attacks and strokes. According to Rosenthal (2000), the optimal content of cholesterol is below 100 mg/dl. The best growth rate is obtained from the value of the growth rate during maintenance.

Table 3. Ratio of saturated fatty acids and unsaturated fatty acids, cholesterol and growth Rates

Treatments	Saturated Fatty Acids	Unsaturated Fatty Acids	Ratio
P_0	59.13	40.87	1.45:1
P_1	58.41	41.59	1.41:1
P_2	57.71	42.29	1.36:1
P_3	55.81	44.18	1.26:1
P_4	54.81	45.14	1.21:1

Table 4. Total cholesterol content of giant prawns with/without dietary supplementation of cod liver oil

Adding Cod Liver Oil (%)	Total Cholesterol Content (mg/dl)
0	385.03
3	307.30
6	228.90
9	161.91
12	88.31

From these data (Table 4) the addition of cod liver oil as many as 12% obtained a ratio of 1.21:1 which is a suitable ratio in terms of total cholesterol content. It is known that giant prawns that are fed commonly with the addition of 12% cod liver oil to meat, have a total cholesterol of 88.31 mg/dl, while giant prawns that are fed the same common feed but without the addition of cod liver oil cholesterol is 385.03 mg/dl. The total cholesterol content can be seen in table 4. Through table 4, it is seems that the smaller the ratio, will resulted with better the cholesterol value. This is adjusted to the addition of unsaturated fatty acids (UFA) that can reduce total cholesterol because it is neutral against LDL (does not decrease or increase), but can increase HDL lipoprotein (Mora and Selpas, 2013). In accordance with the value of the growth rate which was the best ratio obtained 1.21:1, the supplementation of cod liver oil by 12% is the best rate of growth, while feed whose composition of cod liver oil is reduced has decreased growth rate (Sobirin, 2016).

The small ratio of saturated fatty acids (SFA) and unsaturated fatty acids (UFA), the better the rate of growth, because saturated fatty acids are inversely proportional to UFA. This is in accordance with conditions where the ratio of saturated and UFA of 33.32:66.68 has a growth rate of 3.53 gr/day (Das et al., 2007). Lack of UFA will cause growth disturbance (Pangkey, 2011). As a matter of fact, consuming foods that contain lots of palmitate stearate can cause heart attacks due to clogged arteries. Consequently, the distribution of nutrients is inhibited and affects growth. This is

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according to the fact that nutrition is very important so that fish have the ability to survive from disease and grow as desired (Pangkey, 2011).

CONCLUSION

Based on results, it can be concluded that, the supplementation of cod liver oil in commercial feed does not affect the ratio of the saturated and unsaturated fatty acids in giant prawn meat. The optimum ratio of saturated fatty acids to unsaturated fatty acids is 1.21:1 with cholesterol content of 88.34 mg/dl.

REFERENCES

Allen KM, Habte-Tsion H, and Thompson KR (2019). Freshwater microalgae (*Schizochytrium sp*.) as a substitute to fish oil for shrimp feed. Scientific Report, 9**:** 6178. DOI: https://doi.org/10.1038/s41598-019-41020-8

Arifin EZ (1998). Dasar-dasar penulisan karangan ilmiah, Jakarta: PT Gramedia Widiasarana Indonesia (Grasindo). ISI Press, Surakarta. Available at: http://repository.isi-ska.ac.id/1395/3/Dasar-Dasar%20Penulisan%20Ilmiah.pdf

Arthur JR (2004). 'Pathogen and Ecological Risk Analysis for the Introduction of Giant River Prawn, Macrobrachium Rosenbergii, from Fiji to Cook Islands', A consultancy report prepared for the Secretariat of the Pacific Community, Noumea Cedex, New Caledonia, under Contract Pro, 7(54): 8. Available at: file:///C:/Users/ok/Downloads/600905cbffbec3ce7902423a2633f8bf.pdf

Bragagnolo N, and Rodriguez-Amaya DB (2001). Total lipid, cholesterol, and fatty acids of farmed freshwater prawn (Macrobrachium rosenbergii) and wild marine shrimp (Penaeus brasiliensis, Penaeus schimitti, Xiphopenaeus kroyeri). Journal of Food Composition and Analysis, Pp. 359-369. DOI: https://doi.org/10.1006/jfca.2000.0981

Das SK, Tiwari VK, Venkateshwarlu G, Reddy AK, Parhi J, Sharma P, and Chettri JK (2007). Growth, survival and fatty acid composition of Macrobrachium rosenbergii (de Man, 1879) post larvae fed HUFA-enriched Moina micrura. Aquaculture, 269(1–4): 464-475. DOI: https://doi.org/10.1016/j.aquaculture.2007.04.069

de Carvalho C, and Caramujo MJ (2018). The Various Roles of Fatty Acids. Molecules (Basel, Switzerland), 23(10): 2583. DOI: https://doi.org/10.3390/molecules23102583

Erlangga E (2012). Meraup Untung dari Budi Daya Udang Galah pada Kolam Air Tawar', Pustaka Agro Mandiri. Tangerang Selatan. hal, pp. 6-49. Available at: http://perpustakaan.kkp.go.id/union/index.php?p=show_detail&id=40370

Faradilah F (2015). 'Pengaruh Cod Liver Oil pada Pakan Komersial terhadap Kolesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) Daging Udang Vanamei', Skripsi. Budidaya Perairan. Fakultas Perikanan dan Kelautan. Universitas Airlangga, p. 70. Available at: http://repository.unair.ac.id/57134/

Fatagar SH (2014). Jumlah Konsumsi Pakan Udang Galah (Macrobrachium rosenbergii) yang Diberi Pakan Atraktan Berbeda', Skripsi. Budidaya Perairan. Fakultas Perikanan dan Ilmu Kelauta. Institut Pertanian Bogor. Bogor, p. 3. Availble at: https://adoc.pub/jumlah-konsumsi-pakan-udang-galah-macrobrachium-rosenbergii-.html

Indonesia Report (2013). Sentra Produksi Budidaya Udang Galah di Indonesia', Statistik Perikanan Budidaya Indonesia. Indonesia. Pp. 1-35. Available at: http://kkp.go.id/an-component/media/upload-gambar-pendukung/kkp/LAPORAN/Laporan%20Tahunan%20KKP%202017%20(FINAL%20OK).pdf

Kamarudin MS, Jones A, Vay L, and Zainal Abidin A (1994). Ontogenetic change in digestive enzyme activity during larval development of Macrobrachium rosenbergii, Aquaculture, 123(3-4): 323-333. DOI: https://doi.org/10.1016/0044-8486(94)90068-X

Kusriningrum RS (2008). Perancangan percobaan, Universitas Airlangga. Surabaya. hal, p.82.

Legowo AM (2004). Pengembangan produk ternak rendah lemak dan tinggi asam lemak tidak jenuh', Journal of The Indonesian Tropical Animal Agriculture, 29(4): 225-233. Available at: http://www.jppt.undip.ac.id/pdf/29(4)2004p225-233.pdf

Lodish H, Berk A, and Zipursky SL (2000). Molecular cell biology. 4th edition. New York: W. H. Freeman, Section 16.1, oxidation of glucose and fatty acids to CO2. Available at: https://www.ncbi.nlm.nih.gov/books/NBK21624/

Melianawati R, and Suwirya K (2010). Optimasi tingkat pemberian pakan terhadap benih kerapu sunu (Plectropomus leopardus), in Prosiding Forum Inovasi Teknologi Akuakultur, pp. 659-665. Available at: https://adoc.pub/optimasi-tingkat-pemberian-pakan-terhadap-benih-kerapu-sunu-.html

Meliandasari D, Dwiloka B, and Suprijatna E (2016). Optimasi daun kayambang (Salvinia molesta) untuk penurunan kolesterol daging dan peningkatan kualitas asam lemak esensial. Jurnal Aplikasi Teknologi Pangan, 4(1): 22-27. Available at: http://journal.ift.or.id/files/Optimasi%20Daun%20Kayambang%20(Salvinia%20molesta)%20untuk%20Penurunan%20Kolesterol%20Daging%20dan%20Peningkatan%20Kualitas%20Asam%20Lemak%20Esensial.pdf

Mora E, and Selpas N (2013). Isolasi dan Karakterisasi Asam Oleat dari Kulit Buah Kelapa Sawit (Elais guinensis Jacq.). Penelitian Farmasi Indonesia, 1(2): 47-51. Availble at: https://pfi.ejournal.unri.ac.id/index.php/FPFI/article/viewFile/1245/1236

Pangkey H (2011). Kebutuhan asam lemak esensial pada ikan laut', Jurnal Perikanan dan Kelautan Tropis, 7(2): 93-102. DOI: https://dx.doi.org/10.35800/jpkt.7.2.2011.185

Rosenthal RL (2000). Effectiveness of altering serum cholesterol levels without drugs. Proceedings (Baylor University, Medical Center), 13(4): 351-355. DOI: https://doi.org/10.1080/08998280.2000.11927704

Rustan AC, and Drevon CA (2005). Fatty Acids: Structures and Properties. In eLS Edition, Wiley, Uk, Pp. 1-8. DOI: https://doi.org/10.1038/npg.els.0003894

Sartika RAD (2008). Pengaruh asam lemak jenuh, tidak jenuh dan asam lemak trans terhadap kesehatan. Kesmas: National Public Health Journal, 2(4): 154-160. DOI: http://dx.doi.org/10.21109/kesmas.v2i4.258

To cite this paper: Marzuki L, Agustono and Rahardja BS (2020). The Effect of Dietary Supplementation of Cod Liver Oil on Ratio of Saturated and Unsaturated Fatty Acids in Giant Prawn *(Macrobrachium rosenbergii)* Meat. *World Vet. J.*, 10 (4): 665-669. DOI: https://dx.doi.org/10.54203/scil.2020.wvj80

Sobirin M (2016). Pengaruh penambahan cod liver oil (CLO) pada pakan komersial terhadap laju pertumbuhan, rasio konversi pakan dan efisiensi pakan udang galah (Macrobrachium rosenbergii)'. Universitas Airlangga. Available at: http://repository.unair.ac.id/57129/

Stoneham TR, Kuhn DD, Taylor DP, Neilson AP, Smith SA, Gatlin DM, Chu H, and O'Keefe SF (2018). Production of omega-3 enriched tilapia through the dietary use of algae meal or fish oil: Improved nutrient value of fillet and offal. PloS One, 13(4): e0194241. DOI: https://doi.org/10.1371/journal.pone.0194241

Tartrakoon W, Tartrakoon T, ad Kitsupee N (2016). Effects of the ratio of unsaturated fatty acid to saturated fatty acid on the growth performance, carcass and meat quality of finishing pigs. Animal nutrition (Zhongguo xu mu shou yi xue hui), 2: 79-85. DOI: https://doi.org/10.1016/j.aninu.2016.03.004

Turchini GM, Torstensen BE, and Ng WK (2009). Fish oil replacement in finfish nutrition. Reviews in Aquaculture, 1: 10-57. DOI: https://www.doi.org/10.1111/j.1753-5131.2008.01001.x

Venugopal V, and Gopakumar K (2017). Shellfish: Nutritive Value, Health Benefits, and Consumer Safety. Comprehensive Reviews in Food Science and Food Safety, 16: 1219-1242. DOI: https://www.doi.org/10.1111/1541-4337.12312

To cite this paper: Marzuki L, Agustono and Rahardja BS (2020). The Effect of Dietary Supplementation of Cod Liver Oil on Ratio of Saturated and Unsaturated Fatty Acids in Giant Prawn *(Macrobrachium rosenbergii)* Meat. *World Vet. J.*, 10 (4): 665-669. DOI: https://dx.doi.org/10.54203/scil.2020.wvj80

2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(4): 670-677, December 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2020.wvj81

Prevalence of Virulence Genes and Antifungal Resistance in *Candida albicans* Isolated from Raw Goat Milk

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ORGINAL ARTICLE
pii: S232245682000081-10
Received: 14 Oct 2020
Accepted: 28 Nov 2020

ABSTRACT

The contamination of goat milk with pathogenic fungi can cause health hazards for the consumers either they consume it raw or even in the processed form. Since there are few studies concerning yeasts in raw goat milk, the present study aimed to determine the prevalence of yeasts and isolate *Candida albicans* from raw goat milk samples. Also, this study determined the distribution of virulence genes and the antifungal susceptibility profile of *Candida albicans* isolates. A total of 30 goat milk samples (collected from free-grazing goats) were mycologically examined. The confirmed *Candida albicans* isolates were subjected to PCR assay to detect the virulence genes (SAP4, RAS1, ALS1, HWP1, and PLB1). Also, antifungal sensitivity testing was performed against the commercially available antifungal agents and probiotics (*Lactobacillus acidophilus* and *Lactobacillus plantarum*). The mycological examination revealed that 14 out of 30 (46.7%) goat milk samples were positive for yeasts and only 4 (13.3%) isolates were confirmed as *Candida albicans*. The results from the PCR assay showed that RAS1 and ALS1 were found in 4 (100%) isolates, HWP1 and SAP4 were found in 2 (50%) isolates, while PLB1 was not detected in tested *Candida albicans* isolates (0%). Antifungal sensitivity testing results showed that ketoconazole gave the best activity against *Candida albicans* isolates, followed by fluconazole, nystatin, and itraconazole. All isolates were resistant to terbinafine. Moreover, both *Lactobacillus acidophilus* and *Lactobacillus plantarum* showed antifungal effects against *Candida albicans*, but *Lactobacillus plantarum* was more effective than *Lactobacillus acidophilus*. Antifungal resistance is a major problem that can lead to failure of candidiasis treatment. Regular antifungal sensitivity testing and searching for an alternative bio-eco-friendly approach for proper control and treatment of candidiasis are strongly needed to prevent treatment failure and emergence of resistant isolates.

Keywords: Antifungal sensitivity testing, *Candida albicans*, Goat milk, Virulence genes, Probiotics.

INTRODUCTION

Yeasts are considered an important component of the microflora of dairy products and are usually found in large counts in milk because it is rich in proteins, sugars, lipids, and organic acids. Yeasts can cause biochemical deterioration in milk, leading to a serious impact on public health (Spanamberg et al., 2009). Fungi also affect milk quality and shelf life (Hasan and Yassein, 2018).

Genus *Candida* contains approximately 200 yeasts, of which *Candida albicans* is the most commonly isolated one. *Candida* species are opportunistic pathogens that are commensally found in the oral cavity, digestive system, and vagina. However, they can also cause systemic infections especially in immunocompromised and hospitalized persons (Hizlisoy et al., 2020). In *Candida albicans* pathogenesis, the adhesion and biofilm formation are assumed to be under the control of the hyphal wall protein1 (Hwp1) that is found on the surface of yeast hyphae (Sundstrom et al., 2002). Hwp1 encoding gene is part of a core of eight genes that are induced during the filamentation process of *Candida albicans*. The adhesion process of *Candida albicans* is also induced by agglutinin-like sequence 1 (ALS1) that facilitates the adherence to the endothelial cells of host and also it is important for hyphal development in addition to adherence to endothelial cells of the host (Fu et al., 2002). ALS1 and ALS3 are two genes with similar sequences and functions. Furthermore, phospholipase B (PLB1) helps in the pathogenesis of *Candida albicans* as it can mediate the systemic gastrointestinal tract manifestations (Samaranayake et al., 2005). The secreted aspartyl proteinases (Saps) help in the hydrolysis of peptide bonds of the host proteins, supposed to be a part of the virulence mechanism of *Candida albicans* (Naglik et al., 2003).

The existence of virulence genes and the elevation in the prevalence of resistance against antifungal agents have been incriminated in the pathogenesis of *Candida albicans*. The antifungal resistance is increasing due to the use of inadequate doses of the selective therapies as well as the frequent use of antifungal agents for fungal infection prophylaxis in both humans and animals (Mendes et al., 2018). However, the relationship between virulence genes and the resistance profiles of *Candida albicans* has not been sufficiently investigated, particularly in deep *Candida albicans* infections (Shrief et al., 2019). In recent years, alternative approaches to proper control and treatment of fungal diseases have been explored.

To cite this paper: Soliman MMH, Kandil MM, Elnemr SA and Abuelnaga ASM (2020). Prevalence of Virulence Genes and Antifungal Resistance in *Candida albicans* Isolated from Raw Goat Milk. *World Vet. J.*, 10 (4): 670-677. DOI: https://dx.doi.org/10.54203/scil.2020.wvj81

Probiotic bacteria are utilized in animal and human feeding to stimulate the balance of intestinal microbiota of the body. They also improve digestion, strengthen the immune system, and promote the production of vitamins. The use of probiotics can reduce the use of antibiotics and enhance animal growth (De Baets et al., 2009). *Lactobacillus acidophilus* and *Lactobacillus plantarum* are considered the commonly isolated species of *Lactobacillus* in the gastrointestinal tract and are known as probiotics (Gudadappanavar et al., 2017). *Lactobacillus* species have the potential to produce different antimicrobial compounds such as acetic acid, hydrogen peroxide, lactic acid, and different types of bacteriocins such as small heat-tolerant lantibiotics (SHSL), non-lanthionine-incorporating membrane-active peptides (MAP), larger heat-sensitive proteins (LHLP), and complex bacteriocins that include one or various chemical constituents. Due to their capability to produce variable antimicrobial compounds, these probiotics can be used for treatment and control of various manifestations (Spinler et al., 2008).

The present work aimed to isolate *Candida albicans* from raw goat milk, detect virulence genes and perform the antifungal sensitivity testing against the commercially available antifungal agents (fluconazole, itraconazole, ketoconazole, terbinafine, and nystatin) as well as investigate the ability of probiotics (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) to suppress the growth of *Candida albicans.*

MATERIALS AND METHODS

Candida isolation and identification

A total of 30 goat milk samples were collected from free-grazing goats, subcultured on Sabouraud dextrose agar plates, and incubated at 30 °C for 72 h. Pure colonies were picked to make morphological, differential biochemical identification tests and germ tube test according to (Deorukhkar and Roushani, 2018).

Virulence genes detection

DNA extraction

DNA extraction was done using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's instructions. In brief, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56 °C for 10 min. After the end of the incubation period, 200 µl of 100% ethanol was added to this mixture. Washing and centrifugation of the sample were done according to the manufacturer's recommendations. The elution of fungal nucleic acid was done with 100 µl elution buffer provided with the extraction kit.

Oligonucleotide primers

Primers (Table 1) were supplied from Metabion (Germany).

Table 1. Sequences of primers, target genes, product sizes, and conditions of PCR cycles

Target genes	Primers sequences (5'-3')	Amplicon size (base pair)	Amplification cycles (35 cycles)				Final extension	References
			Primary denaturation	Secondary denaturation	Annealing	Extension		
RAS1	(Forward) CCCAACTATTGAGGATTCTTATCGTAAA (Reverse)TCTCATGGCCAGATATTCTTCTTG	106	94˚C 5 min.	94˚C 30 sec.	60˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	Tsang et al. (2012)
ALS1	(Forward) GAC TAG TGA ACC AAC AAA TAC CAG A (Reverse) CCA GAA GAA ACA GCA GGT GA	318	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 40 sec.	72˚C 7 min.	Inci et al. (2013)
HWP1	(Forward) ATG ACT CCA GCT GGT TC (Reverse) TAG ATC AAG AAT GCA GC	572	94˚C 5 min.	94˚C 30 sec.	45˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	
PLB1	(Forward) ATGATTTTGCATCATTTG (Reverse) AGTATCTGGAGCTCTACC	751	94˚C 5 min.	94˚C 30 sec.	50˚C 1 min.	72˚C 1 min.	72˚C 10 min.	Mukherjee et al. (2001)
SAP4	(Forward) GCT CTT GCT ATT GCT TTA TTA (Reverse) TAG GAA CCG TTA TTC TTA CA	394	94˚C 5 min.	94˚C 30 sec.	49˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	Sikora et al. (2011)

PCR amplification

The PCR reaction was performed in a 25-µl reaction mixture containing 12.5 µl of master mix (EmeraldAmp Max, Takara, Japan), 1 µl of each primer (20 pmol concentration), 4.5 µl of distilled water, and finally 6 µl of template DNA. The reaction was done in the thermal cycler (Applied biosystem 2720, Germany).

Analysis of PCR products

Electrophoresis of the PCR products was performed on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at ambient temperature using gradients of 5 V/cm. For gel electrophoresis, 15 µl of the products was loaded in each gel slot. Gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the product size. Photographing the agarose gel was performed using a gel documentation system (Alpha Innotech, Biometra, Germany) and the data was analyzed through computer software.

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Antifungal susceptibility testing

Cultures preparation

The suspension of each *Candida albicans* isolate was prepared in sterile distilled water and was adjusted to 0.5 McFarland turbidity standard to obtain a concentration of 10^6 CFU/ml. *Lactobacillus acidophilus* DSMZ 20079 and *Lactobacillus plantarum* DSM 20179 were purchased from MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Both strains were activated on de Man, Rogosa, and Sharp (MRS) broth (Biolife, Italy) at 37 °C for 24 h. Serial dilutions were prepared to obtain a concentration of 10^6 CFU/ml.

Well diffusion method

The antifungal sensitivity testing was performed on *Candida albicans* isolates. The adjusted spore suspensions were evenly spread on the surface of Sabouraud dextrose agar plates. Holes were made on the medium by using a 6 mm cork borer. The commercial antifungal drugs, including fluconazole (25 µg), itraconazole (8 µg), ketoconazole (15 µg), terbinafine (1 µg), nystatin (100 IU). A volume of 100 µl of the antifungal agent was placed into each well. Also, *Lactobacillus acidophilus* and *Lactobacillus plantarum* (100 µl/well of 10^6 CFU/ml suspension) were placed into the wells. The plates were then incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeters; the inhibition zone is the area surrounding the hole where there is no growth of inoculated fungus. All assays were carried out in triplicates to calculate the mean results (Abdullah et al., 2013).

RESULTS AND DISCUSSION

In the last decades, *Candida* species especially *Candida albicans* have been considered an important healthcare-associated infection (Cox, 1993). The discrimination of various *Candida* species mostly relies on different biochemical identifications, germ tube test, and the unique culture characteristics on specific media (Vijayalakshmi et al., 2016).

In the present study, 14 out of 30 (46.7%) goat milk samples were positive for yeasts. Dos Santos and Marin (2005) isolated fungi from 32% of tested bovine milk specimens, of which 17.3% were *Candida* species. Dworecka-Kaszak et al. (2012) isolated fungi from only 14% of the tested samples also Krukowski et al. (2001) detected fungi in 9.6% of the tested milk specimens in the Lublin district located in Poland. Dos Santos and Marin (2005) declared that the percentage of fungal isolation in surveys performed in many countries varies significantly, with 6.1% rates as described in Egypt by Awad et al. (1980), 1.3% in Denmark (Aalbek et al., 1994), and 12.07% in Brazil (Costa et al., 1993).

Candida is mostly known as an opportunistic mycotic infection, and the origin of infection may come from the surface of the udder, milking machines, milker's palms, animal feed, straw, ground, sanitary agents, remedies, and other utensils. In immunosuppressive circumstances, the balance of pathogens population size may be altered, and the mycotic elements along with the other pathogens are capable of defeating the udder defensive techniques. Although the distribution of *Candida* species exhibits large diversity in different localities, it is crucial to give attention toward the increased cases of mammary gland infections caused by *Candida* species in the last decade (Dworecka-Kaszak et al., 2012). In the present work, only 4 (13.3%) isolates were confirmed as *Candida albicans.* Spanamberg et al. (2014) isolated *Candida albicans, Candida glabrata, Candida tropicalis*, and *Pichia guilliermondii* from normal and mastitic ewe milk specimens. *Candida albicans* was the most frequently isolated species in a study performed by Costa et al. (1993) but Dworecka-Kaszak et al. (2012) isolated mostly *Candida parapsilosis*. Krukowski et al. (2001) stated that *Candida kefyr*, *Candida cirferi*, and *Candida krusei* were more frequently isolated from cow milk in Poland.

In the present work, the virulence genes were detected in the four isolates of *Candida albicans* (Table 2 and Figures 1-5). The results revealed that RAS1 and ALS1 were found in 4 (100%) isolates, HWP1 and SAP4 were found in 2 (50%) isolates but PLB1 was not detected in any of the four isolates (0%). Similar results were obtained by Vijayalakshmi et al. (2016) who detected the virulence genes of HWP1 in 77%, INT1 in 72%, ALS1 in 65%, SAP1 in 65%, and PLB1 in 52% of multi-drug resistant *Candida albicans*. Also, Abdul-Lateef et al. (2015) detected the virulence genes of INT1, ALS1 in a higher frequency (100%), HWP1 in 90.9%, SAP1 in 59.09%, and PLB1 in 13.63% of tested *Candida albicans* isolates. However, Inci et al. (2013) detected the ALS1 gene in 53.9% of tested *Candida albicans* isolates, while the HWP1 gene was found in only 5.3% of tested isolates. The diversity in the prevalence percentages of virulence genes may attribute to various issues including the number of specimens under investigation and the difference in the isolation origins of *Candida albicans* (Vijayalakshmi et al., 2016).

Table 2. The distribution of the virulence genes in *Candida albicans* isolates recovered from goat milk

Isolates	RAS1	ALS1	HWP1	PLB1	SAP4
1	+	+	-	-	-
2	+	+	+	-	+
3	+	+	+	-	+
4	+	+	-	-	-

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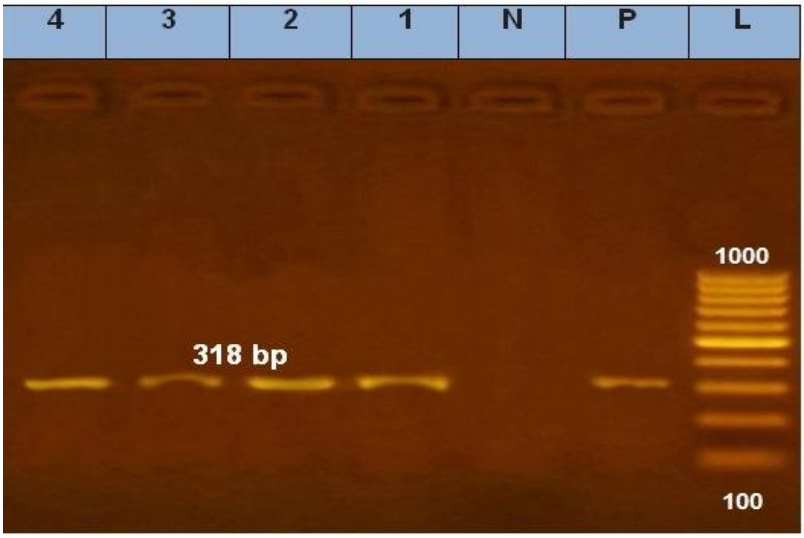

Figure 1. Agarose gel electrophoresis showing PCR-amplified product of ALS1 gene of *Candida albicans* derived from goat milk. (Lane L: 100-bp DNA ladder, Lane P: positive control, Lane N: negative control, Lanes 1-4: tested *Candida albicans* isolates).

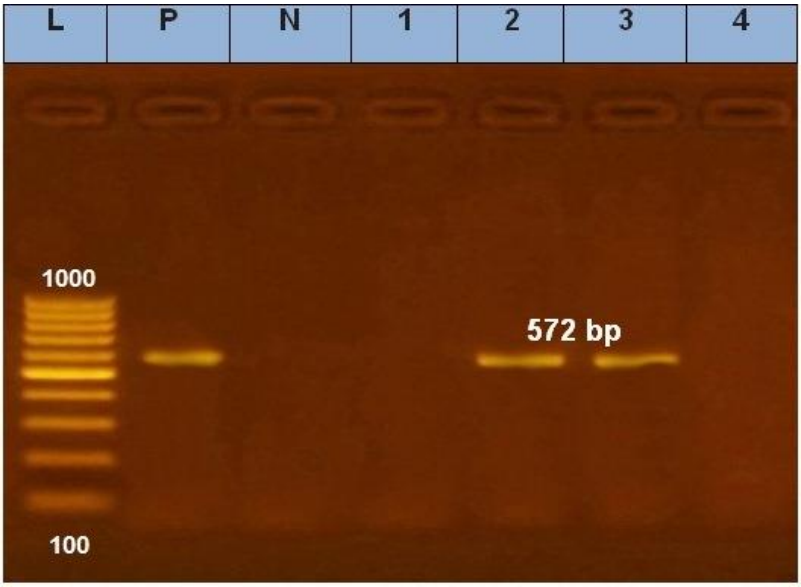

Figure 2. Agarose gel electrophoresis showing PCR-amplified product of HWP1 gene of *Candida albicans* derived from goat milk. (Lane L: 100-bp DNA ladder, Lane P: positive control, Lane N: negative control, Lanes 1-4: tested *Candida albicans* isolates).

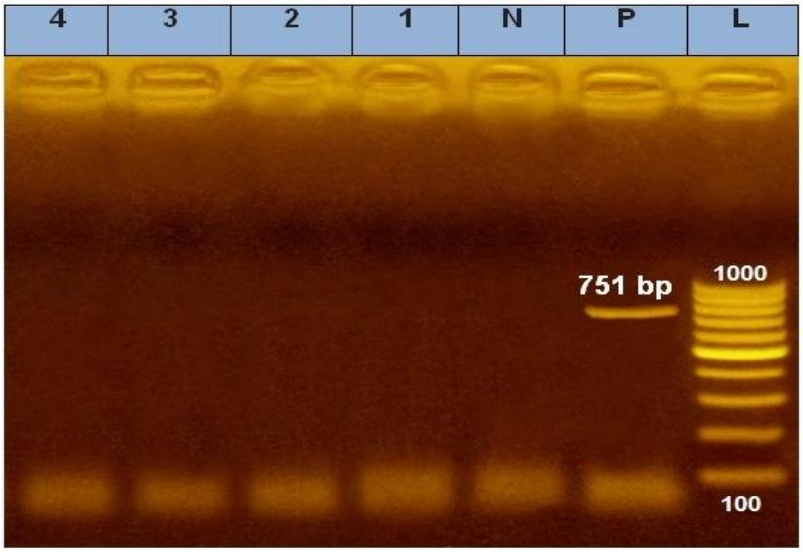

Figure 3 Agarose gel electrophoresis showing PCR-amplified product of PLB1 gene of *Candida albicans* derived from goat milk. (Lane L: 100-bp DNA ladder, Lane P: positive control, Lane N: negative control, Lanes 1-4: tested *Candida albicans* isolates).

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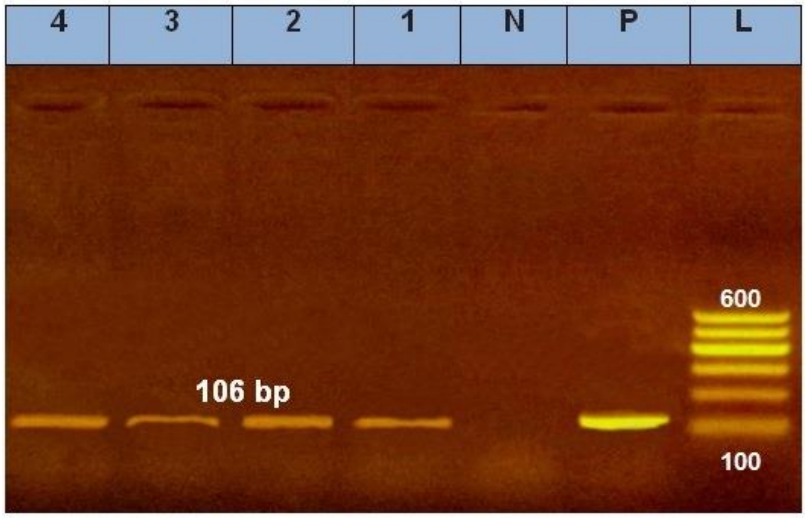

Figure 4. Agarose gel electrophoresis showing PCR-amplified product of RAS1 gene of *Candida albicans* derived from goat milk. (Lane L: 100-bp DNA ladder, Lane P: positive control, Lane N: negative control, Lanes 1-4: tested *Candida albicans* isolates).

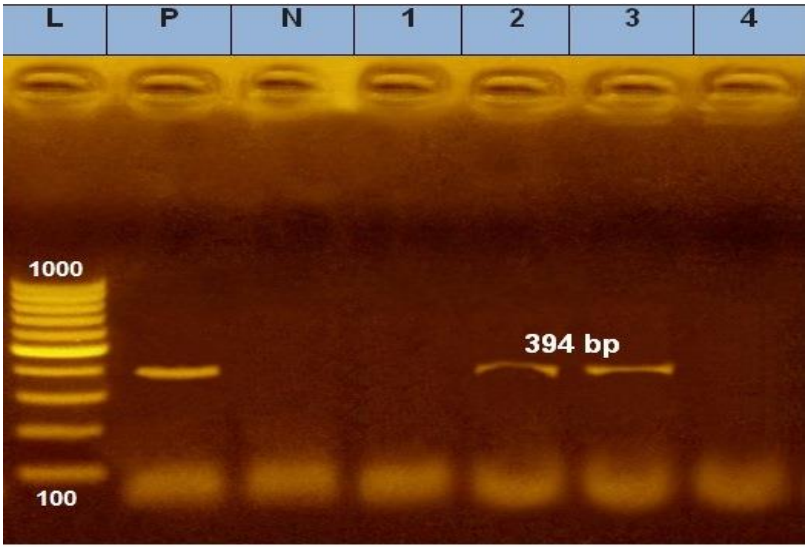

Figure 5. PCR results for the amplification of SAp4 gene of *Candida albicans* derived from goat milk. (Lane L: 100-bp DNA ladder, Lane P: positive control, Lane N: negative control, Lanes 1-4: tested *Candida albicans* isolates).

In recent years, antimycotic agents have shown an increase in usage as the fungal infections have also increased, leading to the suppression of endogenous fungal microflora, and the emergence of more resistant isolates due to the inhibition of susceptible ones (Koç, 2003). Some *Candida* species are naturally resistant to some antimycotics and it was revealed that many *Candida* isolates possess resistance against amphotericin B and others developed resistance against ketoconazole (Gunes et al., 2001). In the present study, ketoconazole showed the best activity against *Candida albicans*, followed by fluconazole. While nystatin and itraconazole showed lower activity against *Candida albicans*. All isolates were resistant to terbinafine (Table 3 and Figure 6).

Table 3. Results of antifungal sensitivity of *Candida albicans* isolates derived from goat milk against different antifungal agents and probiotics

***Candida albicans* isolates**	**Inhibition zone diameter (mm)**						
	Fluconazole (25 µg)	**Itraconazole (8 µg)**	**Ketoconazole (15 µg)**	**Terbinafine (1 µg)**	**Nystatin (100 IU)**	***Lactobacillus acidophilus* (10^6)**	***Lactobacillus plantarum* (10^6)**
1	22.7±1.5	19. 3±0. 7	33.3±0.9	Resistant	22.3±0.3	14. 7±0.3	16.3±0.9
2	37.3±1.5	21. 7±0.9	32.3±1.5	Resistant	22±1	12.3±0.3	14.7±0.3
3	36.3±0.9	21.3±0.9	35. 7±0.3	Resistant	22.7±1.5	13±0.6	15±1.6
4	29±2.1	18.3±0.9	31. 7±0.9	Resistant	21±0.6	11. 7±0.9	13.3±0.9

Data are expressed as Mean ± Standard Error

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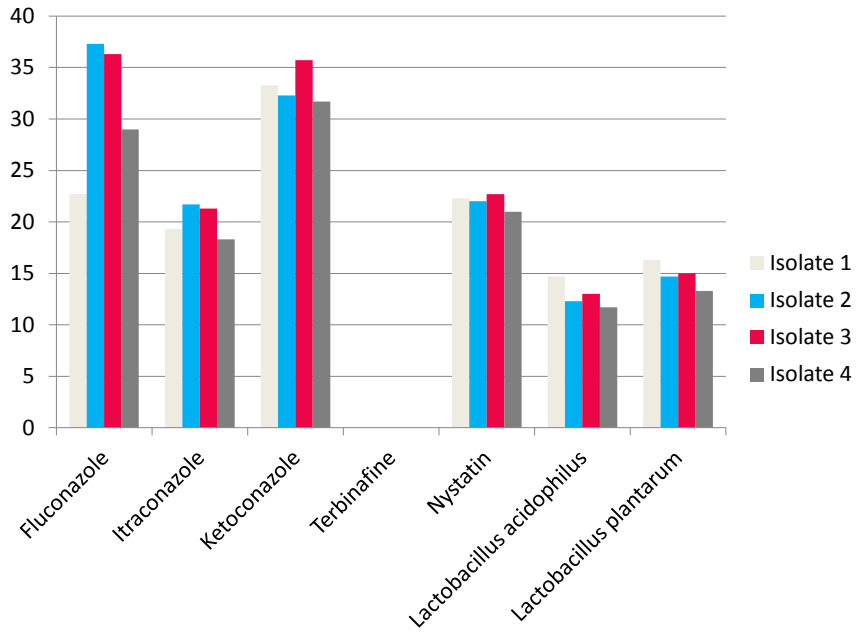

Figure 6. Comparison of the antifungal sensitivity results of *Candida albicans* isolates against commercially available antifungal agents and probiotics.

Sonmez and Erbas (2017) found high resistance rate (100%) to fluconazole, miconazole, amphotericin B, and flucytosine while high susceptibility to ketoconazole. Two (20%) *Candida albicans* isolates were found to be highly susceptible to nystatin while others were of medium susceptibility hence Sonmez and Erbas (2017) concluded that ketoconazole is the drug of choice in treating *Candida* infections. Lyon et al. (2010) found that the susceptibility of fluconazole significantly increased from 87.5% in 2005 to 97.4% in 2007. Furthermore, Monroy-Pérez et al. (2016) stated that all strains of *Candida albicans* were sensitive to nystatin, and 37 (94.9%) and 38 (97.4%) strains were resistant to fluconazole and ketoconazole, respectively. Shrief et al. (2019) declared that the resistance against antifungal drugs including itraconazole, fluconazole, and caspofungin was 8% for each one and 9% for amphotericin B. Moreover, Dos Santos Abrantes et al. (2014) reported that more than 50% of *Candida albicans* isolates obtained from South Africa and Cameroon showed resistance against fluconazole. However, an earlier study conducted in South Africa revealed 100% susceptibility of *Candida albicans* to fluconazole because this study was performed before the introduction of fluconazole to HIV-AIDS patients as the fluconazole resistance was not developed yet (Blignaut et al., 2002). In the last years, the increase in clinical manifestations due to *Candida* species and the different sensitivity patterns against the used antifungal agents highlight the importance of *in vitro* susceptibility testing in selecting appropriate antifungal agents (Sonmez and Erbas, 2017). Antifungal susceptibility testing is a great tool that may specify clinical response, help in the efficient selection of antifungal agents, and predict antifungal treatment failure. Antifungal susceptibility testing of *Candida* species and tracing the emergence of resistant isolates is of great importance in order to make information available to the clinicians for a proper therapeutic outcome (Khan et al., 2018).

Due to the elevated prevalence of candidiasis in immunodeficient individuals, the emergence of resistance in *Candida* species to current antimycotics, the treatment failures and the frequent relapse of candidiasis, the use of some beneficial and harmless compounds such as probiotics for the treatment and control of this disease can be recommended as an interesting safe medicinal way (Silva et al., 2016). Probiotics can be used to effectively combat pathogens with no adverse effect on normal microbiota. In this concern, the use of probiotics can be a substitute in the food and pharmaceutical industries (Abdhul et al., 2015). In the current study, both *Lactobacillus plantarum* and *Lactobacillus acidophilus* showed antifungal activity against *Candida albicans* but *Lactobacillus plantarum* showed higher activity in comparison to *Lactobacillus acidophilus*. These results were similar to those obtained by Hasslöf et al. (2010) who revealed that *Lactobacillus acidophilus* had weaker inhibition activity in comparison with the other probiotic strains. Also, Strus et al. (2005) assured that tested probiotics, including *Lactobacillus plantarum* and *Lactobacillus acidophilus*, suppressed the growth of *Candida albicans* to a certain degree. Kovachev and Vatcheva-Dobrevska (2015) revealed that local application of probiotics such as *Lactobacillus acidophilus* may improve the effectiveness of conventional antimycotics and prevent recurrent infection in women with *Candida albicans* vaginal manifestation. Jiang et al. (2014) revealed that *Candida albicans* was the most susceptible yeast to lactobacilli but another study conducted by Salari and Almani (2020) found that cell concentrations of 10^2 to 10^{10} CFU/ml for both *Lactobacillus acidophilus* and *Lactobacillus plantarum* were able to suppress the growth of most of the tested *Candida* species, except for *Candida albicans*, however, *Candida albicans* displayed very high susceptibility to cell-free supernatants of two *Lactobacillus* species. The differences in the results of various studies may attribute to differences in the clinical isolates of *Candida*

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albicans, the examined *Lactobacillus* strains, the investigations used for testing the antifungal susceptibility, tested *Candida* species, the initial counts of *Lactobacillus* species, the length of the incubation period, and the source of the *Candida* species isolation.

CONCLUSION

In the current study, both probiotics *Lactobacillus acidophilus* and *Lactobacillus plantarum* exhibited antifungal effects against *Candida albicans*. However, *Lactobacillus plantarum* was higher in the activity than *Lactobacillus acidophilus*. Fungal mastitis cases due to *Candida* species are spreading and candidiasis cases in immunocompromised individuals are also increasing along with the development of resistant strains that can lead to treatment failures. Plus the synthetic antimycotics have serious side effects. Therefore, further studies on experimentally infected animals are strongly recommended to evaluate the antifungal activity of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and other *Lactobacillus* species and determine their precise mode of action.

DECLARATIONS

Authors' contribution

Mona MH Soliman designed the plan of the study, shared in performing the experiments and writing of the manuscript. Mai M Kandil shared in performing the experiments and manuscript writing. Elnemr SA shared in performing the experiments and shared in analyzing the data. Azza SM Abuelnaga shared in performing the experiments and analyzing the data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

Aalbek B, Stenderup J, Jensen HE, Valbak J, Nylin B, and Huda A (1994). Mycotic and algal bovine mastitis in Denmark. Acta Pathologica, Microbiologica et Immunologica, 102(6): 451-456. DOI: https://doi.org/10.1111/j.1699-0463.1994.tb04898.x

Abdhul K, Ganesh M, Shanmughapriya S, Vanithamani S, Kanagavel M, Anbarasu K, and Natarajaseenivasan K (2015). Bacteriocinogenic potential of a probiotic strain *Bacillus coagulans* [BDU3] from Ngari. International Journal of Biological Macromolecules, 79: 800-806. DOI: https://doi.org/10.1016/j.ijbiomac.2015.06.005

Abdullah K, Soliman M, and Mohamed M (2013). Susceptibility of *Candida albicans* clinical isolates to some plant extracts in Saudi Arabia. Zagazig University Medical Journal, 19(5): 1-6. DOI: https://doi.org/10.21608/zumj.2013.4279

Abdul-Lateef LAR, AL- Kafhaji KA, and Al-Saddam ASK (2015). Genetic identification of some virulence factors of *Candida* albicans in candidal intertrigo of diabetic patients in Hilla Province/ Iraq. Australian Journal of Basic and Applied Sciences, 9: 66-72.

Awad FI, El Molla A, Fayed A, Abd el-Halim M, and Refai M (1980). Studies of mycotic mastitis in Egypt. Journal of Egyptian Veterinary Medical Association, 40(3): 35-41. Available at: https://www.worldcat.org/title/journal-of-the-egyptian-veterinary-medical-association/oclc/3610675

Blignaut E, Messer S, Hollis RJ, and Pfaller MA (2002). Antifungal susceptibility of South African oral yeast isolates from HIV/AIDS patients and healthy individuals. Diagnostic Microbiology and Infectious Disease, 44(2): 169-174. DOI: https://doi.org/10.1016/s0732-8893(02)00440-6

Costa EO, Gandra CR, Pires MF, Coutinho SD, Castilho W, and Teixeira CM (1993). Survey of bovine mycotic mastitis in dairy herds in the State of São Paulo, Brazil. Mycopathologia, 124(1): 13-17. DOI: https://doi.org/10.1007/bf01103051

Cox G (1993). Fungal infections. Current Opinion in Infectious Disease, 6: 422-426. Available at: https://journals.lww.com/co-infectiousdiseases/pages/default.aspx

De Baets L, Van Iwaarden P, Meeus N, Schimmel H, Philipp W, and Emons H (2009). First certified reference materials for molecular fingerprinting of two approved probiotic *Bacillus* strains. International Journal of Food Microbiology, 129(1): 16-20. DOI: https://doi.org/10.1016/j.ijfoodmicro.2008.10.028

Deorukhkar SC, and Roushani S (2018). Identification of *Candida* species: conventional methods in the era of molecular diagnosis. Annals of Microbiology and Immunology, 1(1): 1002-1008. Available at: http://www.remedypublications.com/annals-of-microbiology-and-immunology-home.php

Dos Santos Abrantes PM, McArthur CP, and Africa CW (2014). Multi-drug resistant oral *Candida* species isolated from HIV-positive patients in South Africa and Cameroon. Diagnostic Microbiology and Infectious Disease, 79(2): 222-227. DOI: http://dx.doi.org/10.1016/j.diagmicrobio.2013.09.016

Dos Santos RDC, and Marin JM (2005). Isolation of *Candida* spp. from mastitic bovine milk in Brazil, Mycopathologia, 159(2): 251-253. DOI: https://DOI.org/10.1007/s11046-004-2229-2

Dworecka-Kaszak B, Krutkiewicz A, Szopa D, Kleczkowski M, and Biegańska M (2012). High prevalence of *Candida* yeast in milk samples from cows suffering from mastitis in Poland. The Scientific World Journal, Article ID 196347. DOI: https://doi.org/10.1100/2012/196347

Fu Y, Ibrahim AS, Sheppard DC, Chen Y, French SW, Cutler JE, Filler SG, and Edwards JE (2002). *Candida* albicans Als1p: An adhesin that is a downstream effector of the EFG1 filamentation pathway. Molecular Microbiology, 44(1): 61-72. DOI: https://doi.org/10.1046/j.1365-2958.2002.02873.x

Gudadappanavar AM, Hombal PR, Timashetti SS, and Javali SB (2017). Influence of *Lactobacillus acidophilus* and *Lactobacillus plantarum* on wound healing in male Wistar rats- an experimental study. International Journal of Applied and Basic Medical Research, 7: 233-238. DOI: https://doi.org/10.4103/ijabmr.ijabmr_329_16

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Gunes I, Kalkanci A, and Kustimur S (2001). Comparison of three different commercial kits with conventional methods for the identification of *Candida* strains to species level. Mikrobiyoloji Bulteni, 35: 559-564. Available at: http://www.mikrobiyolbul.org/managete/fu_folder/2001-04/2001-35-04-559-564.pdf

Hasan KAM, and Yassein SN (2018). Prevalence and type of fungi in milk from goats with subclinical mastitis. Online Journal of Veterinary Research, 22(8): 669-674.

Hasslöf P, Hedberg M, Twetman S, and Stecksen-Blicks C (2010). Growth inhibition of oral mutans streptococci and *Candida* by commercial probiotic lactobacilli—an *in vitro* study. BMC Oral Health, 10: 18. DOI: https://doi.org/10.1186/1472-6831-10-18

Hizlisoy H, Onmaz NE, Al S, Karadal F, Yildirim Y, Gonulalan Z, Semih Gumussoy K, Aydemir GD, Tekinsen FK and Dinc G (2020). Clonal diversity and antifungal susceptibility of *Candida* spp. recovered from cow milk. Mljekarstvo/Dairy, 70(1): 40-49. DOI: https://doi.org/10.15567/mljekarstvo.2020.0104

İnci M , Atalay MA, Özer B, Evirgen Ö, Duran N, Motor VK, Koç AN, Önlen Y, Kilinç C, and Durmaz S (2013). Investigations of ALS1 and HWP1 genes in clinical isolates of *Candida albicans*. Turkish Journal of Medical Sciences, 43: 125-130. Available at: https://journals.tubitak.gov.tr/medical/index.htm

Jiang Q, Stamatova I, Kari K, and Meurman JH (2014). Inhibitory activity in vitro of probiotic *lactobacilli* against oral *Candida* under different fermentation conditions. Beneficial Microbes, 6: 361-368. DOI: https://doi.org/10.3920/bm2014.0054

Khan M, Ahmed J, Gul A, Ikram A, and Lalani FK (2018). Antifungal susceptibility testing of vulvovaginal *Candida* species among women attending antenatal clinic in tertiary care hospitals of Peshawar. Infection and Drug Resistance, 11: 447. DOI: https://doi.org/10.2147/idr.s153116

Koç N (2003). Antifungal resistance in our country. 3. National Fungal Diseases and Clinical Mycology Congress (27-30 May 2003) Congress Book. Bornova, Izmir, Turkey, pp. 285-300. Available at: http://ecmm.eu/ECMM_N06_1-2003_A3.pdf

Kovachev SM, and Vatcheva-Dobrevska RS (2015). Local probiotic therapy for vaginal *Candida albicans* infections. Probiotics and Antimicrobial Proteins, 7: 38-44. Available at: https://doi.org/10.1007/s12602-014-9176-0

Krukowski H, Tietze M, Majewski T, and Rózański P (2001). Survey of yeast mastitis in dairy herds of small-type farms in the Lublin region, Poland. Mycopathologia, 150(1): 5-7. DOI: https://doi.org/10.1023/a:1011047829414

Lyon GM, Karatela S, Sunay S, and Adiri Y (2010). Antifungal susceptibility testing of *Candida* isolates from the *Candida* surveillance study. Journal of Clinical Microbiology, 48(4): 1270-1275. DOI: https://doi.org/10.1128/jcm.02363-09

Mendes JF, Gonçalves CL, Ferreira GFD, Esteves IA, Freitas CHD, Villarreal JPV, Mello JRBD, Meireles MCA, and Nascente PDS (2018). Antifungal susceptibility profile of different yeasts isolates from wild animals, cow's milk with subclinical mastitis and hospital environment. Brazilian Journal of Biology, 78(1): 68-75. DOI: https://doi.org/10.1590/1519-6984.04916

Monroy-Pérez E, Paniagua-Contreras GL, Rodríguez-Purata P, Vaca-Paniagua F, Vázquez-Villaseñor M, Díaz-Velásquez C, Uribe-García A, and Vaca S (2016). High virulence and antifungal resistance in clinical strains of *Candida* albicans. Canadian Journal of Infectious Diseases and Medical Microbiology, pp. 1-7. DOI: https://doi.org/10.1155/2016/5930489

Mukherjee PK, Seshan KR, Leidich SD, Chandra J, Cole GT, and Ghannoum MA (2001). Reintroduction of the PLB1 gene into *Candida albicans* restores virulence *in vivo*. Microbiology, 147: 2585-2597. DOI: https://doi.org/10.1099/00221287-147-9-2585

Naglik JR, Challacombe SJ, and Hube B (2003). *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. Microbiology and Molecular Biology Reviews, 67(3): 400-428. DOI: https://doi.org/10.1128/mmbr.67.3.400-428.2003

Salari S, and Almani PGN (2020). Antifungal effects of *Lactobacillus acidophilus* and *Lactobacillus plantarum* against different oral *Candida* species isolated from HIV/AIDS patients: an in vitro study. Journal of Oral Microbiology, 12(1): 1769386. DOI: https://doi.org/10.1080/20002297.2020.1769386

Samaranayake YH, Dassanayake RS, Jayatilake JAMS, Cheung BPK, Yau JYY, Yeung KWS, and Samaranayake LP (2005). Phospholipase B enzyme expression is not associated with other virulence attributes in *Candida albicans* isolates from patients with human immunodeficiency virus infection. Journal of Medical Microbiology, 54(6): 583-593. DOI: https://doi.org/10.1099/jmm.0.45762-0

Shrief R, Zaki, MES, El-Sehsah EM, Ghaleb S, and Mofreh M (2019). Study of antifungal susceptibility, virulence genes and biofilm formation in *Candida albicans*. The Open Microbiology Journal, 13(1): 241-248. DOI: https://doi.org/10.2174/1874285801913010241

Sikora M, Dabkowska M, Swoboda-Kopec E, Jarzynka S, Netsvyetayeva I, Jaworska-Zaremba M, Pertkiewicz M, and Mlynarczyk G (2011). Differences in proteolytic activity and gene profiles of fungal strains isolated from the total parenteral nutrition patients. Folia Microbiologica, 56: 143-148. DOI: https://doi.org/10.1007/s12223-011-0023-3

Silva MP, Rossoni RD, Junqueira JC, and Jorge AOC (2016). Probiotics for Prevention and Treatment of Candidiasis and Other Infectious Diseases: *Lactobacillus* spp. and Other Potential Bacterial Species. In: Rao V, Rao L, eds. Probiotics and Prebiotics in Human Nutrition and Health. IntechOpen, London, UK, pp. 242-262. DOI: https://doi.org/10.5772/64093

Sonmez M, and Erbas G (2017). Isolation and identification of *Candida* spp. from mastitis cattle milk and determination of antifungal susceptibilities. International Journal of Veterinary Science, 6(2): 104-107.

Spanamberg A, Fraga CF, Ferreiro L, Aguinsky MS, Sanches EMC, Roehe C, Lautert C, and Santurio JM (2014). Yeasts in the Raw Ewe's Milk. Acta Scientiae Veterinariae, 42: 1236. DOI: https://doi.org/10.22456/1679-9216.16897

Spanamberg A, Ramos JP, Leoncini O, Alves SH, and Valente P (2009). High frequency of potentially pathogenic yeast species in goat's raw milk and creamed cheese in Southern Brazil. Acta Scientiae Veterinariae, 37(2): 133-141. DOI: https://doi.org/10.22456/1679-9216.16239

Spinler JK, Taweechotipatr M, Rognerud CL, Ou CN, Tumwasorn S, and Versalovic J (2008). Human-derived probiotic *Lactobacillus reuteri* demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. Anaerobe, 14: 166-171. DOI: https://doi.org/10.1016/j.anaerobe.2008.02.001

Strus M, Kucharska A, Kukla G, Brzychczy-Wloch M, Maresz K, and Heczko PB (2005). The *in vitro* activity of vaginal *Lactobacillus* with probiotic properties against *Candida*. Infectious Diseases in Obstetrics and Gynecology, 13: 69-75. DOI: https://doi.org/10.1080/10647440400028136

Sundstrom P, Balish E, and Allen CM (2002). Essential role of the *Candida albicans* transglutaminase substrate, hyphal wall protein 1, in lethal oroesophageal candidiasis in immunodeficient mice. The Journal of Infectious Diseases, 185(4): 521-530. DOI: https://doi.org/10.1086/338836

Tsang PWK, Bandara HMHN, and Fong WP (2012). Purpurin suppresses *Candida albicans* biofilm formation and hyphal development. PLoS One, 7(11): e50866. DOI: https://doi.org/10.1371/journal.pone.0050866

Vijayalakshmi P, Thenmozhi S, and Rajeswari P (2016). The Evaluation of the virulence factors of clinical *Candida* isolates and the anti-biofilm activity of Elettaria cardamomum against multi-drug resistant *Candida albicans*. Current Medical Mycology, 2(2): 8-15. DOI: https://doi.org/10.18869/acadpub.cmm.2.2.3

To cite this paper: Soliman MMH, Kandil MM, Elnemr SA and Abuelnaga ASM (2020). Prevalence of Virulence Genes and Antifungal Resistance in *Candida albicans* Isolated from Raw Goat Milk. *World Vet. J.*, 10 (4): 670-677. DOI: https://dx.doi.org/10.54203/scil.2020.wvj81

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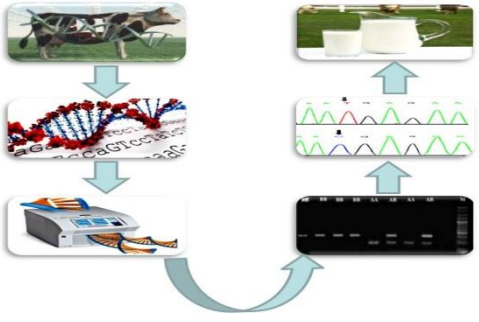

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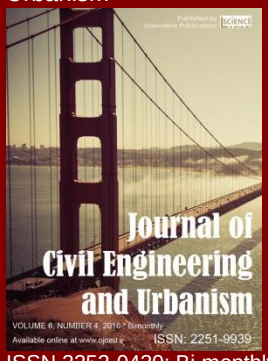

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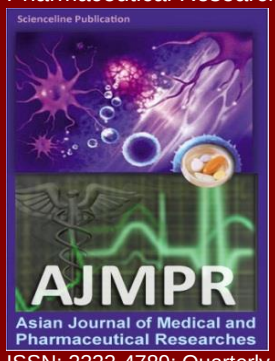

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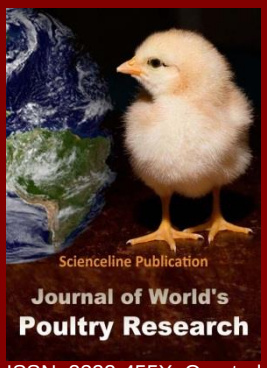

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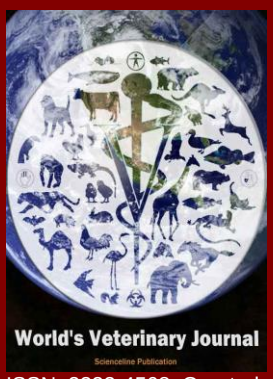

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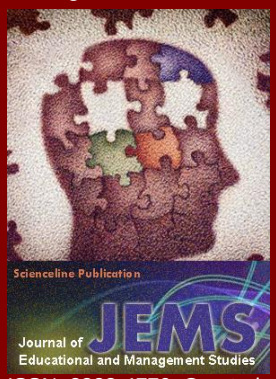

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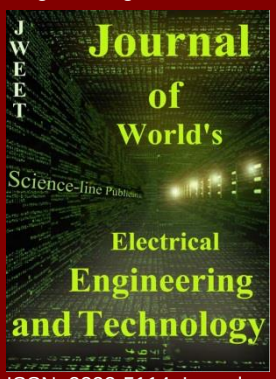

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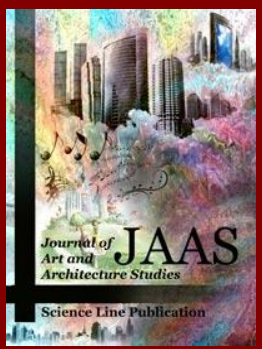

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