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Review

Forage for Pre-weaning Calves: An Update

Nikkhah A and Alimirzaei M.

World Vet. J. 12(2): 123-127, 2022; pii:S232245682200015-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj15

ABSTRACT: Forage nutrition for pre-weaning calves hosts numerous practical questions and on-farm challenges. The present review article aimed to update and address the biological consequences of forage provision to pre-weaned dairy calves. Health, nutrient intake (milk plus solid feed), and rumen development are the most important factors related to calf growth in pre- and post-weaning periods. A growing body of evidence suggests that the health and growth performance of dairy calves in the pre-weaning period are associated with their later performance as dairy cows. It seems that starter feeding strategies, including grain type, processing method, feed texture, and forage inclusion during the critical pre-weaning period may have profound effects on rumen function and calf performance. It is well understood that grain fermentation by-products are essential for increased growth and absorptive capacity of the rumen papillae. Forage provision as a part of a starter diet has been a topic of recent research. The rumen pH is the main factor altering the fate of fermentation and eventually animal health. In the pre-weaned calf, two major hypotheses exist regarding forage feeding. The first hypothesis describes that the rumen is not completely developed in pre-weaned calves and forage provision during this period might increase gut fill, and hence, decrease starter intake. It is believed that depressed starter intake may limit energy intake and finally suppress calf growth rate. The second hypothesis indicates that the rumen pH may decline as calves age and starter intake increases. Accordingly, forage inclusion in calf starter diets could prevent further rumen pH decline and subsequent negative consequences while improving starter intake and calf growth. Research data regarding these hypotheses are controversial. Many factors, such as milk feeding method, grain, forage type, and experimental conditions could affect calf responses to dietary forage. The current review focused on the biological consequence of forage provision to young calves to provide a practical framework for better use of forages in pre-weaned calves feeding programs.

Keywords: Forage, Growth, Pre-weaned calf, Rumen development

[Full text-PDF]

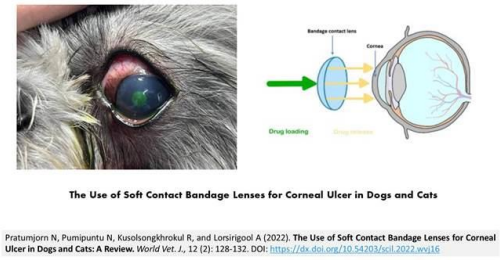
Review

The Use of Soft Contact Bandage Lenses for Corneal Ulcer in Dogs and Cats: A Review

Pratumjorn N, Pumipuntu N, Kusolsongkhrokul R, and Lorsirigool A.

World Vet. J. 12(2): 128-132, 2022; pii:S232245682200016-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj16



ABSTRACT: A corneal ulcer is the characteristic of the destruction of the corneal epithelium layer and loss of the stroma layer at various depths. At present time, soft bandage contact lenses are used in many countries for corneal ulcers in dogs and cats to protect their cornea, increase contact time with topical eyes solutions, and support corneal reepithelialization. This article aimed to review information on the use of soft contact bandage lenses to treat corneal ulcers in dogs and cats interms of their efficacy and precaution. The results of the present review have revealed that soft contact bandage lenses are used to protect the cornea, enhance contact time with topical eye treatments, reduce median healing time, and provide comfort in dogs and cats with corneal ulcers. In the case of serious infections and dry eyes, soft contact bandage lenses are not recommended.

Keywords: Corneal ulcer, Incidence, Soft contact bandage lenses, Treatment

[Full text-PDF]

Research Paper

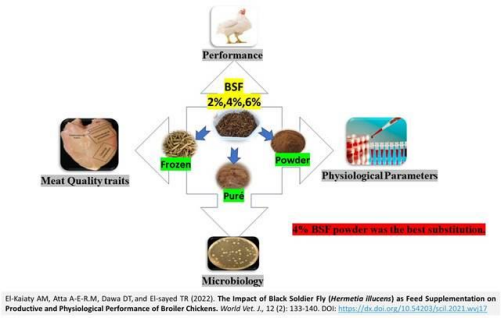
The Impact of Black Soldier Fly (*Hermetia illucens*) as Feed Supplementation on Productive and Physiological Performance of Broiler Chickens

El-Kaiaty AM, Atta A-E-R.M, Dawa DT, and El-sayed TR.

World Vet. J. 12(2): 133-140, 2022; pii:S232245682200017-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj17

ABSTRACT: A total of 450 broiler chicks (Ross 308) were used to evaluate the effect of different inclusion levels of a partially black soldier fly (BSF), BSF Powder (BSFP), BSF Puré (BSFPr), and BSF frozen whole larvae (BSFL) on the growth performance, blood parameters, humoral immune response, and intestinal bacterial count of broiler chickens. The chickens were reared from day 1 to 35 and assigned to the control and 9 dietary groups with different forms of BSF (3 replicates per group with 15 chicks). Black soldier fly was included at levels of 2%, 4%, and 6% for BSFP, BSFPr, and BSFL, respectively, in the starter and growing diets. The results indicated similar body weight, weight gain, and the growth rate in chickens fed 4% BSFP, and 2% BSFPr during the experiment. There was a marked difference in blood parameters due to the different BSF forms and included percentages. The humoral immunity antibody titers against the Newcastle disease virus fluctuated among the experimental groups of different ages. Finally, it could be concluded that the BSF can be incorporated at a level of 4% in the form of powder and Puré in a broiler diet which seemed to be adequate to achieve the favorable results in growth performance, blood parameters, immunity, and bacteriological examination.



Keywords: Black soldier fly, Insects, Black solidier fly powder, Humoral immune response, Soybean substitution

[Full text-PDF]

Research Paper

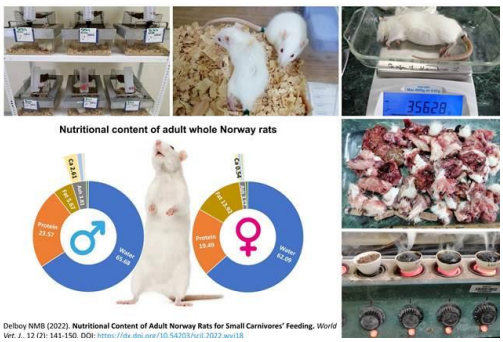
Nutritional Content of Adult Norway Rats for Small Carnivores' Feeding

Delboy NMB.

World Vet. J. 12(2): 141-150, 2022; pii:S232245682200018-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj18

ABSTRACT: Rats are the natural diet of many free-ranging carnivores. They are also fed to small carnivore mammals, raptor birds, and reptiles in captivity as a sole or partial diet, however, little is known about the nutrients that a rat can provide as animal feed. This study aimed to determine the nutritional content of the whole captive-bred Norway rats. A total of 12 randomly selected weaned male and female Wistar Norway rats were fed ad libitum with a local dry dog food diet. The rats were weighed weekly until an average weight of 300 g was reached. Biochemical and mineral analyses were carried out for each rat. The results of the study showed significant differences between male and female rats in terms of growth rate, crude protein, total fat, and calcium concentrations. Males presented a faster growth rate and reached the desired weight in around half the time (6 weeks), compared to females (13 weeks). Moreover, males had a higher percentage of crude protein (23.57%) on a fed matter basis, calcium (2.61%), and phosphorus (0.98%). Females showed higher total fat (13.92%) and lower crude protein (19.49%), calcium (0.54%), and phosphorus (0.47%), compared to males. The results of this research may be used to determine whether a whole rat can provide all the necessary nutrients to carnivore animals commonly kept in captivity. Present findings indicated that rats could provide the necessary nutrients, however, if given as a sole diet, they could not be enough to supply the nutritional requirements of animals in the long term.



Keywords: Carnivore nutrition, Norway rats, Nutritional Content, Wistar rat

[Full text-PDF]

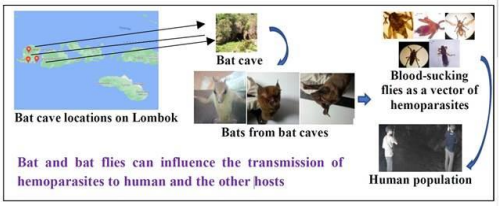
Research Paper

Identification of Blood-sucking Flies of Bats in Lombok Island, Indonesia

Kholik Kh, Atma CD, and Ningtyas NSI.

World Vet. J. 12(2): 151-155, 2022; pii:S232245682200019-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj19



Kholik Kh, Atma CD, and Ningtyas NSI (2022). **Identification of Blood-sucking Flies of Bats in Lombok Island, Indonesia.** *World Vet. J.*, 12 (2): 151-155. DOI: https://dx.doi.org/10.54203/scil.2022.wvj19

ABSTRACT: Bats and blood-sucking bat flies have an important role in transmitting several hemoparasites. Bat flies have been identified as vectors transmitting hemoparasites from wild bats. The purpose of the present study was to identify bats and their blood-sucking flies as vectors of hemoparasites in bat caves located at Lombok Island, Indonesia. In the course of the study, a survey was conducted on three bat caves from September to December 2018. The bats were captured by a net trap and the species of bats and bat flies were identified. A total of 66 captured bats were identified as *Hipposideros* species (n = 28), *Eonycteris spelaea* (n = 23), and *Taphozouss* species (n = 15). The blood-sucking flies were identified as *Eucampsipoda sundaica* on *Eonycteris spelaea,* and *Stylidia* cf. *euxesta, Brachytarsina* species*, Raymondia* species*,* and *Megastrebla nigriceps* on *Hipposideros* species*.* The results showed that five species of blood-sucking flies were present in captured bats. The bat and blood-sucking flies can influence the transmission of *Polychromophilus* species, *Babesia* species, *Plasmodium* species, and *Trypanosoma* species to humans and other hosts.
Keywords: Bats, Blood-sucking flies, Hemoparasites, Lombok, Vector

[Full text-PDF]

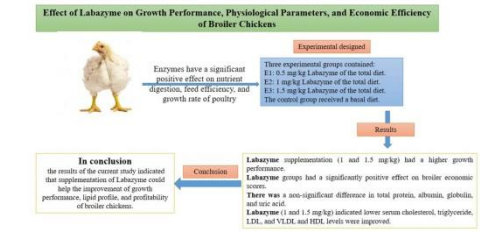
Research Paper

Effect of Labazyme on Growth Performance, Physiological Parameters, and Economic Efficiency of Broiler Chickens

Abdulwahid AS, Mohammed AB, Raouf SM, and Aljumaily TKhH.

World Vet. J. 12(2): 156-163, 2022; pii:S232245682200020-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj20



Abdulwahid AS, Mohammed AB, Raouf SM, and Aljumaily TKhH (2022). **Effect of Labazyme on Growth Performance, Physiological Parameters, and Economic Efficiency of Broiler Chickens**. *World Vet. J.*, 12 (2): 156-163. DOI: https://dx.doi.org/10.54203/scil.2022.wvj20

ABSTRACT: Enzymes have a significant positive effect on nutrient digestion, feed efficiency, and growth rate of poultry. The current experiment aimed to determine the optimal dosage levels of Labazyme as feed additives. A total of 240 one-day-old broiler chickens (Ross 308) were randomly assigned to four groups with three replicates. The feeding experiment was carried out from hatching to day 42 of age. Three experimental groups contained Labazyme at 0.5, 1, and 1.5 mg/kg of the total diet. The control group received a basal diet. Growth performance, European performance efficiency index (EPEI), production index (PI), biochemical and lipid profiles, as well as antioxidant parameters were then measured. The results showed that chickens fed Labazyme supplementation (1 and 1.5 mg/kg) had a higher growth performance than those in the control group. Nonetheless, there was a significant difference between the Labazyme and the control group in terms of feed intake. In addition, Labazyme groups had a significantly positive effect on broiler economic scores. The EPEI and PI of the Labazyme-fed chickens were both higher than the control. There was a non-significant difference in total protein, albumin, globulin, and uric acid. The serum glucose level of the chickens fed Labazyme (1 and 1.5 mg/kg) was lower, compared to the control group. In contrast, chickens that consumed a diet supplemented with Labazyme 1 and 1.5 mg/kg indicated lower serum cholesterol, triglyceride, low-density lipoprotein, and very-low-density lipoprotein levels in broilers, compared to the control group. Serum high-density lipoprotein levels were improved and more pronounced in chickens fed Labazyme, compared to the control group. In conclusion, the results of the current study indicated that supplementation of Labazyme could help the improvement of growth performance, lipid profile, and profitability of broiler chickens.
Keywords: Broiler, Labazyme, Lipid profiles, Production index

[Full text-PDF]

Research Paper

Molecular Diversity and Histopathological Findings of Novel Bovine Viral Diarrhea Virus Strains Isolated from Bull Semen

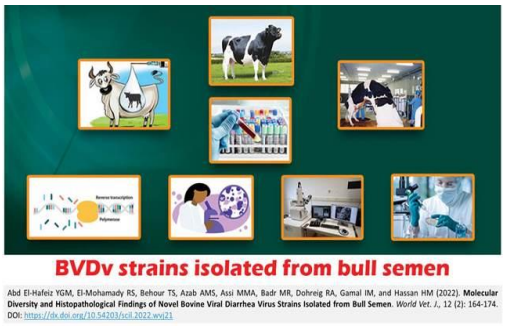
Abd El-Hafeiz YGM, El-Mohamady RS, Behour TS, Azab AMS, Assi MMA, Badr MR, Dohreig RA, Gamal IM, and Hassan HM.

World Vet. J. 12(2): 164-174, 2022; pii:S232245682200021-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj21

ABSTRACT: Bovine viral diarrhea virus (BVDV) is one of the most common viral pathogens affecting the cattle industry worldwide. The present study aimed to molecularly characterize BVDV isolates that are currently circulating in breeding bulls farmed with cattle suffering from reproductive disorders, and also to assess the consequences of BVDV infection on

bulls' semen quality and conception, and its pathological effects on the structure of testicular tissue and spermatozoa. For this purpose, semen, serum, and testicular samples were collected from four breeding bulls in four private dairy farms in the governorates of Kafr-El Sheik, Beni-Suef, Giza, and Assuit, in Egypt from April 2019 to May 2020. An evaluation of sperm abnormalities was carried out by assessing the integrity of the plasma and acrosomal membranes where severe damage and abnormalities were found. Ultrastructure analysis of the spermatozoa by transmission electron microscopy revealed the presence of a swollen plasma membrane with segmented outer acrosomal membrane of spermatozoa and vacuolar degenerated mitochondria. Histopathological examination of testicular and epididymal tissues indicated moderate to severe degenerative effects of virus infection on seminiferous tubules with hypospermatogenesis. By detection of virus antigen in the serum samples using ELISA, bulls were identified as persistently infected with BVDV. Virus isolation revealed four noncytopathic (NCP-BVDV) strains that were confirmed by fluorescent antibody technique (FAT) and amplification of the 5′ untranslated genomic region (5′UTR) and molecularly typed by amplification of the Erns glycoprotein region. Isolates' Phylogenetic analysis revealed two subgenotypes: BVDV-1b (Genbank accession numbers; LC634512, LC634513, LC634515) and BVDV-1d (LC634516). According to the knowledge of the authors of the present study, the circulation of the BVDV-1d subgenotype is not reported in Egypt. Therefore, it would be of great importance to track circulating strains in specific countries for successful vaccination programs or accurate diagnostic tests, and this necessitates regular updates.



Abd El-Hafeiz YGM, El-Mohamady RS, Behour TS, Azab AMS, Assi MMA, Badr MR, Dohreig RA, Gamal IM, and Hassan HM (2022). **Molecular Diversity and Histopathological Findings of Novel Bovine Viral Diarrhea Virus Strains Isolated from Bull Semen**. *World Vet. J.*, 12 (2): 164-174. DOI: https://dx.doi.org/10.54203/scil.2022.wvj21

Keywords: BVDV, Isolation, Spermatozoa ultrastructure, Sperm abnormalities, Testicular histopathology

[Full text-PDF]

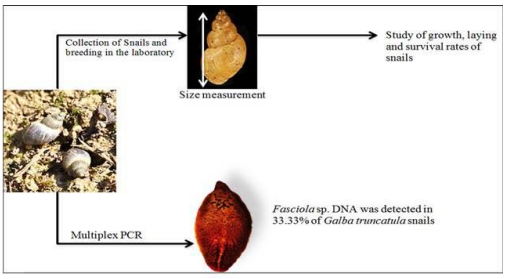
Research Paper

Growth, Laying, and Survival Rates of the *Galba truncatula* Snails Infected with Fasciola hepatica

Aimeur F, Mekroud M, Khelifi-Touhami NA, Titi A, Ouchene N, and Mekroud A.

World Vet. J. 12(2): 175-180, 2022; pii:S232245682200022-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj22



Aimeur F, Mekroud M, Khelifi-Touhami NA, Titi A, Ouchene N, and Mekroud A (2022). **Growth, Laying, and Survival Rates of the *Galba truncatula* Snails Infected with *Fasciola hepatica***. *World Vet. J.*, 12 (2): 175-180. DOI: https://dx.doi.org/10.54203/scil.2021.wvj22

ABSTRACT: Fasciolosis is one of the most important parasitic diseases in ruminants in Algeria, of which the intermediate host is *Galba truncatula* (*G. truncatula*) snail. The current study aimed to investigate the prevalence of *Fasciola* sp. in naturally infected *G. truncatula* snails using multiplex PCR. Secondly, it was targeted toward examining the rate of growth, survival, and laying of the snails in experimental conditions during 6 weeks of rearing in three tanks. This study was conducted in two different regions of Algeria, namely El Tarf and Constantine. The investigated tanks 1, 2, and 3 consisted of 12 (size 3-4 mm), 30 (size 5-6 mm), and 30 (size 7-8 mm) snails, respectively. *Fasciola* sp. DNA was detected in 33.33% of *G. truncatula* snails (25% in Constantine and 42.85% in El Tarf). The total survival rates in the first, second, and third tanks were 50%, 43.3%, and 40%, respectively. The obtained results indicated that the growth rate of the snail depended on its initial size (the smaller the initial size, the higher the weekly growth rate). The total growth rates were 3, 1.7, and 1.1 mm in tanks 1, 2, and 3, respectively. The use of multiplex PCR indicated a relatively high level of infestation of the snails by *Fasciola* sp. Snails larger than 7 mm had the highest lay rate. Further studies are needed to investigate other snails that may be infested with *Fasciola* sp.

Keywords: *Fasciola*, *Galba truncatula*, PCR multiplex, Snail

[Full text-PDF]

Short Communication

Parascaris equorum in Horses of Payakumbuh City, West Sumatra, Indonesia

Zelpina E, Sujatmiko S, Silvia Noor P, and Lefiana D.

World Vet. J. 12(2): 181-185, 2022; pii:S232245682200023-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj23

Zelpina E, Sujatmiko S, Silvia Noor P, and Lefiana D (2022). ***Parascaris equorum* in Horses of Payakumbuh City, West Sumatra, Indonesia**. *World Vet. J.*, 12 (2): 181-185. DOI: https://dx.doi.org/10.54203/scil.2022.wvj23

ABSTRACT: *Parascaris equorum* is a species of the *Ascarididae* family which belongs to the phylum nematoda (roundworms) and is a type of parasite that affects equine health, performance, and production. The current study was carried out from April to August 2021 to determine the prevalence of equine *Parascaris equorum* in horses residing in Payakumbuh City, Indonesia. A total number of 128 fecal samples from horses were examined in the current study. Coprological examination was performed for the detection of

Parascaris equorum eggs inside the amassed samples. The overall occurrence of *Parascaris equorum* was 14.06% (18 out of 128). The prevalence rates of sub-districts differed with the highest in East at 18.18% (8/44), followed by North, South, and West at 16.66% (6/36), 14.06% (2/22), and 7.7% (2/26), respectively. The obtained results indicated a significant difference in the prevalence rate of *Parascaris equorum* between males and females as well as those horses aged < 5 years (26.22%) and > 5 years (3%). Therefore, it is critical to not only enhance horse health management, maintenance, and health but also to provide anti-parasitic medications on a regular basis.
Keywords: Gastrointestinal nematode, Horse, *Parascaris equorum*, Prevalence

[Full text-PDF]

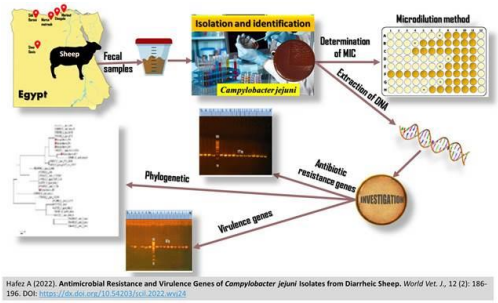
Research Paper

Antimicrobial Resistance and Virulence Genes of Campylobacter jejuni Isolates from Diarrheic Sheep

Hafez A.

World Vet. J. 12(2): 186-196, 2022; pii:S232245682200024-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj24



ABSTRACT: One of the important agents causing gastroenteritis worldwide is *Campylobacter jejuni (C. jejuni)*. The current study aimed to detect five virulence genes (flaA, virB11, ciaB, iam, and dnaJ) and two antibiotic resistance genes (gyrA and tetO) in *C. jejuni* obtained from sheep stool. The virulence genes were detected by PCR in 64 *C. jejuni* strains. The phenotypic resistance to five selected antibiotics (Ciprofloxacin, Erythromycin, Gentamycin, Streptomycin, and Tetracycline) was screened with the microdilution method. The isolates with antibiograms were tested for detection of *gyrA and tetO* genes via PCR using specific primers. The virulence genes *flaA* (32%) and *dnaJ* (29%) had the highest prevalence. The tested isolates of *C. jejuni* revealed high resistance to both quinolone (68.3%) and tetracycline groups (48.4%) with an increased prevalence of antibiotic resistance of gyrA and tetO genes. Gentamycin and erythromycin offered better alternative drugs for the treatment of campylobacteriosis. To generalize the findings, extensive profiling that involves more virulence genes is required in several strains of *Campylobacter*.
Keywords: Antibiotic resistance, *Campylobacter jejuni*, Sheep, Virulence genes

[Full text-PDF]

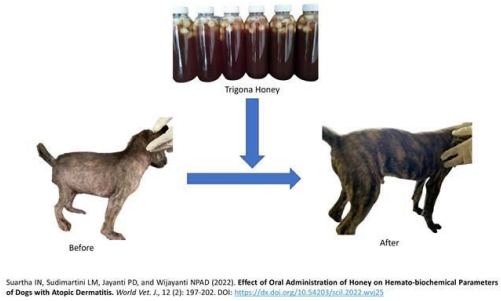
Research Paper

Effect of Oral Administration of Honey on Hemato-biochemical Parameters of Dogs with Atopic Dermatitis

Suartha IN, Sudimartini LM, Jayanti PD, and Wijayanti NPAD.

World Vet. J. 12(2): 197-202, 2022; pii:S232245682200025-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj25



ABSTRACT: Honey from *Trigona* species is widely used as herbal medicine in humans due to its antimicrobial, anti-inflammatory, and antioxidant effects as well as the potential to increase body resistance and boost blood formation. The current study aimed to determine the hemato-biochemical profile of dogs with atopic dermatitis treated with *Trigona* honey. The hematology profile included the measurement of erythrocytes, hemoglobin, hematocrit, and erythrocyte index, as well as blood biochemical parameters, including aspartate aminotransferase (AST), alanine transaminase (ALT), and blood sugar. A total of 12 local dogs aged 4 months old were divided into two treatment groups, namely the control group (G1) and treatment with liquid *Trigona* honey at a dosage of 5 ml/dog/day (G2) for 35 days. Then, blood was collected and tested for routine and chemical blood assay. The results showed that the administration of fresh *Trigona* honey (5 ml/day for 5 weeks) exhibited a significant increase in most of hematological variables of dogs with atopic dermatitis, compared to G1. The results of blood biochemical profiles (AST, ALT, and blood glucose) remained unaffected by the treatment of *Trigona* honey. It can be concluded that honey from *Trigona* spp. was safe to be given to the dogs with dermatitis and no adverse physiological effects were observed during the present study.

Keywords: Blood, Dermatitis, Dog, Hemato-biochemical, *Trigona* species honey

[Full text-PDF]

Research Paper

The Transmission Pattern of Amoebiasis in Bale Zone, South East Ethiopia

Jilo SA, Kadir MA, Hussein JA, and Nair SP.

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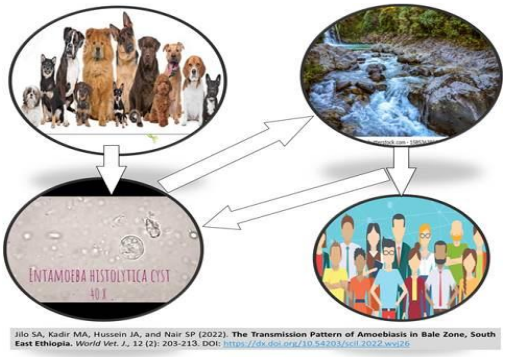

Jilo SA, Kadir MA, Hussein JA, and Nair SP (2022). **The Transmission Pattern of Amoebiasis in Bale Zone, South East Ethiopia.** *World Vet. J.*, 12 (2): 203-213. DOI: https://dx.doi.org/10.54203/scil.2022.wvj26

ABSTRACT: Amoebiasis is a primarily zoonotic disease, mainly transferred through the fecal-oral route and waterborne. Amoebiasis is still a big challenge for human and animal health and is a major cause of diarrhea in developing countries, including Ethiopia. Therefore, the study was conducted to assess the epidemiology of the disease in humans, dogs, and the occurrence of the parasite in water bodies. A prospective cross-sectional study was conducted in selected districts of the Bale zone in southeastern Ethiopia. Pet owners were selected randomly. Socio-demographic data were collected using a questionnaire and fecal samples were used to perform microscopic examination. A total of 383 fecal samples of humans, 383 fecal samples of dogs, and 58 water samples were studied from December 2019 to July 2020. Of 383 humans, 179 were males and 186 were females, while 94 individuals were grouped as children younger than 8 years, 164 were grouped as youth within the age range of 8-18 years, and 125 were grouped as adults who were older than 18 years. Of 383 local breeds, dogs were grouped as 87 puppies younger than one year, 192 young dogs with the age range of 1-2 years, and 104 adult dogs who were older than 2 years. Fecal samples were taken from 173 male and 210 female dogs. The water samples were taken randomly from the water sources (river, lake, pond, or water tank) at different sites where dogs and humans can easily contact water to use for different purposes. Of the total samples, 70 humans (18.3%), 63 dogs (16.5%), and 16 water samples (27.6%) were contaminated with the parasite. The major risk factors for the transmissions of parasites were contaminated drinking water, large family size, open-air defecation, and improper handwashing. The present study revealed that the human reservoir was a major risk factor for the spread and transmission of amoebiasis in dogs. The high prevalence of the disease might be due to open-air defecation, unhygienic health practices, domestic animals inside the houses, and using local water bodies as a drinking source.

Keywords: Amoeba, Dog, Human, Transmission, Water

[Full text-PDF]

Review

Diagnosis and Control of Peste des Petits Ruminants Disease in Small Ruminants; A Review

Mahmoud MA, Ghazy AA, and Shaapan RM.

World Vet. J. 12(2): 214-220, 2022; pii:S232245682200027-12

DOI: https://dx.doi.org/10.54203/scil.2022.wvj27

Mahmoud MA, Ghazy AA, and Shaapan RM (2022). Diagnosis and Control of Peste des Petits Ruminants Disease in Small Ruminants; A Review. *World Vet. J.*, 12 (2): 214-220. DOI: https://dx.doi.org/10.54203/scil.2022.wvj27

ABSTRACT: Peste des Petits Ruminants (PPR) is an acute highly contagious febrile disease of sheep and goats characterized by erosive and necrotizing stomatitis and associated with severe pneumo-enteritis and bronchopneumonia ended by recovery or death. The aim of the present study was to throw light on the diagnosis and control of PPR. Diagnosis of PPR depends on clinical signs, pathological lesions, and specific detection of the viral antigen, viral genome, or specific antibodies by serological tests and nucleic acid-based assays. The most commonly used diagnostic techniques are cell culture isolation, agar gel immunodiffusion, hemagglutination tests, immunocapture ELISA, and competitive ELISA. In addition to the abovementioned techniques, virus neutralization tests and reverse transcriptase PCR are used. Peste des Petits Ruminants is characterized by high fever associated with watery nasal and ocular discharges, mucopurulent stomatitis, and broncho-pneumonia. Moreover, severe bloody diarrhea and the disease associated with high levels of mortality reached up to 90%. The diagnosis of viral diseases is important in determining the control strategies. Therefore, it can be concluded that recent diagnostic tools are urgently needed not only for the diagnosis but also for following-up combating programs and control of viral diseases. Early and rapid complete identification of infectious viral agents in small ruminants as well as in the surrounding environment is recommended for effective control of PPR. The control program depends mainly on vaccination, hygiene and sanitation measures, and effective quarantine measures.

Keywords: Control, Dairy Ruminants, Diagnosis, Goat, Peste des Petits, Sheep

[Full text-PDF]

Review

Bats and Antibiotic Resistance: A Culprit or a Victim?

Garcês A.

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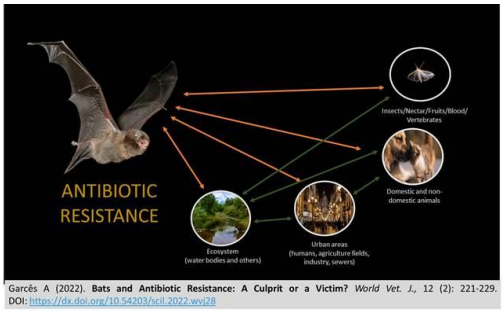
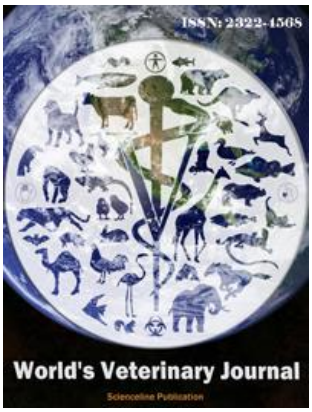


Garcês A (2022). **Bats and Antibiotic Resistance: A Culprit or a Victim?** *World Vet. J.*, 12 (2): 221-229. DOI: https://dx.doi.org/10.54203/scil.2022.wvj28

ABSTRACT: In the last decades, the increase of antimicrobial resistance bacteria has become a concern for public health. Bats' ability to fly, form colonies for a long lifespan, and inhabit a variety of diverse ecological niches make them successful species in terms of adaptation and distribution on earth. Moreover, these characteristics let them act as the potential natural reservoir of numerous zoonotic pathogens (bacteria, viruses, fungi). Bat bacteriome knowledge is still very scarce, but a few studies have indicated that bats are hosts of antimicrobial resistance and play an important role in the dispersion of resistance in the environment. Moreover, bats are vulnerable to acquiring these pathogens since they sometimes live in close contact with humans and domestic animals. Therefore, the present study aimed to compile the latest studies that describe the presence of antibiotic-resistant in bats. Based on the papers analyzed for this review, it is possible to conclude that bats are hosts of pathogenic bacteria that carry numerous antibiotic resistance. Extended-spectrum b-lactamases (ESBLs) or Methicillin-resistant *Staphylococcus* which nowadays days are a great public health concern, have already been reported in these animals, with some isolated strains being of Human origin. Although not completely understood regarding the dynamics and transmission routes, bats seem to have an important role in the dissemination and acquisition of antibiotic resistance in the environment. They can be contaminated by bacteria with antibiotic resistance and disperse through the environment. However, they also can be the host of bacteria that carry antibiotic resistance.

Keywords: Antibiotic-Resistant, Bacteria, Bat, Chiropters, Zoonoses

[Full text-PDF]



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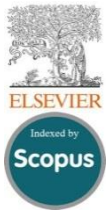

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Forage for Pre-weaning Calves: An Update

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REVIEW ARTICLE
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ABSTRACT

Forage nutrition for pre-weaning calves hosts numerous practical questions and on-farm challenges. The present review article aimed to update and address the biological consequences of forage provision to pre-weaned dairy calves. Health, nutrient intake (milk plus solid feed), and rumen development are the most important factors related to calf growth in pre- and post-weaning periods. A growing body of evidence suggests that the health and growth performance of dairy calves in the pre-weaning period are associated with their later performance as dairy cows. It seems that starter feeding strategies, including grain type, processing method, feed texture, and forage inclusion during the critical pre-weaning period may have profound effects on rumen function and calf performance. It is well understood that grain fermentation by-products are essential for increased growth and absorptive capacity of the rumen papillae. Forage provision as a part of a starter diet has been a topic of recent research. The rumen pH is the main factor altering the fate of fermentation and eventually animal health. In the pre-weaned calf, two major hypotheses exist regarding forage feeding. The first hypothesis describes that the rumen is not completely developed in pre-weaned calves and forage provision during this period might increase gut fill, and hence, decrease starter intake. It is believed that depressed starter intake may limit energy intake and finally suppress calf growth rate. The second hypothesis indicates that the rumen pH may decline as calves age and starter intake increases. Accordingly, forage inclusion in calf starter diets could prevent further rumen pH decline and subsequent negative consequences while improving starter intake and calf growth. Research data regarding these hypotheses are controversial. Many factors, such as milk feeding method, grain, forage type, and experimental conditions could affect calf responses to dietary forage. The current review focused on the biological consequence of forage provision to young calves to provide a practical framework for better use of forages in pre-weaned calves feeding programs.

Keywords: Forage, Growth, Pre-weaned calf, Rumen development

INTRODUCTION

Raising dairy heifers with a proper growth rate (800-900 g/d) for calving between 22-24 months of age is the main goal of commercial dairy heifer raising programs (Akins, 2016). Greater milk yield in the first lactation is partly attributed to higher growth rates in the early stages of life (Soberon et al., 2012). Reaching these goals is mainly dependent on calf feeding strategies during pre- and post-weaning periods. In the last two decades, most calf studies have focused on milk feeding strategies to increase the average daily gain (ADG) during the pre-weaning period. However, it should be considered that solid feed intake and the resulting rumen development are key factors determining calf growth in both pre- and post-weaning periods (Khan et al., 2016). Solid feed consumption in restricted milk-fed calves, has a critical role in supplying energy and protein to the growing calf. Also, in intensified milk-fed calves, enough starter feed intake is essential for successful weaning and post-weaning performance (Khan et al., 2011a). As a result, the quantity, quality, and chemical composition of starter feed provided to dairy heifers could affect their feed intake, rumen development, and subsequent performance.

In addition to concentrates (grain and protein supplements), forages in calf diets can influence rumen development and calf performance. It is believed that forage provision to young calves increases rumen muscularity, volume, and motility needed for optimal rumen development (Beiranvand et al., 2014). In terms of calf performance, feeding forages to pre-weaned calves may have controversial consequences. There is some evidence that forage inclusion in calf diets up to 5% limits feed intake and decreases the growth of calves from 28-56 d of age (Hill et al., 2008). In weaned calves, lower ADG was observed for calves fed 15% hay versus those fed 4.5 to 5% dietary roughage (Hill et al., 2009). In contrast, desirable ruminal pH and improved growth performance were observed when calves were fed different sources of forages as a free choice or mixed rations (Overvest et al., 2015; Omidi-Mirzaei et al., 2018). The contradictory results observed in such research can be partly attributed to the milk feeding method and the age at which the calves were

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weaned. Since milk and solid feed intakes are negatively correlated, feeding different amounts of milk or milk replacer may affect calf response to dietary fiber. On the other hand, as calves age, feed intake increases over time; hence, the duration of the pre-weaning period and weaning time could affect nutrient intake. It can be hypothesized that differences in the duration of solid feed intake may alter the rumen environment, causing different metabolic responses. Additionally, it has been reported that forage source, level, and feeding methods as well as the physical form of the starter diet affect rumen development and calf performance (Diao et al., 2019). Consequently, calf response to dietary fiber is not arbitrary and depends on a variety of nutritional factors. It is important to note that forage intake and rumen pH are interrelated. Thus, forage provision may play a critical role in stabilizing rumen pH and promoting optimal rumen health and calf performance (Terre et al., 2015).

Rumen pH is an important factor affecting the host ruminant health and performance (Laarman et al., 2011). It seems that the ruminal acidosis during pre-weaning and weaning transition periods occurs in a way similar to dairy cows (Galsinger et al., 2020). Moreover, its signs are also similar. Providing forages depending on their source, level (5-15%, in general), and method of feeding has the potential to modulate rumen pH and promote starter intake and growth of pre-weaned calves (Imani et al., 2017). However, in a study investigating the physical form of the starter feed on calf performance, significant amounts of fine starter particles were reported as the main factor affecting intake and ADG (Bateman et al., 2009). It has been recommended that to prevent rumen parakeratosis and bloat, 75% of starter particle size should exceed 1190 µm in diameter (Porter et al., 2007). The variability of rumen pH and occurrence of ruminal acidosis in response to different dietary treatments can be more complicated given that salivary glands are not completely functional in young calves (Khan et al., 2016). For example, parotid glands' ability to produce sufficient amounts of saliva is still low even by 4 weeks of age (Kay, 1960). Consequently, the age at which forages are provided to the calves may be an important factor influencing their metabolic and productive responses. Overall, new studies suggest that fibrous sources such as oat hay provision to pre-weaned calves can improve rumen fermentation parameters, allowing calves to pass the weaning transition successfully (Gasiorek et al., 2020).

Because calf performance in the pre-weaning period is associated with post-weaning growth performance and later dairy herd productivity, the physiological effects of forage feeding on the digestive tract of pre-weaned calves will be reviewed in this article also. Determining optimal types, levels and particle sizes of forages offered in the pre-weaning period requires much future research.

DIETARY FIBER, RUMEN PHYSIOLOGY, AND CALF GROWTH

Weaning is an important period in a dairy calf's life in which milk dependency is disrupted and calves have to meet their energy and protein requirements through solid feed consumption. Calves suffer much from the weaning distress unless the digestive tract adapts properly to ferment and assimilate solid feeds eaten before weaning (Baldwin et al., 2004). Rumen development is a key factor in allowing calves to wean successfully (Nikkhah and Alimirzaei, 2022). Just after birth, the rumen is inactive, but it undergoes extensive metabolic and physical changes over time by initiating solid feed consumption. The type, physical form, and chemical composition of concentrates and forages offered to calves can differently affect rumen development and fermentation patterns. However, in general, starter feeds rich in grains stimulate the rumen epithelium and papillae growth, leading to increased absorptive capacity (Baldwin et al., 2000). Stimulatory effects of grain feeding on the rumen epithelial growth are related to their fermentation end-products profile. It has been shown that volatile fatty acids (VFA; mainly butyric, propionic, and acetic acids) produced by the ruminal anaerobic metabolism are responsible for the proliferation and differentiation of the rumen papillae (Warner et al., 1956). Amongst VFAs, butyric acid is exclusively metabolized in the rumen epithelial cells and is considered a major promoter of papillae differentiation (Baldwin et al., 2004). Butyric acid accompanied by propionic and acetic acids have a direct role in the expression of enzymes involved in the rumen epithelial cells' growth and differentiation (Connor et al., 2013). With these findings, pre-weaning calves can benefit from feeding high grain diets. On the other hand, in a study conducted to evaluate the rumen mucosal absorptive capacity, increased acid absorption was observed when the solution pH decreased from 7.5-8 to 5-5.5 (Sutton et al., 1963). Starch sources and levels in starter diets as well as feed processing methods could affect molar proportions of VFA in the rumen, resulting in altered rumen pH (Lesmeister and Heinrichs, 2004). The accumulation of fermentation products and subsequent lower ruminal pH increases rumen fluid osmolality, causing bacterial death and finally decreased feed intake (Carter et al., 1990). In addition, lower ruminal pH can impair rumen motility and induce papillae keratinization, resulting in lower blood flow to the rumen papillae, and thus, decreased nutrient absorption (Khan et al., 2016).

In adult dairy cows, adequate dietary fiber is required for preventing durable rumen pH decline and acidosis. Although the actual need for dietary fiber has not yet been determined well in young calves, forage provision is recommended to avoid acidosis (McBurney et al., 1983; Castells et al., 2012). Additionally, it has been illustrated that the severity of sub-acute ruminal acidosis can be mitigated by including small amounts of forages in calf diets (Laarman et al., 2011). In another study, increased ruminal pH in pre- and post-weaning periods was reported by feeding forages

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(Khan et al., 2011b). Dietary fiber, especially from forage sources, potentially promotes chewing activity and saliva flow to the rumen which can neutralize protons produced in the rumen and increase ruminal pH (Lin et al., 2018). In addition to the modulatory effect of dietary fiber on rumen pH, forage provision impacts rumen muscularity and physical structure (Heinrichs, 2005). In this regard, larger particle size and higher amounts of dietary effective fiber increase rumen motility. Overall, rumen maturation from metabolic and physical perspectives is essential for subsequent calf growth and development in the post-weaning period.

The growth performance of young calves may be affected by dietary fiber. As discussed above, calves respond differently to forage provision depending on starter feeds and forages' chemical as well as physical features. Interaction of dietary forage with other diet ingredients may cause differences in calf performance. For instance, the supplemental fat source may interact with dietary forage (Karimi et al., 2021). Calves fed soybean oil containing 51% linoleic acid (C18:2) had the lowest starter intake and ADG during the pre-weaning period. Decreased feed intake and growth rate reported in studies concerning forage provision to pre-weaned calves may be associated with gut fill and limited energy intake (Xiao, 2020). It seems that the time at which forages are included in the diet could influence calf performance. In a recent study conducted to determine the optimal time of forage feeding, results indicated that the provision of wheat straw on d 21 had a significant effect on calves weaning body weight (Ghahremani et al., 2021). Forage provision should be considered with starter feed particle size, as well. It seems that forage effects can be more profound when starter feed is too fine and readily fermentable in the rumen, which can elevate acid production and reduce the rumen pH. In a recent study, starter intake decreased by including forage in whole corn grain-based starters, implying that forage provision may be more effective when diet ingredients are finely ground (Gholizadeh et al., 2021). More research is needed to understand how much roughage should be included in the dairy calf diet and what the best forage particle sizes are for pre- and post-weaning calves.

CONCLUSION

A successful post-weaning performance is dependent on proper rumen development in the pre-weaning period. Providing forages to young calves may promote rumen development needed for optimal feed intake, rumination, and nutrient absorption. The level and particle size of forage supplied to the young calf should be orchestrated in accordance with the gain source in the starter diet. These will require future research to be optimally determined.

DECLARATIONS

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Authors' contribution

The authors contributed equally to this work including conceptualization, review strategic contemplation, writing development, editing, and revising. Akbar Nikkhah led the project.

Competing interests

None.

Ethical considerations

Ethical considerations (e.g., plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been made by the authors.

REFERENCES

Akins MS (2016). Dairy heifer development and nutrition management. Veterinary Clinics of North America: Food Animal practice, 32: 303-317. DOI: http://dx.doi.org/10.1016/j.cvfa.2016.01.004

Baldwin RL and McLeod KR (2000). Effects of diet forage: concentrate ratio and metabolizable energy intake on isolated rumen epithelial cell metabolism In vitro. Journal of Animal Science, 78: 771-73. DOI: https://doi.org/10.2527/2000.783771x

Baldwin RL, Mcleod KR, Klots JL, and Heitmann RN (2004). Rumen development, intestinal growth and hepatic metabolism in the pre- and post-weaning ruminants. Journal of Dairy Science, 87: E55-E65. DOI: http://dx.doi.org/10.3168/jds.S0022-0302(04)70061-2

Bateman HG, Hill TM, Aldrich JM, and Schlotterbeck RL (2009). Effects of corn processing, particle size, and diet form on performance of calves in bedded pens. Journal of Dairy Science, 92: 782-789. DOI: https://doi.org/10.3168/jds.2008-1242

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Beiranvand H, Ghorbani GR, Khorvash M, Nabipour A, Dehghan-Banadaki M, Homayouni A, and Kargar S (2014). Interactions of alfalfa hay and sodium propionate on dairy calf performance and rumen development. Journal of Dairy Science, 97: 2270-2280. DOI: http://dx.doi.org/10.3168/jds.2012-6332

Carter RR and Grovum WL (1990). A review of the physiological significance of hypertonic body fluids on feed intake and ruminal function: salivation, motility and microbes. Journal of Animal Science, 68: 2811-2832. DOI: https://doi.org/10.2527/1990.6892811x

Castells L, Bach A, Araujo G, Montoro C, and Terre M (2012). Effect of different forage sources on performance and feeding behavior of Holstein calves. Journal of Dairy Science, 90: 5226-5236. DOI: https://doi.org/10.3168/jds.2011-4405

Connor EE, Baldwin VI RL, Li CJ, Li RW, and Chung H (2013). Gene expression in bovine rumen epithelium during weaning identifies molecular regulators of rumen development and growth. Functional & Integrative Genomics, 13: 133-142. DOI: https://doi.org/10.1007/s10142-012-0308-x

Diao Q, Zhang R, and Fu T (2019). Review of strategies to promote rumen development in calves. Animals, 9: 490. DOI: https://doi.org/10.3390/ani9080490

Galsinger SL, Coblentz WK, Zanton GI, Ogden RK, and Akins MS (2020). Physiological effects of starter-induced ruminal acidosis in calves before, during, and after weaning. Journal of Dairy Science, 103: 2762-2772. DOI: https://doi.org/10.3168/jds.2019-17494

Gasiorek M, Stefanska B, Pruszynska-Oszmalek E, Taciak M, Komisarek J, and Nowak W (2020). Effect of oat hay provision method on growth performance, rumen fermentation and blood metabolites of dairy calves during pre-weaning and post-weaning periods. Animal, 14: 2054-2062. DOI: https://doi.org/10.1017/S1751731120000774

Ghahremani A, Mahjoubi E, Hossein Yazdi M, Chamani M, Bahrami M, and Patton RA (2021). Forage inclusion in calf starter has the best outcome when it is supplemented since 21 days after birth in Holstein calves. Tropical Animal Health and Production, 53: 203. DOI: https://doi.org/10.1007/s11250-021-02635-0

Gholizadeh A, Hossein yazdi M, Mahjoubi E, and Habibi-Tanha MZ (2021). Potential interactive effects between corn grain forms (ground vs. whole) of starter feed and forage provision: Effects on growth and health status of intensified milk-fed Holstein calves. Animal Feed Science and Technology, 271: 114680. DOI: https://doi.org/10.1016/j.anifeedsci.2020.114689

Heinrichs J (2005). Rumen development in the dairy calf. Advances in Dairy Technology, 17: 53-65. Available at: http://www.das.psu.edu/dcn

Hill TM, Bateman HG, Aldrich JM, and Schlotterbeck RL (2008). Effects of the amounts of chopped hay or cottonseed hulls in a textured calf starter on young calf performance. Journal of Dairy Science, 91: 2684-2693. DOI: https://doi.org/10.3168/jds.2007-0935

Hill TM, Bateman HG, Aldrich JM, and Schlotterbeck RL (2009). Roughage for diets fed to weaned dairy calves. Professional Animal Science, 25: 283-288. https://doi.org/10.15232/S1080-7446(15)30719-1

Imani M, Mirzae M, Baghbanzadeh-Nobari B, and Ghaffari MH (2017). Effects of forage provision to dairy calves on growth performance and rumen fermentation: A meta-analysis and meta-regression. Journal of Dairy Science, 100:1136-115. DOI: https://doi.org/10.3168/jds.2016-11561

Karimi A, Alijoo YA, Kazemi-Bonchenari M, Mirzaei M, and Sadri H (2021). Effects of supplemental fat sources and forage feeding levels on growth performance, nutrient digestibility, ruminal fermentation, and nitrogen utilization in dairy calves, Animal, 15: 100179. DOI: https://doi.org/10.1016/j.animal.2021.100179

Kay RNB (1960). The rate of flow and composition of various salivary secretions in sheep and calves. Journal of Physiology, 150: 515-537. DOI: https://doi.org/10.1113/jphysiol.1960.sp006402

Khan MA, Bach A, Weary DM, and Von Keyserlingk MAG (2016). Invited review: Transitioning from milk to solid feed in dairy heifers. Journal of Dairy Science, 99: 885-902. DOI: https://doi.org/10.3168/jds.2015-9975

Khan MA, Weary DM, and Von Keyserlingk MAG (2011a). Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. Journal of Dairy Science, 94:1071-1081. DOI: https://doi.org/10.3168/jds.2010-3733

Khan MA, Weary DM, and Von Keyserlingk MAG (2011b). Hay intake improves performance and rumen development of calves fed higher quantities of milk. Journal of Dairy Science, 94: 3547-3553. DOI: https://doi.org/10.3168/jds.2010-3871

Laarman AH and Oba M (2011). Short communication: Effect of calf starter on rumen pH of Holstein dairy calves. Journal of Dairy Science, 94: 5661-5664. DOI: https://doi.org/10.3168/jds.2011-4273

Lesmeister KE and Heinrichs AJ (2004). Effects of corn processing on growth characteristics, rumen development, and rumen parameters in neonatal airy calves. Journal of Dairy Science, 87: 3439-3450. DOI: https://doi.org/10.3168/jds.S0022-0302(04)73479-7

Lin XY, Wang Y, Wang J, Hou QL, Hu ZY, Shi KR, Yan ZG, and Wang ZH (2018). Effect of initial time of forage supply on growth and rumen development in pre-weaning calves. Animal Production Science, 58: 2288-2299. DOI: https://doi.org/10.1071/AN16667

McBurney MI, Van Soest PJ, and Chase LE (1983). Cation exchange capacity and buffering capacity of neutral detergent fiber. Journal of the Science of Food and Agriculture, 34: 910-916. DOI: https://doi.org/10.1002/jsfa.2740340903

Nikkhah A and Alimirzaei M (2022). Timing of transfer from individual boxes to group pens in newly weaned Holstein calves: Matters?. Archives of Animal Science, 1: 101-104. Available at: http://scientificeminencegroup.com/articles/Timing-of-Transfer-from-Individual.pdf

Omidi-Mirzaei H, Azarfar A, Mirzaei M, Kiani A, and Ghaffari MH (2018). Effects of forage source and forage particle size as a free-choice provision on growth performance, rumen fermentation, and behavior of dairy calves fed texturized starters. Journal of Dairy Science, 101: 4143-4157. DOI: https://doi.org/10.3168/jds.2017-13990

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Overvest MA, Bergeron R, Haley DB, and DeVries TJ (2015). Effect of feed type and method of presentation on feeding behavior, intake, and growth of dairy calves fed a high level of milk. Journal of Dairy Science, 99: 317-327. DOI: https://doi.org/10.3168/jds.2015-9997

Porter JC, Warner RG, and Kertz AF (2007). Effects fiber level and physical form of starter on growth and development of dairy calves fed no forage. Professional Animal Science, 23: 395-400. DOI: https://doi.org/10.15232/S1080-7446(15)30994-3

Soberon F, Raffrenato E, Everett RW, and Van Amburgh ME (2012). Early life milk replacer intake and effects on long term productivity of dairy calves. Journal of Dairy Science, 95: 783-793. DOI: https://doi.org/10.3168/jds.2011-4391

Sutton JD, McGilliard AD, and Jacobson NL (1963). Functional development of rumen mucosa. I. Absorptive ability. Journal of Dairy Science, 46: 426-436. DOI: https://doi.org/10.3168/jds.S0022-0302(63)89068-2

Terre M, Castells L, Khan MA, and Bach A (2015). Interaction between the physical form of the starter feed and straw provision on growth performance of Holstein calves. Journal of Dairy Science, 98: 1101-1109. DOI: https://doi.org/10.3168/jds.2014-8151

Xiao J, Maswayi Alugongo G, Li J, Wang Y, Li S, and Cao Z (2020). Review: How forage feeding early in life influences the growth rate, ruminal environment, and the establishment of feeding behavior in pre-weaned calves. Animals, 10:188. DOI: https://doi.org/10.3390/ani10020188

Warner RG, Flatt WP, and Loosli JK (1956). Dietary factors influencing the development of the ruminant stomach. Journal of Agricultural and Food Chemistry, 4: 788-792. DOI: https://doi.org/10.1021/jf60067a003

To cite this paper: Nikkhah A and Alimirzaei M (2022). Forage for Pre-weaning Calves: An Update. *World Vet. J.*, 12 (2): 123-127. DOI: https://dx.doi.org/10.54203/scil.2022.wvj15

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The Use of Soft Contact Bandage Lenses for Corneal Ulcer in Dogs and Cats: A Review

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ABSTRACT

A corneal ulcer is the characteristic of the destruction of the corneal epithelium layer and loss of the stroma layer at various depths. At present time, soft bandage contact lenses are used in many countries for corneal ulcers in dogs and cats to protect their cornea, increase contact time with topical eyes solutions, and support corneal reepithelialization. This article aimed to review information on the use of soft contact bandage lenses to treat corneal ulcers in dogs and cats interms of their efficacy and precaution. The results of the present review have revealed that soft contact bandage lenses are used to protect the cornea, enhance contact time with topical eye treatments, reduce median healing time, and provide comfort in dogs and cats with corneal ulcers. In the case of serious infections and dry eyes, soft contact bandage lenses are not recommended.

Keywords: Corneal ulcer, Incidence, Soft contact bandage lenses, Treatment

INTRODUCTION

The cornea is the front part of the eyes covering the iris, pupil, and anterior chamber (Farghali et al., 2021). A corneal ulcer is characterized by damage to the corneal epithelium layer (superficial corneal ulcer) and loss of the stroma layer at various depths (deep corneal ulcer) (Kartashov et al., 2019). The severity of a corneal ulcer depends on the size, depth, edema, the appearance of keratomalacia, and present hypopyon or hyphae at the anterior chamber (Ekapopphan et al., 2018). There are various risk factors for corneal ulcers in dogs and cats, including congenital abnormalities, the anatomical structure of the eyes (some breeds risk of dry eyes, such as Pug and Shih-Tzu), trauma (fighting, accidents), infections (herpes virus, parasites), and systemic diseases such as diabetes mellitus and infectious canine hepatitis (Croix et al., 2001; Good et al., 2003; Sykes, 2014; Kartashov et al., 2019; Patel et al., 2020).

Diagnosis of corneal ulcer

Methods for diagnosis of corneal ulcer begin with taking into account the history of the patient and general physical examinations. Pets with corneal ulcers often squint with the painful eye and experience tearing, redness, and increased corneal opacity (Patel et al., 2020; Kim et al., 2021). A complete ophthalmic examination should be performed on both eyes, including the Schirmer tear test (STT) to measure the level of tears, intraocular pressure to measure the fluid pressure of the eyes, a fluorescein dye test to detect the injury to the cornea, and menace response-pupillary light reflex-dazzle reflex, and slit-lamp examination to recognize the neuromuscular system and inside the eye (Kartashov et al., 2019; Kim et al., 2021). A fluorescein dye positive test means fluorescein stain will be maintained in the stroma layer and appear to be green color, indicating corneal epithelium layer devastation, in which case checks should be made to determine the size, depth, and infiltration of white blood cells or red blood cells to evaluate the severity (Patel et al., 2020). Moreover, matrix metalloproteinases (MMPs) are endopeptidases found in almost every eye tissue (Kaya et al., 2021). Matrix metalloproteinases, particularly collagenases, are essential for maintaining corneal integrity and clarity (Kaya et al., 2021). It was suggested that an imbalance in collagenase levels could trigger the keratolytic process, which would destroy the basement membrane structure and cause corneal ulcers (Kaya et al., 2021). Dry eyes, trauma, and infection are examples of conditions that cause MMPs imbalance (Kaya et al., 2021).

Incidence, causative factor, and microbial isolation

There have been reports of the incidence of corneal ulcers in dogs in India, which mainly involve Pug, Pomeranian, mongrel, and brachycephalic dog breeds (Patel et al., 2020). Additionally, corneal ulcers have been reported in cats in

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Russia, mostly involving British and Scottish cat breeds (Kartashov et al., 2019). Moreover, previous reports suggested that brachycephalic cats are predisposed to developing non-healing corneal ulcers (Croix et al., 2001). This sore have been reported in both dogs and cats, specially the younger ones and males (Kartashov et al., 2019; Patel et al., 2020). Some of the causative factors of corneal ulcers reported in dogs include traumatic injury, keratoconjunctivitis sicca, entropion, chemical injury, and distichiasis (Bossuyt, 2016; Patel et al., 2020). For cats, the dermoid, crystalline keratopathy, traumatic injury, entropion, and feline herpesvirus 1 (FHV-1) have been reported as causative factors (Moore, 2005; Bossuyt, 2016; Kartashov et al., 2019). Ekapopphan et al. (2018) surveyed bacteria isolated from corneal ulcers of dogs in Thailand, in which the most commonly found were *Staphylococcus spp*., *Pseudomonas aeruginosa*, *Enterobacteriaciae*, *Streptococcus spp*., *Aeromonas spp*., and *Acinetobacter lwoffii*. Hewitt et al. (2020) studied bacteria isolated from corneal ulcers of dogs in the United States, among which *Staphylococcus pseudintermedius*, *Streptococcus canis*, and *Pseudomonas aeruginosa* were the most common ones. In cats, *Staphylococcus spp*., *Streptococcus spp*., *Corynebacterium spp*., *Escherichia coli*, *Proteus spp*., *Pseudomonas aeruginosa*, and *Klebsiella spp.* have been reported as bacteria isolated from corneal ulcers (Moore, 2005). In addition, other microorganisms, including *Candida spp*., have been reportedly detected in corneal ulcers in dogs (Ekapopphan et al., 2018)

Treatment of corneal ulcers

Treatment of corneal ulcers depends on the progress of the lesion. Superficial corneal ulcer treatment typically utilizes topical eye drops and systemic drugs (Williams et al., 2017). Meanwhile, severe deep corneal ulcer cases are often unresponsive or respond slowly to topical or systemic drugs, and therefore, require surgery (Jaksz and Busse, 2017). At present, the treatment method for corneal ulcers in dogs and cats has been developed to reduce surgical treatment methods while providing results that have similar or better healing outcomes, such as using soft contact bandage lenses (Grinninger et al., 2015; Bossuyt, 2016; Kim et al., 2021). This article gathers information on the use of soft contact bandage lenses to treat corneal ulcers in dogs and cats.

Surgical treatment

Severe deep corneal ulcers, such as descemetocele, corneal dystrophy, ulcerative keratitis, indolent corneal ulcer, and chronic corneal ulcer may require surgical management (Mezzadri et al., 2021). The surgical methods include debridement epithelium, conjunctival pedicle graft, grid or punctate keratotomy, third eyelid flap, superficial keratectomy, and temporary tarsorrhaphy (Croix et al., 2001; Pandey et al., 2018).

Non-surgical treatment

Several reports of nonsurgical treatment include the use of topical eye drops with triple antibiotic (neomycin-polymyxin-bacitracin) prophylactic antibiotic therapy in dogs with superficial corneal ulcers (Hewitt et al., 2020), a combination of chloramphenicol and ciprofloxacin in suspected infected corneal ulcers (Hewitt et al., 2020), and pain control with non-steroidal anti-inflammatory drugs)NSAIDs(or systemic opioids (Jaksz and Busse, 2017). Uveitis cases should use atropine for mydriatic to reduce accompanying painful ciliary muscle spasms (Jaksz and Busse, 2017). Autologous serum, ethylenediaminetetraacetic acid, and acetylcysteine are used in keratomalacia to inhibit proteolytic enzymes (Jaksz and Busse, 2017). In dogs with keratoconjunctivitis sicca, a cross-linked thiolated carboxymethylated hyaluronic acid (xCMHA-S) hydrogel was utilized to close nonhealing stromal ulcers (Williams et al., 2017). Injection of autologous platelet-rich plasma into the conjunctiva of dogs and cats with corneal ulcers was found to be efficient, yet the procedure is time-consuming (Farghali et al., 2021). At present, bandage contact lenses are used in many countries in cases of corneal ulcers in dogs and cats, including Georgia, Germany, Korea, the Netherlands, the United Kingdom, and the United States (Grinninger et al., 2015; Bossuyt, 2016; Braus et al., 2018; Sukjong et al., 2018; Diehl et al., 2019; Kim et al., 2021). Bandage contact lenses have been reported to have properties that promote healing, deliver medicine, and maintain hydration (Sukjong et al., 2018). Previous studies report that the use of soft bandage contact lenses in corneal ulcers in dogs decreases healing time, compared with dogs not treated with soft bandages (Wooff and Norman, 2015).

Contact bandage lenses

Contact lenses have been used in humans since 1887 (Loh and Agarwal, 2010). Different types of contact lenses include hard lenses (firm, reduce oxygen flow to the cornea) and soft lenses (soft, made of hydrogel, and permit oxygen to the cornea, Loh and Agarwal, 2010). Soft contact lenses are used as bandages to preserve the cornea, improve contact duration with topical eye treatments that enhance the cornea-drug interaction over time, and encourage corneal reepithelialization (Sukjong et al., 2018, Figure 1). In veterinary uses, soft contact bandage lenses designed for both humans and animals have been adopted (Diehl et al., 2019). In 2018, both types of lenses were recommended for canine eyes (Braus et al., 2018). Each product brand has a different design component inside the contact lens (Table 1). Before using soft contact bandage lenses, the animal should be tested via STT to measure dry eye conditions, observe purulent discharge, and check cases with suspected bacterial keratitis since some previous reports do not recommend the use of lenses in such instances (Bossuyt, 2016).

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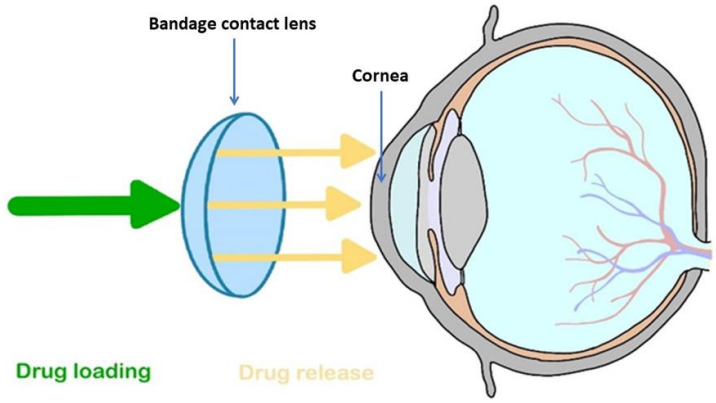

Figure 1. The soft contact bandage lenses help to protect the cornea, increase contact time with topical eyes solutions and increase the cornea-drug interaction for a long time.

Table 1. Soft contact bandage lenses used in animals and human

Trade name	Materials	Designed for species	References
SofLens® (Bausch & Lomb)	Hydroxyethyl methacrylate and ethylene glycol dimethacrylate	Human	Schmidt et al. (1977)
Softcon® (Lombart)	Hydroxyethyl methacrylate and polyvinyl pyrrolidone	Human	Schmidt et al. (1977)
PureVision®2 (Bausch & Lomb)	Silicone vinyl carbamate, N-vinylpyrrolidone, a siloxane crosslinker, and a vinyl alanine	Human	Braus et al. (2018)
Acrivet® (Bausch & Lomb)	2–hydroxyethyl methacrylate and vinylpyrrolidone	Veterinary (Dog)	Braus et al. (2018); Wooff and Norman (2015)
HydroBlues™18 (Keragenix)	Hydrophilic polymers	Veterinary (Dog)	Diehl et al. (2019)
AnimaLens™HRT 78 (Keragenix)	Hydrophilic polymers	Veterinary (Dog)	Diehl et al. (2019)
Acuvue® Oasys™ with Hydraclear™ Plus (Johnson and Johnson)	Silicone hydrogel senofilc	Human	Diehl et al. (2019)

Table 2. Treatment of corneal ulcers by using soft contact bandage lenses in dogs and cats

Abnormality	Methods	Average or median of healing times (days)	Complications	References
Spontaneous chronic corneal epithelial defects (Dog)	debridement + SCBL	14	Blepharospasm	Grinninger et al. (2015)
Spontaneous chronic corneal epithelial defects (Dog)	linear grid keratotomy + SCBL	7	Not found	Wooff and Norman (2015)
Acute bullous keratopathy (Cat)	SCBL	44	Not found	Sukjong et al. (2018)
Symblepharon (Cat)	superficial keratectomy + SCBL	22	Not found	Kim et al. (2021)
Superficial corneal ulcer (Dog)	SCBL	10.6	Blepharospasm, premature loss of the contact lens	Bossuyt (2016)
Superficial corneal ulcer (Cat)	SCBL	10	Blepharospasm, premature loss of the contact lens	Bossuyt (2016)

SCBL: Soft contact bandage lenses

Use of soft contact bandage lenses for dogs and cats with corneal ulcers

The size of the contact bandage lens should be measured using the Jameson caliper or based on recommendations from the product to get an appropriate size for the eyes of the animal (Wooff and Norman, 2015). Using an inappropriately sized bandage lens could result in dislocation of the lens (Bossuyt, 2016). Wooff et al. (2015) studied the use of soft contact bandage lenses in combination with linear grid keratotomy (LGK) in Boxer dog breed with spontaneous chronic corneal epithelial defects (SCCEDs). Wooff and Norman (2015) found a significant reduction in median healing time, compared with LGK alone. Meanwhile, Bossuyt et al. (2016) studied the use of soft contact

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bandage lenses in corneal ulcers in Lhasa apso, Pug, Boxer, Shih Tzu, mixed dog breeds, and domestic shorthair cats, with the lenses found to provide comfort and protection in the primary healing phase until the ulcer had healed, which occurred faster than without a contact lens. Additionally, Braus et al. (2018) recommended using soft contact bandage lenses in the Beagle dog breed for around 14 days although cytology indicated a mild inflammatory reaction. Kim et al. (2021) reported the use of superficial keratectomy and soft contact lens with partial temporary tarsorrhaphy in symblepharon in the Persian cat breed. The result found no reappearance of symblepharon when rechecked 347 days post-operation. Additional studies involving the use of soft contact bandage lenses in dogs and cats with corneal ulcers are presented in Table 2.

CONCLUSION

The use of soft contact bandage lenses in corneal ulcers in dogs and cats helps to protect the cornea, increase contact time with topical eyes solutions, decrease median healing time, and provide comfort. However, soft contact bandage lenses are not recommended in cases of dry eye conditions and severe infections. Furthermore, soft contact bandage lenses have been found to improve treatment responses when used in combination with other surgical procedures.

DECLARATIONS

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Authors' contribution

Nattapat Pratumjorn, Natapol Pumipuntu, and Ratchanon Kusolsongkhrokul collected data. Athip Lorsirigool wrote the final draft of the manuscript and performed the submission. All authors read and approved the final revised manuscript.

Competing interests

The authors declared that they have no conflict of interest.

Ethical consideration

All authors check plagiarism, fabrication and/or falsification, double publication and/or submission, and redundancy already.

REFERENCES

Bossuyt SM .(2016) The use of therapeutic soft contact bandage lenses in the dog and the cat: a series of 41 cases. Vlaams Diergeneeskundig Tijdschrift, 85: 343-348. DOI: https://www.doi.org//10.21825vdt.v85i6.16308

Braus BK, Riedler D, Tichy A, Spergser J, and Schwendenwein I . (2018)The effects of two different types of bandage contact lenses on the healthy canine eye. Veterinary ophthalmology, 21: 477-486. DOI: https://www.doi.org/10.1111/vop.12535

Croix NC, Woerdt A, and Olivero DK .(2001)Nonhealing corneal ulcers in cats: 29 cases (1991–1999). Journal of the American Veterinary Medical Association, 218: 733-735. DOI: https://www.doi.org/10.2460/javma.2001.218.733

Diehl KA, Bowden AC, and Knudsen D . (2019)Bandage contact lens retention in dogs-A pilot study. Veterinary ophthalmology, 22: 584-590. DOI: https://www.doi.org/10.1111/vop.12626

Ekapopphan D, Srisutthakarn A, Moonarmart W, Buddhirongawatr R, and Bangphoomi N . (2018)Identification and antimicrobial susceptibility of microorganisms isolated from severe corneal ulcers of dogs in Thailand. Journal of Veterinary Medical Science, 80 (8): 1259-1265. DOI: https://www.doi.org/10.1292/jvms.18-0045

Farghali HA, AbdElKader NA, AbuBakr HO, Ramadan ES, Khattab MS, Salem NY, and Emam IA . (2021)Corneal Ulcer in Dogs and Cats: Novel Clinical Application of Regenerative Therapy Using Subconjunctival Injection of Autologous Platelet-Rich Plasma. PloS One, 8: 123. DOI: https://www.doi.org/10.3389/fvets.2021.641265

Good KL, Maggs DJ, Hollingsworth SR, Scagliotti RH, and Nelson RW . (2003)Corneal sensitivity in dogs with diabetes mellitus. American Journal of Veterinary Research, 64: 7-11. DOI: https://www.doi.org//10.2460ajvr.2003.64.7

Grinninger P, Verbruggen A, Kraijer-Huver I, Djajadiningrat-Laanen S, Teske E, and Boevé M . (2015)Use of bandage contact lenses for treatment of spontaneous chronic corneal epithelial defects in dogs. Journal of Small Animal Practice, 56: 446-449. DOI: https://www.doi.org/10.1111/jsap.12360

Hewitt JS, Allbaugh RA, Kenne DE, and Sebbag L .((2020Prevalence and antibiotic susceptibility of bacterial isolates from dogs with ulcerative keratitis in Midwestern United States. Frontiers in Veterinary Science, 7: 583965. DOI: https://www.doi.org/10.3389/fvets.2020.583965

Jaksz M and Busse C (.(2017Treating corneal ulceration in dogs part 2: deep ulcers, In: Recuperado el. Available at: https://www.vettimes.co.uk/app/uploads/wp-post-to-pdf-enhanced-cache//1treating-corneal-ulceration-in-dogs-part--2deep-ulcers.pdf

Kartashov S, Kartashova E, Butenkov A, Rakityanskaya A, Petrova M, Oboeva M, and Sultanova M .(2019)Corneal ulcers associated with FHV-1 in cats. IOP Conference Series: Earth and Environmental Science, 403: 012024. DOI: https://www.doi.org//10.1051e3sconf/201913501072

Kaya SG, Inanc-Surer S, Cakan-Akdogan G, Oktay G, Utine CA, and Kalyoncu S . (2021)Roles of matrix metalloproteinases in the cornea: A special focus on macular corneal dystrophy. Medicine in Drug Discovery, .100095 :11 DOI: https://www.doi.org/10.1016/j.medidd.2021.100095

Kim Y, Kang S, and Seo K . (2021)Application of superficial keratectomy and soft contact lens for the treatment of symblepharon in a cat: a case report. Journal of Veterinary Science, 22 (2): e19. DOI: https://www.doi.org/10.4142/jvs.2021.22.e19

Loh K and Agarwal P . (2010)Contact lens related corneal ulcer. Malaysian family physician: the official journal of the Academy of Family Physicians of Malaysia, 5 (1): 46. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4170383/

Mezzadri V, Crotti A, Nardi S, and Barsotti G . (2021)Surgical treatment of canine and feline descemetoceles, deep and perforated corneal ulcers with autologous buccal mucous membrane grafts. Veterinary Ophthalmology, 24: 599-609. DOI: https://www.doi.org//10.1111vop.12907

Moore P (2005). Feline corneal disease. Clinical Techniques in Small Animal Practice, 20: 83-93. DOI: https://doi.org/10.1053/j.ctsap.2004.12.012

Pandey P, Shahi A, Kumar D, and Shukla M . (2018)Clinical Evaluation of Surgical Procedures for Corneal Ulcer in Dogs. The Indian Journal of Veterinary Sciences and Biotechnology, 13: 95-99. DOI: https://doi.org/10.21887/ijvsbt.v13i4.11569

Patel K, Parikh P, Mahla J, Ashwath S, and Kelawala D .(2020)Incidence of Corneal Ulcer in Dogs-A Retrospective Study. Tamilnadu Journal of Veterinary and Animal Sciences, 8: 3174-3179. DOI: https://www.doi.org//10.20546ijcmas.2020.908.361

Schmidt G, Blanchard G, and Keller W . (1997)The use of hydrophilic contact lenses in corneal diseases of the dog and cat: a preliminary report. Journal of Small Animal Practice, 18: 773-777. DOI: https://www.doi.org/10.1111/j.1748-5827.1977.tb05854.x

Sukjong Y, Saejong Y, and Hwi-Yool K . (2018)Nonsurgical treatment involving a contact lens and hyperosmotic solution for acute bullous keratopathy in a cat. Turkish Journal of Veterinary & Animal Sciences, 42. DOI: https://www.doi.org/10.3906/vet-1706-16

Sykes JE .(2014) Infectious Canine Hepatitis. Canine and Feline Infectious Diseases, 182-186. DOI: https://www.doi.org/10.1016/B978-1-4377-0795-3.00018-1

Williams DL, Wirostko BM, Gum G, and Mann B . (2017)Topical cross-linked HA-based hydrogel accelerates closure of corneal epithelial defects and repair of stromal ulceration in companion animals. Investigative Ophthalmology & Visual Science, 58: 4616-4622. DOI: https://www.doi.org/10.1167/iovs.16-20848

Wooff PJ and Norman JC . (2015)Effect of corneal contact lens wear on healing time and comfort post LGK for treatment of SCCEDs in boxers. Veterinary Ophthalmology, 18: 364-370. DOI: https://www.doi.org//10.1111vop.12202

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The Impact of Black Soldier Fly (*Hermetia illucens*) as Feed Supplementation on Productive and Physiological Performance of Broiler Chickens

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ABSTRACT

A total of 450 broiler chicks (Ross 308) were used to evaluate the effect of different inclusion levels of a partially black soldier fly (BSF), BSF Powder (BSFP), BSF Puré (BSFPr), and BSF frozen whole larvae (BSFL) on the growth performance, blood parameters, humoral immune response, and intestinal bacterial count of broiler chickens. The chickens were reared from day 1 to 35 and assigned to the control and 9 dietary groups with different forms of BSF (3 replicates per group with 15 chicks). Black soldier fly was included at levels of 2%, 4%, and 6% for BSFP, BSFPr, and BSFL, respectively, in the starter and growing diets. The results indicated similar body weight, weight gain, and the growth rate in chickens fed 4% BSFP, and 2% BSFPr during the experiment. There was a marked difference in blood parameters due to the different BSF forms and included percentages. The humoral immunity antibody titers against the Newcastle disease virus fluctuated among the experimental groups of different ages. Finally, it could be concluded that the BSF can be incorporated at a level of 4% in the form of powder and Puré in a broiler diet which seemed to be adequate to achieve the favorable results in growth performance, blood parameters, immunity, and bacteriological examination.

Keywords: Black soldier fly, Insects, Black solidier fly powder, Humoral immune response, Soybean substitution

INTRODUCTION

The world population growth has led to a food shortage, water wars, climate change, and soil erosion. Accordingly, the United Nations launched the sustainability initiative with 17 goals some of which are no hunger, zero poverty, good health, and well-being, and climate action (Viana et al., 2022). In this regard, all countries sought to reinforce sustainability in all life aspects, which places more pressure to find a creative solution to increase protein production so that, insects are expected to be human beings' food by 2035 (UNDRR, 2015). For hundreds of years, mankind has tried to combat insects by spending billions of dollars to eradicate insects in order to protect their crops. To extract a small amount of plant protein, they are used to killing a rich source of protein. As insects contain up to 75% protein wherever crops contain up to 14% protein (Premalatha et al., 2011).

Generally, humans hate eating insects, which prevents them from using insects as feed. However, residents of some countries have eaten insects for thousands of years, and this practice is called entomophagy. Black soldier fly is used as a partial substitution for soybean meal in commercial diets and it is a cheap replacer because they can be raised on plant or food byproducts (Schiavone et al., 2017a). Black soldier fly larvae (BSFL) are rich in essential amino acids, such as lysine and methionine, which are important for poultry production (Spranghers et al., 2017). There are more than 470 edible insect species in Africa (Ayieko et al., 2010). As a result, people can benefit from insects by using them in poultry feed (Spranghers et al., 2017).

Poultry production represents a huge sector in the animal production scale and it is of significant importance to pay close attention to poultry nutrition since 70% of the production cost is for. The soybean is going expensive day by day, and this increases the poultry production cost besides reducing the revenue of the invested pound Van (2003).

Black soldier flies have also been observed to lessen the mass and nutrient content of pig manure with similar efficiency to those of poultry manure (Li et al., 2011; Zhou et al., 2013). The meat quality of chickens fed BSF has been similar to that of the chickens fed commercial diet, therefore, they can be used as a partial substitution for soybean meal (Pieterse et al., 2019). As a result, it is well documented that BSFL may be used to feed a wide range of vertebrates (Tomberlin et al., 2015). Black soldier fly larvae also utilize a variety of vertebrate byproducts as a substrate, with no

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adverse impacts on the quality of meats grown on BSFL for humans and solution for sustainable and economic agriculture in developing countries (Diener et al., 2011; Nyakeri et al., 2017).

Black soldier fly larvae could be used as a partial replacement for chicken feed by providing additional protein in addition to being raised on chicken excrement. With this in mind, the present study aimed to investigate the effect of different forms of BSF (*Hermetia illucens*) on broiler production performance, physiological parameters, immune responses, and bacteriological examinations.

MATERIALS AND METHODS

Ethical approval

All samples were taken according to the standard protocol without causing any discomfort or injury to the chickens and the study was carried out according to the Institutional Animal Care and Use Committee of Cairo University (CU-IACUC) Medical Veterinary and Agricultural Sciences board, Egypt under approval code CU/II/F/18/21.

Study design

A total of 450 broiler chicks (Ross 308) aged one day with an average weight of 42 ± 1 g were used for the current study. The chicks were wing-banded, and then divided randomly into 9 experimental and control groups (45 chicks each) according to the BSF form (EgyMag company, Egypt), each group had 3 replicates (15 chickens per each) using a completely randomized design. Chicks from 9 groups were fed a partially replaced-soybean meal diet with different forms of BSF, including BSFP, BSFpr, and BSFL. The first three groups received BSFP at levels of 2%, 4%, and 6%. Groups 4-6 received BSFpr and groups 7- 9 gained BSFL at the same levels of 2%, 4%, and 6%.

Chicks were kept in cages (1 m in length, 0.6 m in width, and 0.4 m in height) under similar, standard hygienic, and environmental conditions. Chicks were vaccinated using Hitchner B1 strain, H5N1, and Gumboro vaccines at 6, 10, and 14 days of age, respectively. The samples were revaccinated against Newcastle disease virus (NDV) at 20 days of age according to CEVA animal health company, France (Newcastle, Gumboro, and Influenza). Batteries brooders with electric heaters were used for brooding chicks. The brooding temperature was maintained at 35°C for the first five days, then decreased by 2°C weekly until the end of week five. The lighting program was a 24-hour light for the first five days, then decreased to 22 hours from day 6 to 35. Chicks were fed starter (1- 14 days) and grower (15-35 days) diets as shown in Table 1. Feed and water were offered *ad libitum* (Okasha, 2021).

Table 1. Composition and chemical analyses of starter and grower diets of broiler chickens

Ingredients (%)	Starter (1-14 days)	Grower (15-35 days)
Yellow corn	56	59.89
Soybean meal (46% protein)	32	28.42
Corn gluten	6.05	4.95
Soya oil	1.5	2.53
Mono-calcium phosphate	1.55	1.38
Limestone	1.75	1.7
Premix (Vitamin+Mineral)*	0.2	0.2
D.L. Methionine	0.22	0.22
L. Lysine Hcl	0.25	0.25
Salt	0.40	0.40
Chemical analyses		
Choline Chloride	0.06	0.06
Crude protein	23	21
Metabolizable energy (kcal/kg)	3000	3100
Calcium	1.0	0.94
Available phosphorus	0.49	0.44
Lysine	1.4	1.3
Methionine	0.67	0.61
Methionine + Cystine	1.04	0.95
Sodium	0.18	1.8
Total	100	100

*Each 2 grams of premix mixture contained Vitamin A (trans-retinyl acetate, 9000 IU), Vitamin D3 (cholecalciferol, 2600 IU), Vitamin E (dl-α-tocopherol acetate, 16 mg), Vitamin B1 (1.6 mg), Vitamin B2 (6.5 mg), vitamin B6 (2.2 mg), Vitamin B12 (cyanocobalamin, 0.015 mg), Vitamin K3 (2.5mg), choline (choline chloride, 300 mg), nicotinic acid (30 mg), pantothenic acid (d-calcium pantothenate, 10 mg), folic acid (0.6 mg), d-biotin (0.07 mg), manganese (MnO, 70 mg), zinc (ZnO, 60 mg), iron (FeSO4 H2O, 40 mg), copper (CuSO4 5H2O, 7 mg), iodine ([Ca(IO3)2], 0.7 mg) selenium (Na2SeO3, 0.3 mg)

Estimated parameters and data collection

Production performance

Each chicken was weighed biweekly. Weight gain and growth rate were calculated according to Broody (1949).

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Blood plasma constituents

Three heparinized blood samples from each treatment were drowned randomly from the wing vein for chemical analyses at the end of the experiment. The chemical analyses were carried out by colorimetric method using a commercial kit (Biodiagnostic company, Egypt) for determining plasma Protein (total protein and albumin), lipid profile (triglycerides, total cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL), and Kidney function (uric acid).

Humoral immune response

The coagulation test was used to determine antibody titer against the Newcastle disease virus following Swayne's method (1998). The serum samples were randomly selected from the wing veins of chicks in each treatment at 18, 23, and 28 days of age. Serum samples were subjected to the hemagglutination inhibition assays using a method described by Oberländer et al. (2020). The geometric means of serum hemagglutination inhibition titers obtained from each group were defined as the reciprocal logarithm in a base of 2 of the highest serum dilutions completely inhibiting agglutination.

Intestinal bacteriological count

Bacteriological examinations were carried out at 3 and 5 weeks of age using standard methods for aerobic bacteria (Brown and Smith, 2014). Microbiological analyses were detected at the department of microbiology, Cairo University, Egypt. Under complete aseptic conditions, 5 grams of broiler intestine samples with a length of 2 cm were separated and cut-opened before weighted and transferred into sterile 50 ml Falcon tubes containing 30 ml of sterile saline solution (0.85% NaCl). Samples were vigorously mixed by vortexing for 1 minute at maximum speed, tenfold serial dilutions were prepared from each sample using the same saline solution, and finally, the dilutions were used for the detection and enumeration of different bacterial groups. To begin, 1 ml from each of the previously prepared dilutions was transferred into two separate sterile Petri-dishes to which approximately 15 ml of sterile melted and cooled plate count agar were added. After mixing, the inoculated plates, they were incubated in an incubator (Thermo Scientific 3951 Large Capacity, Germany) at 30°C for 48 hours. Total bacterial counts per gram were calculated on plates with 30-300 colonies and each count was recorded. In the next step, 1 ml from each of the previously prepared dilutions was transferred into two separate sterile plates to which approximately 15 ml of sterile melted and cooled Eosin methylene blue agar (EMB) medium was added. After mixing, the inoculated plates were incubated at 37°C for 48 hours. *Escherichia coli (E. coli)* spices, *Proteus* spices, and *Enterobacter* spices counts per gram were calculated based on the differential counts of green, colorless and pink colonies, respectively (Downes and Ito, 2001). Xylose Lysine Deoxycholate Agar (XLD) medium was used for the detection of *Salmonella* and red colonies with the black center were recorded for *Salmonella* species.

Statistical analysis

One-way analysis of variance was used to determine the effect of adding BSFP, BSFpr, and BSFL at levels of 2%, 4%, and 6% to the broilers' diet on chickens' growth performance, blood parameters, and humoral immunity. Data were statistically analyzed by the General Linear Model Procedure of the SAS software (SAS, 2004). Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) and significant differences were defined at $p < 0.05$.

RESULTS AND DISCUSSION

Productive performance

Table 2 shows the influence of inclusions levels and the form of BSF larvae on broiler performance. Both black soldier fly puré and BSFP supplementation improved broiler performance throughout the experimental period. Groups with 4% BSFP and 6% BSFPr had the highest body weight, while 6% BSFL had the lowest values ($p < 0.05$). At 3 and 5 weeks of age, chicks in the 4% BSFP group had the highest body weight, while different levels of BSFL recorded significantly lower values ($p < 0.05$). A similar pattern was recorded in body weight gain throughout all periods of the study. Concerning growth rate, 4% BSFP also had the highest percentage throughout the first 3 weeks and the entire experiment, but 4% BSFL recorded the highest percentage between 3-5 weeks of age. The previous research attributed the improvement in chicks' performance to the amino acid profile and higher protein content, compared to vegetable protein sources, which makes BSFP a desirable feed. However, the nutrient composition of BSFP fluctuates (Wang and Shelomi, 2017) since larvae are raised on a variety of sub-states, such as organic waste streams, by-products, chicken faces, and kitchen wastes leading to a wide range of nutritive value, crud protein (33-55%), ether extract (18-32%), calcium (2.4-5.8%), lysine (1.9-2.7%), and methionine (1.9-2.7%, Finke, 2013; Nguyen et al., 2015; Shumo et al., 2019). Results in the present study agree with those reported by Dabbou et al. (2018) who found an improvement in broiler performance with BSF inclusion. The positive modulation of live body weight (LBW) by dietary BSF inclusion partially agrees with what was reported by Oluokun (2000) and Loponte et al. (2017) who observed improved growth rate and higher LBW in chicks and Barbary partridges fed with *hermetia illucens* meal as a component of a complete diet and as a partial replacement (25% or 50%) of soybean meal. This is also in agreement with the successful use of BSF and prepupae grown on swine manure or kitchen waste as feed additives in young chicks. Attivi et al. (2020) found lower body weight gain (BWG) at 2%, 4%, and 6% BSF inclusion while the higher BWG was observed at 8% BSF inclusion.

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Table 2. The influence of different forms and levels of black soldier fly on body weight, body weight gain, and growth ratio of broiler chickens

Treatment	Body weight (g)			Body weight gain (g)			Growth rate (%)		
	1 Weeks	3 Weeks	5 Weeks	1-3 Weeks	3-5 Weeks	1-5 Weeks	1-3 Weeks	3-5 Weeks	1-5 Weeks
BSFP 2%	174.62 ± 1.64	761.86 ± 5.20 ^{f}	1940.98 ± 14.43 ^{d}	586.94 ± 7.05 ^{f}	1168.52 ± 1.97 ^{c}	1765.60 ± 0.73 ^{d}	125.38 ± 1.14 ^{f}	87.24 ± 1.4 ^{d}	166.97 ± 0.13 ^{c}
BSFP 4%	177.96 ± 1.64	831.63 ± 5.76 ^{a}	2000.13 ± 14.61 ^{a}	653.55 ± 7.05 ^{a}	1178.66 ± 1.97 ^{a}	1822.07 ± 10.73 ^{a}	129.49 ± 1.14 ^{a}	82.53 ± 1.4 ^{i}	167.32 ± 0.13 ^{a}
BSFP 6%	173.36 ± 1.64	809.34 ± 6.17 ^{b}	1913.82 ± 14.99 ^{f}	630.30 ± 7.05 ^{b}	1107.56 ± 1.97 ^{h}	1737.85 ± 0.73 ^{f}	128.63 ± 1.14 ^{b}	81.85 ± 1.4 ^{j}	166.73 ± 0.13 ^{e}
BSFPr2%	174.40 ± 2.02	786.59 ± 15.88 ^{d}	1955.66 ± 17.11 ^{b}	611.98 ± 13.66 ^{d}	1168.90 ± 17.05 ^{b}	1780.88 ± 5.75 ^{b}	127.40 ± 1.06 ^{h}	85.27 ± 1.59 ^{g}	167.24 ± 0.21 ^{b}
BSFPr4%	177.96 ± 2.02	793.85 ± 16.28 ^{c}	1945.13 ± 16.89 ^{c}	615.89 ± 13.66 ^{c}	1151.28 ± 17.05 ^{e}	1767.17 ± 5.75 ^{c}	126.75 ± 1.06 ^{d}	84.07 ± 1.59 ^{h}	166.47 ± 0.21 ^{f}
BSFPr6%	173.36 ± 2.02	772.79 ± 15.51 ^{e}	1923.95 ± 16.08 ^{e}	595.84 ± 13.66 ^{e}	1155.18 ± 17.05 ^{d}	1751.02 ± 5.75 ^{e}	126.14 ± 1.06 ^{e}	85.90 ± 1.59 ^{f}	166.94 ± 0.21 ^{d}
BSFL 2%	174.40 ± 7.21	618.08 ± 12.75 ^{h}	1759.36 ± 12.97 ^{h}	443.32 ± 16.79 ^{h}	1141.23 ± 19.06 ^{f}	1584.55 ± 7.37 ^{h}	111.71 ± 2.52 ^{b}	96.09 ± 2.23 ^{b}	163.91 ± 0.86 ^{h}
BSFL 4%	191.73 ± 7.21	589.46 ± 13.09 ^{i}	1691.67 ± 13.50 ^{i}	398.67 ± 16.79 ^{i}	1101.26 ± 19.06 ^{i}	1499.93 ± 7.37 ^{i}	101.84 ± 2.52 ^{c}	96.58 ± 2.23 ^{a}	159.31 ± 0.86 ^{j}
BSFL6%	173.36 ± 7.21	570.00 ± 13.27 ^{j}	1608.29 ± 13.69 ^{j}	396.65 ± 16.79 ^{j}	1038.54 ± 19.06 ^{j}	1435.18 ± 7.37 ^{j}	106.43 ± 2.52 ^{i}	95.50 ± 2.23 ^{c}	161.09 ± 0.86 ^{i}
Control	175.64 ± 2.04	744.86 ± 13.20 ^{g}	1871.81 ± 19.33 ^{g}	568.00 ± 12.56 ^{g}	1126.94 ± 23.60 ^{g}	1694.94 ± 31.52 ^{g}	123.24 ± 3.41 ^{g}	86.13 ± 0.98 ^{e}	165.46 ± 4.06 ^{g}
P-value	0.3283	0.0001	0.0001	0.0001	0.2229	0.0001	0.0003	0.0001	0.7456

Values with different superscripts within a column are significantly different ($p \leq 0.05$). BSFP: Black soldier fly powder, BSFPr: Black soldier fly puré, BSFL: Black soldier fly larvae

Table 3. The influence of different forms and levels of black soldier fly on plasma total protein, total cholesterol, triglycerides, low-density lipoproteins, high-density lipoproteins, uric acid, Triiodothyronine, and Thyroxine hormones of boiler chickens

Treatment	T.P (g/dl)	Al (g/dl)	TCh (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	UA (mg/dl)	T3 (mg/dl)	T4 (mg/dl)
BSFP 2%	7.83^{a}	3.45^{g}	303.36^{a}	88.89^{g}	207.08^{c}	78.51^{a}	3.27^{j}	6.07^{f}	2.56^{c}
BSFP 4%	5.94^{b}	3.16^{i}	286.24^{e}	96.76^{e}	218.27^{a}	48.62^{i}	5.00^{a}	6.53^{c}	2.58^{a}
BSFP 6%	3.37^{j}	3.52^{f}	266.67^{g}	111.11^{b}	182.72^{h}	61.72^{f}	4.00^{e}	7.17^{a}	2.55^{d}
Mean Standard Error	± 0.57	± 0.15	± 7.14	± 8.79	± 6.8	± 3.94	± 0.44	± 0.21	± 0.02
BSFPr 2%	4.42^{f}	3.40^{h}	288.69^{c}	68.52^{i}	202.45^{e}	72.53^{c}	3.43^{h}	5.57^{i}	2.57^{b}
BSFPr 4%	3.87^{i}	3.12^{j}	283.18^{f}	93.98^{f}	207.03^{d}	57.36^{g}	3.52^{g}	5.80^{h}	2.55^{d}
BSFPr 6%	4.23^{g}	3.73^{d}	258.72^{i}	77.78^{h}	201.67^{f}	41.49^{j}	4.73^{b}	6.30^{d}	2.51^{f}
Mean Standard Error	± 0.32	± 0.16	± 14.72	± 6.79	± 13.26	± 4.91	± 0.47	± 0.23	± 0.02
BSFL 2%	4.65^{d}	3.87^{c}	263.61^{h}	106.94^{d}	178.89^{i}	63.33^{e}	4.11^{c}	6.97^{b}	2.42^{i}
BSFL 4%	4.48^{e}	3.90^{b}	245.26^{j}	107.87^{c}	147.82^{j}	75.86^{b}	3.80^{f}	5.33^{j}	2.49^{g}
BSFL 6%	4.98^{c}	3.95^{a}	287.46^{d}	107.87^{c}	199.11^{g}	66.78^{d}	3.36^{i}	6.03^{g}	2.45^{h}
Mean Standard Error	± 0.67	± 0.16	± 8.04	± 9.89	± 10.3	± 3.25	± 0.43	± 0.41	± 0.02
Control	4.12 ± 0.36 ^{h}	3.71 ± 0.09 ^{e}	291.74 ± 13.93 ^{b}	145.83 ± 6.9 ^{a}	207.86 ±6.30 ^{b}	54.71 ± 3.30 ^{h}	4.04 ± 0.22 ^{d}	6.10 ± 0.33 ^{e}	2.52 ± 0.04 ^{e}
P-value	0.0122	0.0004	0.5781	0.0001	0.0033	0.0245	0.7189	0.4912	0.3295

Values with different superscripts within a column are significantly different ($p \leq 0.05$). BSFP: Black soldier fly powder, BSFPr: Black soldier fly puré, BSFL: Black soldier fly larvae; TP: Total protein, AL: Albumin, TCh: Total cholesterol, TG: Triglycerides, UA: Uric acid, T3: Triiodothyronine, T4: Thyroxine, LDL: Low-density lipoproteins, HDL: High-density lipoproteins

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Blood plasma constituents

The influence of different forms of black soldier fly supplementation on blood plasma constituents is shown in Table 3. The substitution of 2% BSFP increased total protein ($p < 0.05$), HDL ($p < 0.05$), and total cholesterol ($p > 0.05$), but decreased uric acid level ($p > 0.05$). Moreover, 4% BSFP substitution increased LDL significantly ($p < 0.05$) and thyroid hormone T4 insignificantly ($p > 0.05$), and decreased HDL level significantly ($p < 0.05$). However, the lowest level of total protein and the highest level of triiodothyronine were observed in 6% BSFP, on the other hand, 4% and 6% BSFPr caused the lowest level of triglycerides ($p < 0.05$) and HDL level, respectively ($p < 0.05$). The highest albumin and triglycerides levels were recorded at 6% BSFL and control groups, respectively ($p < 0.05$). According to the result of Attivi et al. (2020), any BSF meal inclusion level below 8% had a negative impact on protein digestion. The delivery of BSF larval meal to Japanese broiler quails had a substantial impact on physiological indexes ($p < 0.05$, Dörper et al., 2021). The present results are partially agreed with those reported by Marono et al. (2017) and Loponte et al. (2017) that BSF incorporation in the diet has been shown to improve the blood profile of laying hens. On the contrary, Schiavone et al. (2017b) reported the replacement of soybean oil in their diet with 50% or 100% BSF larvae had no effect on the blood profile of Ross 308 broilers at 35 days of age.

Humoral immune response

The effect of dietary various BSF larvae levels on the humoral immunity (HI) antibody titers against Newcastle disease virus is presented in Table 4. The HI antibody titer fluctuated among groups from age to age. At 18 days of age, 2% BSFPr, 4% BSFPr, and 6% BSFL levels had the highest titer. At 23 days of age, 2% BSFP, 4% BSFPr, and 6% BSFPr had the highest titer while 4% BSFP had the lowest. At 28 days of age, the highest values were recorded for 2% BSFL, 4% BSFP, and 4% BSFPr levels. Previous research demonstrates that some amino acids and peptides in BSFL feeding can help the experimental quails' immune systems (Harlystiarini et al., 2020). According to Rumpold and Schlüter (2013), BSFL as an insect should be used as a source of animal protein in chicken production. Insects could be useful in the production of a variety of bioactive chemicals with beneficial qualities. Insects are beneficial not only as a rich source of protein in food and feed supply but also as a source of pharmacological and medicinal compounds that could preserve life and livestock from a variety of ailments (Hirose et al., 2013). For the _ENREF_43 present study no immunostimulant drug was used, therefore, it was assumed that the chicks naturally expressed their antibodies against the Newcastle virus.

Table 4. The influence of different forms and levels of black soldier fly on humoral immunity antibody titer against Newcastle disease virus in broiler chickens

Newcastle disease virus titer / Treatment	18 Days	23 Days	28 Days
BSFP 2%	4.00 ± 0.85 ^{h}	7.00 ± 0.75 ^{a}	6.00 ± 0.63 ^{f}
BSFP 4%	5.00 ± 0.85 ^{g}	4.33 ± 0.75 ^{f}	8.67 ± 0.63 ^{b}
BSFP 6%	5.33 ± 0.85 ^{f}	6.33 ± 0.75 ^{b}	6.67 ± 0.63 ^{e}
BSFPr 2%	9.00 ± 0.81 ^{a}	5.00 ± 0.42 ^{e}	6.00 ± 0.39 ^{f}
BSFPr 4%	8.00 ± 0.81 ^{c}	6.33 ± 0.42 ^{b}	8.00 ± 0.39 ^{c}
BSFPr 6%	7.33 ± 0.81 ^{d}	7.00 ± 0.42 ^{a}	4.67 ± 0.39 ^{h}
BSFL 2%	4.00 ± 0.57 ^{h}	5.00 ± 0.80 ^{e}	9.00 ± 0.37 ^{a}
BSFL 4%	4.00 ± 0.57 ^{h}	5.67 ± 0.80 ^{d}	7.33 ± 0.37 ^{d}
BSFL 6%	8.33 ± 0.57 ^{b}	6.00 ± 0.80 ^{c}	5.33 ± 0.37 ^{g}
Control	5.67 ± 0.42 ^{e}	6.00 ± 0.30 ^{c}	6.67 ± 0.50 ^{e}
P-value	0.0001	0.5889	0.4904

Values with different superscripts within a column are significantly different ($p \leq 0.05$). BSFP: Black soldier fly powder, BSFPr: Black soldier fly puré, BSFL: Black soldier fly larvae, HI: Humoral immunity, NDV: Newcastle disease virus, DS: Days of age.

Intestinal bacteriological counts

Beneficial bacteria and *Proteus* species results are shown in Table 5. Generally, dietary inclusions with BSF larvae significantly promoted the proliferation of benefits, such as bacteria microbiota, except for both 2% BSFP and 2% BSFPr ($p < 0.05$). On the other hand, the inclusion of BSFP 2% inhibits *Proteus* species more than the other groups. Moreover, the inclusion of 6% BSFP, 2% BSFPr, 4% BSFPr, 6% BSFPr, 2% BSFL, 4% BSFL, 6% BSFL and control did not significantly inhibit *E. coli* proliferation ($p > 0.05$), at the same time, they significantly inhibited *Enterobacter* species proliferation ($p < 0.05$). However, BSFP at levels of 2% and 4% promoted the proliferation of *E. coli* and *Enterobacter* species, respectively. The antibacterial effectiveness of BSF larvae extracts against *Salmonella* species and *E. coli* species as Gram-negative bacteria was demonstrated by Harlystiarini et al. (2020) who discovered that the methanol extract of BSF larvae was a little more susceptible to *E. coli* bacteria versus Gram-positive bacteria. This

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difference in sensitivity could be due to a change in how ribosomes or other components from bacteria's membrane cells interact with the active chemical in BSFL (Choi et al., 2012). The effect of a pharmaceutical extract of BSFL was studied on *E. coli* by Choi et al. (2012). After the larvae were extracted using various types of organic solvents, antibacterial activity was determined using the agar disc diffusion method and turbidimetry assay. The antibacterial activity of the methanol extract (ME) was demonstrated against the proliferation of Gram-negative bacteria, such as *Shigella sonnei*, *Klebsiella pneumoniae*, and *Neisseria gonorrhoeae*. Gram-positive bacteria, including *Bacillus subtilis*, *Streptococcus mutans*, and *Sarcina lutea*, on the other hand, had no antibacterial effect. According to (Pan et al., 2009), the inhibition zone diameter created by BSF larvae extract was over 6 mm, indicating high antibacterial action for both species. Furthermore, BSF larvae extract had slightly better antibacterial activity against *Salmonella* species than it did against *E. coli*. Antimicrobial peptides (AMPs) were synthesized in the fat body and subsequently released into the hemolymph to provide humoral immunity (Tsakas and Marmaras, 2010). The AMPs molecule was detected in all living creatures, including bacteria and humans. Antimicrobial peptides were an element of the innate immune system of insects, among which defensin has been identified in insects (Zasloff, 2002). It was indicated that the creation of a channel in the cytoplasm membrane of bacteria was the general mode of action of insect defensin (Yi et al., 2014). Cardiolipin, the major phospholipid in bacteria, has a strong affinity for defensin. This interaction between defensin and phospholipid may induce microheterogeneity in the lipid membrane, which may be associated with the creation of channels that are responsible for the defensin's biological activity (Yi et al., 2014). Chitin and lauric acid in black solidier fly work as antimicrobial compounds against harmful bacteria thtough their cationic characteristic that hold harm bacteria prevent them to penetrate the call membrane (Je and Kim, 2006; Yi et al., 2014) .

Table 5. The influence of different forms and levels of black soldier fly on intestinal bacterial counts in broiler chickens

Treatment	Beneficial bacteria	*Escherichia coli*	*Proteus* species	*Enterobacter* species
BSFP 2%	9.27 ± 0.15^{c}	6.33 ± 0.16	-	5.49 ± 0.11
BSFP 4%	9.64 ± 0.15^{abc}	6.56 ± 0.16	4.88 ± 0.26	6.18 ± 0.11
BSFP 6%	9.74 ± 0.15^{abc}	-	5.12 ± 0.26	-
BSFPr 2%	8.31 ± 0.15^{d}	-	4.25 ± 0.26	-
BSFPr 4%	9.41 ± 0.15^{bc}	-	5.05 ± 0.26	-
BSFPr 6%	9.91 ± 0.15^{a}	-	4.48 ± 0.26	-
BSFL 2%	9.46 ± 0.15^{abc}	-	4.38 ± 0.26	-
BSFL 4%	9.68 ± 0.15^{abc}	-	4.35 ± 0.26	-
BSFL 6%	9.84 ± 0.15^{ab}	-	4.88 ± 0.26	-
Control	9.35 ± 0.15^{c}	-	5.03 ± 0.26	-
P-value	0.0001	0.3231	0.1245	0.0014

Values with different superscripts within the column are significantly different ($p \leq 0.05$). BSFP: Black soldier fly powder, BSFPr: Black soldier fly puré, BSFL: Black soldier fly larvae.

CONCLUSION

It was concluded that BSF larvae with its two forms (black soldier fly powder and black soldier fly puré) can be used as a protein source rather than soybean meal and the deleterious effects were not seen on broiler chickens during the present study. The two forms of larvae improved immunological status and suppressed *E. coli*, *Enterobacter*, and *protues* count. The BSF larvae could increase the beneficial bacteria in the intestine of broiler chickens notably. The black soldier fly powder at a level of 4% was recommended which can be replaced with soybean content in broiler chicken diets.

DECLARATIONS

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Authors' contribution

Ahmed El-Kaiaty and Abd El-Rahman Atta designed the study and the experiment was carried out by Doha Dawa. The article was written under the supervision of Ahmed El-Kaiaty and Abd El-Rahman Atta. Tarek Ragab El-Sayed made the microbiological examination. The data were analyzed by Hamada Okasha, lecturer of poultry management under the guidance of Abd El-Rahman Atta. All authors checked and confirm the final analyzed data and the revised manuscript.

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Competing interests

The authors state that they all have no potential conflicts in relation to this research, writing, or publishing.

Ethical considerations

The research had all credibility and trust and did not plagiarise or copy from any other papers or ideas. The present findings did not have any fabrication or falsification. The authors consent to publish only in World's Veterinary journal and did not submit this article or any part of the present scientific results in any other journals. All the data carried out from the trial and writing were supervised by the supervisors (Ahmed El-Kaiaty and Abd El-Rahman Atta).

REFERENCES

Attivi K, Agboka K, Mlaga G, Oke O, Teteh A, Onagbesan O, and Tona K (2020). Effect of black soldier fly (Hermetia Illucens) maggots meal as a fgstitute for fish meal on growth performance, biochemical parameters and digestibility of broiler chickens. International Journal of Poultry Science, 19: 75-80. Available at: https://scialert.net/abstract/?doi=ijps.2020.75.80

Ayieko M, Oriaro V, and Nyambuga I (2010). Processed products of termites and lake flies: Improving entomophagy for food security within the Lake Victoria region. African Journal of Food, Agriculture, Nutrition and Development, 10(2): 1-14. DOI: https://www.doi.org/10.4314/ajfand.v10i2.53352

Broody S (1949). Biomergetic and growth. Reinhold publication ctop. New York, NY, pp. 116-117. DOI: https://www.doi.org/10.1002/ajpa.1330040117

Brown A, and Smith H (2014). Benson's Microbiological Applications, Laboratory Manual in General Microbiology, Short Version: McGraw-Hill Education.

Choi WH, Yun JH, Chu JP, and Chu KB (2012). Antibacterial effect of extracts of H ermetia illucens (D iptera: S tratiomyidae) larvae against G ram-negative bacteria. Entomological Research, 42(5): 219-226. DOI: https://www.doi.org/10.1111/j.1748-5967.2012.00465.x

Dabbou S, Gai F, Biasato I, Capucchio MT, Biasibetti E, Dezzutto D, Meneguz M, Plachà I, Gasco L, and Schiavone A (2018). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. Journal of Animal Science and Biotechnology, 9(1): 1-10. DOI: https://www.doi.org/10.1186/s40104-018-0266-9

Diener S, Solano NMS, Gutiérrez FR, Zurbrügg C, and Tockner K (2011). Biological treatment of municipal organic waste using black soldier fly larvae. Waste and Biomass Valorization, 2(4): 357-363. DOI: http://www.doi.org/10.1007/s12649-011-9079-1

Dörper A, Veldkamp T, and Dicke M (2021). Use of black soldier fly and house fly in feed to promote sustainable poultry production. Journal of Insects as Food and Feed, 7(5): 761-780. DOI: https://www.doi.org/10.3920/JIFF2020.0064S

Downes F and Ito H (2001). Compendium of methods for the microbiological examination of foods. Washington: American Public. Health Association (APHA). Available at: https://ajph.aphapublications.org/doi/book/10.2105/MBEF.0222

Duncan DB (1955). Multiple range and multiple F tests. Biometrics, 11(1): 1-42.

Finke MD (2013). Complete nutrient content of four species of feeder insects. Zoo Biology, 32(1): 27-36. DOI: https://www.doi.org/10.1002/zoo.21012

Harlystiarini H, Mutia R, Wibawan I, and Astuti D (2020). Immune responses and egg productions of quails fed rations supplemented with larvae meal of black soldier fly (Hermetia illucens). Tropical Animal Science Journal, 43(1): 43-49. DOI: https://www.doi.org/10.5398/tasj.2020.43.1.43

Hirose Y, Ohta E, Kawai Y, and Ohta S (2013). Dorsamin-A's, glycerolipids carrying a dehydrophenylalanine ester moiety from the seed-eating larvae of the bruchid beetle bruchidius dorsalis. Journal of Natural Products, 76(4): 554-558. DOI: https://www.doi.org/10.1021/np300713c

Je JY and Kim SK (2006). Chitosan derivatives killed bacteria by disrupting the outer and inner membrane. Journal of Agricultural and Food Chemistry, 54(18): 6629-6633. DOI: https://www.doi.org/10.1021/jf061310p

Li Q, Zheng L, Cai H, Garza E, Yu Z, and Zhou S (2011). From organic waste to biodiesel: Black soldier fly, Hermetia illucens, makes it feasible. Fuel, 90(4): 1545-1548. DOI: https://www.doi.org/10.1016/j.fuel.2010.11.016

Loponte R, Nizza S, Bovera F, De Riu N, Fliegerova K, Lombardi P, Vassalotti G, Mastellone V, Nizza A, and Moniello G (2017). Growth performance, blood profiles and carcass traits of Barbary partridge (Alectoris barbara) fed two different insect larvae meals (*Tenebrio molitor* and *Hermetia illucens*). Research in Veterinary Science, 115: 183-188. DOI: https://www.doi.org/10.1016/j.rvsc.2017.04.017

Marono S, Loponte R, Lombardi P, Vassalotti G, Pero ME, Russo F, Gasco L, Parisi G, Piccolo G, Nizza S et al. (2017). Productive performance and blood profiles of laying hens fed Hermetia illucens larvae meal as total replacement of soybean meal from 24 to 45 weeks of age. Poultry Science, 96(6): 1783-1790. DOI: https://www.doi.org/10.3382/ps/pew461

Nguyen TT, Tomberlin JK, and Vanlaerhoven S (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environmental Entomology, 44(2): 406-410. DOI: https://www.doi.org/10.1093/ee/nvv002

Nyakeri E, Ogola H, Ayieko M, and Amimo F (2017). An open system for farming black soldier fly larvae as a source of proteins for smallscale poultry and fish production. Journal of Insects as Food and Feed, 3(1): 51-56. DOI: https://www.doi.org/10.3920/JIFF2016.0030

Oberländer B, Failing K, Jüngst CM, Neuhaus N, Lierz M, and Möller Palau-Ribes F (2020). Evaluation of Newcastle Disease antibody titers in backyard poultry in Germany with a vaccination interval of twelve weeks. Plos One, 15(8): e0238068. DOI: https://www.doi.org/10.1371/journal.pone.0238068

To cite this paper: El-Kaiaty AM, Atta A-E-R.M, Dawa DT, and El-sayed TR (2022). The Impact of Black Soldier Fly (*Hermetia illucens*) as Feed Supplementation on Productive and Physiological Performance of Broiler Chickens. *World Vet. J.*, 12 (2): 133-140. DOI: https://dx.doi.org/10.54203/scil.2022.wvj17

Okasha HM (2021). Effect of flock age, air pressure and litter types on carcass traits and meat quality of broiler chicks. Annals of Agricultural Science, Moshtohor, 59(3): 435-444. DOI: https://doi.org/DOI:10.21608/ASSJM.2021.186382

Oluokun J (2000). Upgrading the nutritive value of full-fat soyabeans meal for broiler production with either fishmeal or black soldier fly larvae meal (*Hermetia illucens*). Nigerian Journal of Animal Science, 3(2): 51-61. Available at: https://www.ajol.info/index.php/tjas/article/view/49768

Pan X, Chen F, Wu T, Tang H, and Zhao Z (2009). The acid, bile tolerance and antimicrobial property of Lactobacillus acidophilus NIT. Food Control, 20(6): 598-602. DOI: https://www.doi.org/10.1016/j.foodcont.2008.08.019

Pieterse E, Erasmus SW, Uushona T, and Hoffman LC (2019). Black soldier fly (Hermetia illucens) pre-pupae meal as a dietary protein source for broiler production ensures a tasty chicken with standard meat quality for every pot. Journal of the Science of Food and Agriculture, 99(2): 893-903. DOI: https://www.doi.org/10.1002/jsfa.9261

Premalatha M, Abbasi T, Abbasi T, and Abbasi S (2011). Energy-efficient food production to reduce global warming and ecodegradation: The use of edible insects. Renewable and Sustainable Energy Reviews, 15(9): 4357-4360. DOI: https://www.doi.org/10.1016/j.rser.2011.07.115

Rumpold BA, and Schlüter OK (2013). Nutritional composition and safety aspects of edible insects. Molecular Nutrition and Food Research, 57(5): 802-823. DOI: https://www.doi.org/10.1002/mnfr.201200735

Schiavone A, Cullere M, De Marco M, Meneguz M, Biasato I, Bergagna S, Dezzutto D, Gai F, Dabbou S, Gasco L et al. (2017a). Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: Effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. Italian Journal of Animal Science, 16(1): 93-100. DOI: https://www.doi.org/10.1080/1828051X.2016.1249968

Schiavone A, De Marco M, Martínez S, Dabbou S, Renna M, Madrid J, Hernandez F, Rotolo L, Costa P, Gai F et al. (2017b). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. Journal of Animal Science and Biotechnology, 8(1): 1-9. Available at: https://link.springer.com/article/10.1186/s40104-017-0181-5

Shumo M, Osuga IM, Khamis FM, Tanga CM, Fiaboe KK, Subramanian S, Ekesi S, van Huis A, and Borgemeister C (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. Scientific Reports, 9(1): 1-13. DOI: https://www.doi.org/10.1038/s41598-019-46603-zS

Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De Meulenaer B, Michiels J, Eeckhout M, De Clercq P, and De Smet S (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. Journal of the Science of Food and Agriculture, 97(8): 2594-2600. DOI: https://www.doi.org/10.1002/jsfa.8081

Swayne DE (1998). Laboratory manual for the isolation and identification of avian pathogens: American association of avian pathologists. University of Pennsylvania, p. 400. Available at: https://www.nhbs.com/a-laboratory-manual-for-the-isolation-identification-and-characterization-of-avian-pathogens-book

Tomberlin JK, Van Huis A, Benbow ME, Jordan H, Astuti DA, Azzollini D, Banks I, Bava V, Borgemeister C, Cammack JA et al. (2015). Protecting the environment through insect farming as a means to produce protein for use as livestock, poultry, and aquaculture feed. Journal of Insects as Food and Feed, 1(4): 307-309. DOI: https://www.doi.org/10.3920/JIFF2015.0098

Tsakas S, and Marmaras V (2010). Insect immunity and its signalling: An overview. Invertebrate Survival Journal, 7(2): 228-238. Available at: https://www.isj.unimore.it/index.php/ISJ/article/view/225

United Nations Office for Disaster Risk Reduction (UNDRR) (2015). Global assessment report on disaster risk reduction 2015: Making development sustainable: The future of disaster risk management. United Nations Office for Disaster Risk Reduction, (4): 316. Available at: https://www.undrr.org/publication/global-assessment-report-disaster-risk-reduction-2015

Van I (2003). Growth and broilers industrialization. Edition 17, Ceres, Bucharest, pp. 235-236.

Viana CM, Freire D, Abrantes P, Rocha J, and Pereira P (2022). Agricultural land systems importance for supporting food security and sustainable development goals: A systematic review. Science of The Total Environment, 806: 150718. DOI: https://www.doi.org/10.1016/j.scitotenv.2021.150718

Wang, and Shelomi M (2017). Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods, 6(10): 91. DOI: https://www.doi.org/10.3390/foods6100091

Yi HY, Chowdhury M, Huang YD, and Yu XQ (2014). Insect antimicrobial peptides and their applications. Applied Microbiology and Biotechnology, 98(13): 5807-5822. DOI: https://www.doi.org/10.1007/s00253-014-5792-6

Zasloff M (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870): 389-395. Available at: https://www.nature.com/articles/415389a

Zhou F, Tomberlin JK, Zheng L, Yu Z, and Zhang J (2013). Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: *Stratiomyidae*) raised on different livestock manures. Journal of Medical Entomology, 50(6): 1224-1230. DOI: https://www.doi.org/10.1603/me13021

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Nutritional Content of Adult Norway Rats for Small Carnivores' Feeding

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ABSTRACT

Rats are the natural diet of many free-ranging carnivores. They are also fed to small carnivore mammals, raptor birds, and reptiles in captivity as a sole or partial diet, however, little is known about the nutrients that a rat can provide as animal feed. This study aimed to determine the nutritional content of the whole captive-bred Norway rats. A total of 12 randomly selected weaned male and female Wistar Norway rats were fed *ad libitum* with a local dry dog food diet. The rats were weighed weekly until an average weight of 300 g was reached. Biochemical and mineral analyses were carried out for each rat. The results of the study showed significant differences between male and female rats in terms of growth rate, crude protein, total fat, and calcium concentrations. Males presented a faster growth rate and reached the desired weight in around half the time (6 weeks), compared to females (13 weeks). Moreover, males had a higher percentage of crude protein (23.57%) on a fed matter basis, calcium (2.61%), and phosphorus (0.98%). Females showed higher total fat (13.92%) and lower crude protein (19.49%), calcium (0.54%), and phosphorus (0.47%), compared to males. The results of this research may be used to determine whether a whole rat can provide all the necessary nutrients to carnivore animals commonly kept in captivity. Present findings indicated that rats could provide the necessary nutrients, however, if given as a sole diet, they could not be enough to supply the nutritional requirements of animals in the long term.

Keywords: Carnivore nutrition, Norway rats, Nutritional Content, Wistar rat

INTRODUCTION

Nutrition affects the health, growth, reproduction, availability of key nutrients, and longevity of different animals (Moraal et al., 2012; Wilder et al., 2013). Wild animals instinctively know what to hunt or forage for to survive. The way free-ranging animals choose their diet is a very complex behavior as they use a wide variety of morphological, physiological, and anatomical adaptations (Fernández et al., 2021). Wild predators mostly select their prey based on macronutrient composition or selectively feed on specific body parts to obtain the nutrients they need (Kohl et al., 2015). However, captive animals should feed the supplied food, and they mostly suffer from nutrient deficiencies. Since the natural diet of wild animals in a captive feeding situation can rarely be supplied, they are given a substitute diet that causes different diseases related to dietary management (Liesegang et al., 2008).

It has to be taken into consideration that no studies have been conducted so far to investigate the exact requirements of captive wild animals concerning their different existing species due to the difficulty in obtaining a large number of blood, urine, and feces samples. Generally, combinations of the nutritional requirements of well-studied domestic carnivores and certain omnivores (minks and foxes), are established as guidelines to feed wild carnivores in captivity (AZA Small Carnivore TAG, 2011). The cat is typically the model species used to establish nutrient requirement guidelines for captive carnivorous, such as big cats, otters, herpestids, and euplerids (AZA Small Carnivore TAG, 2009; AZA Small Carnivore TAG, 2010), while the dog's nutritional requirements are used as a baseline for slightly omnivorous carnivores, including large canids and mustelids (AZA Small Carnivore TAG, 2011).

Small prey animals, such as mice and rats, which are a common natural food source for most reptiles, raptors, and small mammals, are often fed to captive wildlife, as is the case of zoo animals and exotic pets (Kleiman et al., 2010). This is based on the belief that whole preys are similar to the natural diet of some carnivores and are commonly presumed to meet nutrient requirements as long as nearly all soft tissues and some bones or other calcified tissues are consumed (Dierenfeld et al., 2002). For wild animals deprived of their natural ecosystem and free-ranging habits, in terms of nutrition, a whole vertebrate prey contains essential amino acids, vitamins, and other nutrients; in terms of behavior, it provides an important form of environmental enrichment improving their biological functioning by stimulating their natural foraging behavior (Cooper and Williams, 2014).

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Since the 19th century, Norwegian rats (*Rattus norvergicus*) have become domesticated on a large scale and produced various forms of the laboratory rat, adapting to conditions imposed by humans (Barnett, 2002). This has resulted in a genetically different population from the original wild type, which is more docile and tolerant of greater crowding, rarely attacks or escapes, and produces more pups by mating earlier and having a longer life reproductive period (Barnett, 2002). Therefore, many companies breed rats and other prey animals specifically for food to supply the exotic pet market, zoos, and aquaria (Ballard and Cheek, 2016). These rats and mice can be purchased from these companies at different ages as well as in various sizes, including other vertebrates (Ballard and Cheek, 2016).

Generally, rats can be bought as males or females without any distinction. However, some studies have shown that testosterone plays a crucial role in the development of muscle tissue by influencing protein metabolism (Tipton, 2001; Bell, 2018) and bone mass development (Quirós et al., 2020), while estrogen affects body fat distribution and storage (O'Sullivan, 2009; Lizcano and Guzmán, 2014; Bell, 2018). Rats produce low levels of testosterone and estrogen at birth, which slowly increase as they reach puberty (Bell, 2018). In a study by Bell (2018), testosterone and estrogen concentrations were recorded in both males and females from birth. While both genders showed low concentrations of testosterone during the first 19 days of age, testosterone increased dramatically in males as they aged 40-60 days. Similar to testosterone, both sexes produce low levels of estradiol, an estrogen steroid hormone, which then increases in females at puberty and during every estrus cycle. As reported, after a peak of estradiol concentration around day 15 in both sexes, estradiol concentration increased only in females until day 39 and it then begins to fluctuate every 4 to 5 days. It could be concluded that whole post-pubertal male rats have higher protein values due to increased testosterone, which contributes to differences in musculature. On the other hand, estrogen in females enhances their body fat.

Although rats are used so extensively around the world for this purpose and fed to many species of animals, little information is available on the nutritional content of the whole captive-bred Norway rats and whether they can provide all the necessary nutrients to the feeding animal (Kleiman et al., 2010). Therefore, this study aimed to examine the values of moisture, crude protein, total fat, ashes, and minerals found in a whole Norway domesticated rat.

MATERIALS AND METHODS

Ethical approval

The rats in the current research were treated and euthanized following the guidelines passed by the institutional ethics committee for the care of animals and approved by the Postgraduate Bioethics Committee of the Faculty of Veterinary Medicine and Zootechnics at the certificate reference code of EEPVirtual.51.2020. The selection of the experimental animals and use of materials was authorized by the Institutional Committee for the Care and Use of Laboratory Animals of the Faculty of Chemical Sciences and Pharmacy at certificate reference code of CICUAL-CCQQF-03-2020. Both committees are part of the University of San Carlos of Guatemala, in Guatemala City, Guatemala.

Experimental sites

The current study was performed in three different research institutes, all located inside the main campus of the University of San Carlos of Guatemala, in Guatemala City, Guatemala. The sample selection, maintenance, feeding and weighing, and euthanasia of the experimental animals were done in the Amarilis Saravia Bioterium, in the pharmacology research area of the Faculty of Chemical Sciences and Pharmacy. The proximal chemical analysis was carried out in the Laboratory of Bromatology of the Faculty of Veterinary Medicine and Zootechnics. Finally, the chemical analysis of ash was performed in the Soil, Water, and Plant Analysis Laboratory of the Faculty of Agronomy.

Sample selection

A total of 12 captive-bred Norwegian weaned rats of the *Rattus norvergicus albinus* species, Wistar breed, were used in this study. Six males and six females were randomly selected from 12 different litters that were available at the time of the study, with an approximate weight of 50-80 g and an age range of 20-25 days. The rats of the same sex were put together as an experimental group to avoid unwanted reproduction and prevent depression due to social isolation.

Maintenance of experimental animals

Each group was placed in transparent plastic boxes of 12 liters (40 × 28 × 10 cm), with an aluminum lid of 1 mm diameter galvanized wire mesh with openings of 12 × 12 mm, on which the food and the water bottle were placed to avoid contamination with the animals' wastes. Animals were maintained in an air-conditioned room with a constant temperature of 21-23°C, a 12-hour day-night cycle, 45% humidity, and controlled ventilation. Box and bedding changes were made every three days. The boxes were disinfected with soap and chlorine before their use. Thin pine chips from a local sawmill were used as a substrate, fumigated with the pyrethroid cypermethrin (Dismetrina 25 EC, formulated by

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Disagro in Escuintla, Guatemala), and dried in the sun for 4 days to eliminate probable harmful microorganisms and parasites.

Feeding and weighing

All rats were fed the commercial dry food for adult dogs of the brand Rufo by Concentrados Aliansa, made in Guatemala *ad libitum*. This feeding was chosen because small producers of rats and mice in Guatemala commonly use dry commercial dog food to feed rodents sold as prey that are the most inexpensive ones. Rufo and Rambocan made by Concentrados Aliansa, a Central American feed manufacturer based in Guatemala, El Salvador, Honduras, and Costa Rica, are the most used ones for this purpose since the price range from 1.00 to 2.00 US Dollars per kilogram. The average quantity of food provided daily was a one-quarter cup, about 30 g of dry dog food, per rat during the first and second week. This amount increased to half a cup, about 60 g, during weeks three and four. From week five, each rat daily received one cup, about 120 g. All rats were fed until they reached the minimum weight of 300, which took 6 weeks for males and 12 weeks for females. Drinking water was filtered from the tap and placed in rodent water bottle dispensers. The animals were weighed without *nil per os* (NOP) on the first day of every week from the beginning of the experiment until the day of the euthanasia to observe the weight gain. The weights were obtained using a digital kitchen scale with a removable plastic container without the need for anesthesia or restriction of movement. On a weighing day, a physical health examination was also performed to follow the guidelines of the bioethics committee.

Euthanasia

Upon reaching an average weight of 300 g, the rats were euthanized by the researcher according to the guidelines recommended in the IUCN code of ethics and the AVMA guide for the euthanasia of animals. According to Flecknell (2009), lidocaine in rodents should not exceed 10 mg/kg for local and regional anesthesia. With this in mind, an overdose of 1 ml of Lidocaine HCL 2% was injected intraperitoneally into each rat to provide analgesia and anesthesia. Subsequently, intrathecal injection of 0.6 ml of Lidocaine HCL 2% was used through the foramen magnum using a 1 mL syringe, with a 25 gauge, and 16 mm needle. In order to gain proper access to the foramen magnum, the rat's head was bent down about 45 degrees with one hand while the needle was inserted with the skillful hand into the cerebello-medullary cistern through the atlantooccipital junction (Zolhavarieh et al., 2011). Lidocaine causes a relatively rapid loss of cerebrocortical function (brain death) when administered intrathecally to anesthetized animals and leaves relatively low tissue residues and is not expected to pose hazards to scavenging animals that might feed on the carcass (AVMA, 2020). The bodies were identified, individually placed in airtight bags whole, and frozen for 2 months until the laboratory of bromatology reopened its doors to the public after the COVID-19 lockdown of 2020.

Biochemical analysis

The proximate composition analysis was used to determine the percentage of moisture (dry matter), crude protein, ether extract (total fat), crude fiber, ash (total minerals), and nitrogen-free extract (NFE). The analysis was performed for each rat to see if there were marked differences in the nutritional values between individuals. To determine the amount of moisture, oven drying methods were used to obtain the partial and total dry matter. The rats were thawed at room temperature and chopped into pieces with a kitchen ax to reduce the drying time. The pieces were spread on a tempered glass tray and introduced into a forced draft oven, where they remained for 72 hours at 60°C until around 80% of the moisture was removed, obtaining partial dry matter (Gregg, 2016). The samples were weighed daily to see the reduction in humidity. The partial dry matter was ground to a particle size ≤ 2 mm in a hammer mill to obtain a homogeneous sample and stored in individual airtight containers. Subsequently, to obtain the total dry matter, 5 g of the homogeneous sample of each rat were placed in a draft air oven at 105°C for 24 hours (Gregg, 2016).

The macronutrient values were determined following the Association of Official Analytical Chemists methods (AOAC, 2019), except for ether extract. Crude protein determination was carried out using AOAC 976.05 (Kjeldahl Method), with a Kjeldahl Auto 1030 analyzer using 1 g of the partial dry matter sample. The extraction of fat was carried out with 2 g of partial dry matter in accordance with the Randall technique using a Soxhlet SER 148/6 solvent extractor (Gregg, 2016). The crude fiber was obtained using 1 g of the remnants of the ethereal extract according to AOAC 962.09 (Ceramic Fiber Filter Method) in a Fibertec System I. Total ash determination was conducted according to AOAC 942.05, where 5 g of the partial dry matter was introduced into a muffled furnace and heated to 600°C for 2 hours. Finally, to obtain the amount of nitrogen-free extract, the formula introduced by Gregg (2016) was applied: NFE = 100 – (Crude protein % + Total fat % + Crude fiber % + Ash %).

Mineral analysis

For the chemical analysis of minerals, 5 g of the partial dry matter obtained during the biochemical analyses were used to calculate the percentage of macroelements (phosphorus, calcium, potassium, and magnesium) and values in parts per million (ppm) of microelements (copper, zinc, iron, and manganese) for each rat. The destruction of the organic

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matter, for the removal of interferents or water, was performed by the Dry Combustion Method, where the samples were subjected to a high temperature of 450°C in a muffle furnace for 4 hours until they were incinerated (Schumacher, 2002). The minerals were recovered with 1N HCl and then the determinations were made. For phosphorus, the quantification was carried out using the Ultraviolet Reflectance analysis with a Perkin Elmer Lambda 11 UV/VIS Spectrophotometer made in Germany (Kulkarni et al., 2014). The rest of the minerals were determined by Atomic Absorption Spectroscopy (AAS), using a Perkin Elmer AAnalyst 100 Spectrometer made in the United States (JoVE Science Education Database, 2022).

Statistical analysis

Sex differences in biochemical and mineral content and comparisons between them were analyzed using the Mann-Whitney U statistic or the Student's t-test. The means and population standard deviation were calculated for each nutrient of each sample using a population size (N) of 6 for each sex. The confidence intervals were analyzed using the online confidence interval calculator software MathsIsFun.com v0.912 (Pierce, 2021).

RESULTS

Weight gain

The results of weight gain are shown in Graph 1. There was a great difference between the growth rate of the males and the females. All the males reached the desired weight (around 300 g) in only 6 weeks from the start of the experiment (between 62 and 67 days of age) while the group of females took more than twice the time (13 weeks, between 111 and 116 days of age). The heaviest rats on their last day of life were the female rat Number 5 with 360 g and the male rat Number 5 with 349 g. All the rats reached values above 300 g except for one female rat (Number 6), maintaining 273 g from week 12 to week 13. The lightest male rat was Number 3 with 303 g.

Biochemical analysis

The results of the biochemical analysis are presented in Table 1. All data except moisture and dry matter content (DMC) is presented on both a dry and fed matter basis. On a dry matter basis, data contains 0% water. On a fed basis, the data shown includes the moisture percentage. Males presented a higher percentage of moisture and ash than females. They also had more protein (68.64%) and lower lipids (16.55%) than females (51.35% and 36.60%, respectively). Both sexes showed no significant difference in carbohydrate percentage ($p < 0.05$). The confidence intervals, shown in parenthesis, were also smaller in males, indicating more variations between the values of the females.

Mineral analysis

The results of the mineral analysis are presented in Table 2. All the data was obtained based on the partial dry matter of the bromatological analysis. Regarding mineral analysis, the greatest difference between the two groups was found in males as they showed five times more calcium (2.61%) than females (0.54%). While females presented higher levels of potassium and copper, males had a greater quantity of the other minerals. The confidence intervals showed a very small variation in most minerals, except for iron in both males and females at 150.83 ± 23.4 ppm and 114.17 ± 30.9 ppm, respectively.

Requirements for small carnivores

To ensure the nutritional content of whole Norway rats can meet the dietary requirements of some small carnivore animals normally kept in captivity, the necessary information was gathered from multiple sources and summarized in Table 3 for the convenience of the reader. As reported by AZA Raptor TAG (2010), there are no documented dietary needs for raptors or vultures; therefore, target nutrient ranges are developed using a mix of the requirements of a strict carnivore (domestic cat) and big poultry (turkey). Bigger carnivores, such as big cats, larger canids, and hyenas were not included due to their size and the number of rats needed to fulfill their daily intake of nutrients. Carnivore reptiles, such as lizards and snakes, were excluded since no nutritional requirements were found.

According to Table 3, the crude protein percentage of the male rats (23.57%) in the current study on a fed matter basis is within the minimum range of daily needs of most small mammals and the bird of prey (Andean condor), except for domestic cats and otters, which need at least 26% and 24% of crude protein, respectively. Female rats are a better option when it comes to crude fat requirements for most animals. Otters also require a higher percentage of fat (15-30%) than the fat provided by female rats (13.92%). For mink and foxes, the fat percentage was not mentioned in the studies by NRC (1982) about their nutritional requirements, and their study was the only one found in this subject area. The mineral requirements for most of the animals in Table 3 can be supplied with a whole rat. Male rats are best to fulfill calcium, phosphorus, and manganese requirements, while female rats are a better option to provide the potassium, magnesium, copper, and iron nutritional needs. Cats and dogs require higher levels of potassium (0.60%) and zinc (75

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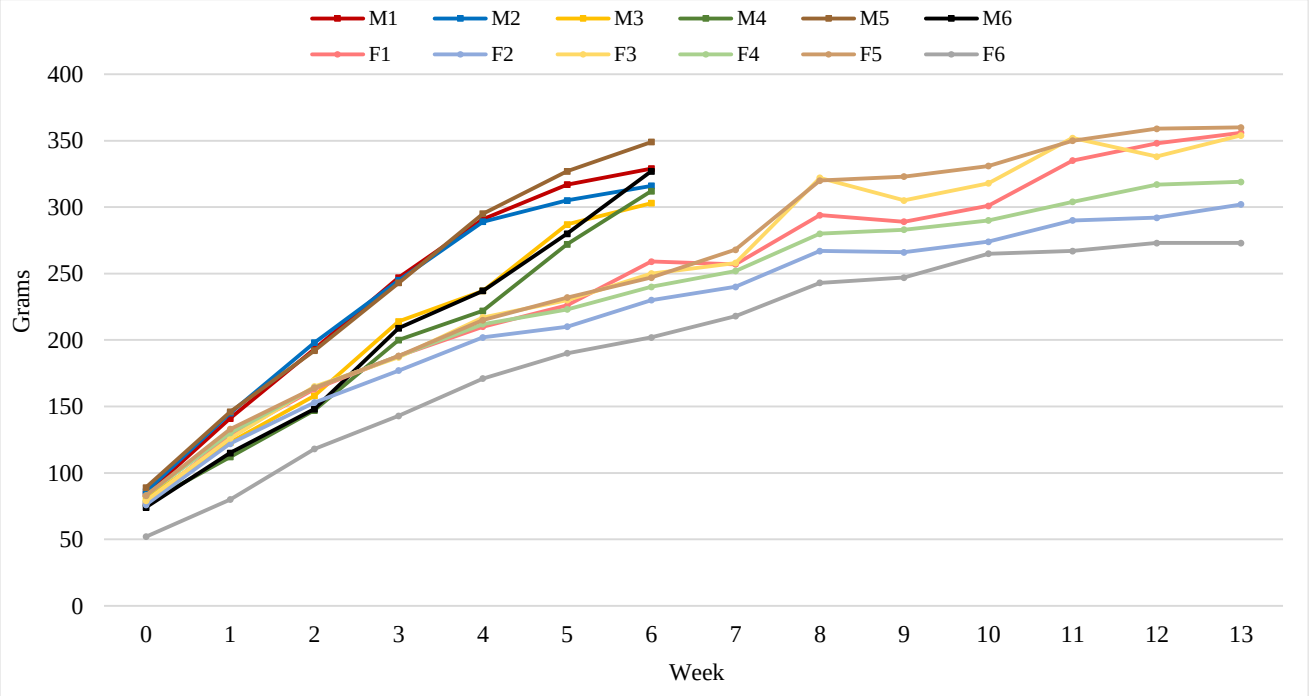
ppm and 80 ppm, respectively) than those provided by rats. The Andean condor needs higher levels of copper (> 9 ppm), zinc (> 75 ppm), and especially manganese, which is more than 67 ppm.



Graph 1. Male and female rats' weight gain in grams. M: Males, F: Female

Table 1. Biochemical analysis of the adults Norway rats

Nutrient (%)	Males - Dry	Males - Fed	Females - Dry	Females - Fed
Moisture	---	65.68 ± 0.66	---	62.09 ± 1.63
DMC	34.32 ± 0.66	---	37.91 ± 1.63	---
Crude protein	68.64 ± 1.35	23.57 ± 0.79	51.35 ± 2.30	19.49 ± 1.35
Total fat	16.55 ± 1.48	5.67 ± 0.41	36.60 ± 2.68	13.92 ± 1.38
Crude fiber	1.78 ± 0.42	0.61 ± 0.15	1.82 ± 0.28	0.69 ± 0.10
Ash	11.11 ± 0.65	3.81 ± 0.22	9.48 ± 2.22	3.57 ± 0.77
NFE	1.93 ± 1.10	---	1.66 ± 1.67	---

All data are means; a confidence interval of 95%, N: 6 for each sex. DMC: Dry matter content, NFE: Nitrogen-free extract

Table 2. Mineral analysis of the adults Norway rats

Mineral	Males	Females
Calcium (%)	2.61 ± 0.41	0.54 ± 0.06
Phosphorus (%)	0.98 ± 0.13	0.47 ± 0.05
Potassium (%)	0.25 ± 0.02	0.46 ± 0.07
Magnesium (%)	0.11 ± 0.01	0.07 ± 0.02
Sodium %()	0.26 ± 0.02	0.20 ± 0.01
Copper (ppm)	3.67 ± 1.51	8.00 ± 0.00
Zinc (ppm)	74.17 ± 5.38	52.17 ± 3.28
Iron (ppm)	150.83 ± 23.40	114.17 ± 30.90
Manganese (ppm)	7.50 ± 2.00	3.67 ± 0.60

All data are means ± the margin of error with a confidence interval of 95%, N: 6 for each sex.

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Table 3. Nutritional content of Norway rats and carnivore requirements

	Nutritional content	Carnivore mammal nutritional requirements								Birds of prey nutritional requirements
Nutrient	**Norway rat**	**Domestic cat [1]**	**Domestic dog [2]**	**Otters [3]**	**Herpestids/ Euplerids [4]**	**Omnivore mustelids[5]**	**Carnivore mustelids [6]**	**Mink [7]**	**Foxes [8]**	**Andean condor [9]**
CP (%)	23.57 M 19.49 F	26.00	18.00	24.00-32.50	19.70-32.50	17.50-26.00	19.70-32.50	21.80-26.00	19.70	> 20.00
Fat (%)	5.67 M 13.92 F	9.00	5.50	15.00-30.00	9.00-30.00	5.00-8.50	9.00-30.00	---	---	> 10.00
Ca (%)	2.61 M 0.54 F	0.60	0.50	0.60-0.80	0.29-1.00	0.50-1.20	0.50-1.00	0.30	0.60	0.80-2.50
P (%)	0.98 M 0.47 F	0.50	0.40	0.60	0.26-0.80	0.50-1.00	0.50-0.80	0.30	0.40	0.39-0.72
K (%)	0.25 M 0.46 F	0.60	0.60	0.20-0.40	0.40-0.60	0.40-0.60	0.40-0.60	---	---	0.40-0.67
Mg (%)	0.11 M 0.07 F	0.04	0.06	0.04-0.07	0.03-0.08	0.04-0.06	0.03-0.08	---	---	0.04-0.06
Na (%)	0.26 M 0.20 F	0.20	0.08	0.04-0.06	0.05-0.40	0.04-0.30	0.05-0.40	0.50	0.50	0.10-0.13
Cu (ppm)	3.67 M 8.00 F	5.00	7.30	5.00-6.25	5.00-8.80	6.00-12.40	5.00-8.80	---	---	> 9.00
Zn (ppm)	74.17 M 52.17 F	75.00	80.00	50.00-94.00	50.00-94.00	50.00-120.00	50.00-94.00	---	---	> 75.00
Fe (ppm)	150.83 M 114.17 F	80.00	40.00	80.00-114.00	80.00-114	30.00-90.00	80.00-114.00	---	---	> 80.00
Mn (ppm)	7.70 M 3.67 F	7.60	5.00	5.00-9.00	---	---	---	---	---	> 67.00

Crude Protein (CP) and Total Fat are presented on a fed matter basis. Minerals are based on a dry matter basis. M: Male rats, F: Female rats.
Nutritional requirements are for adult animals in maintenance. Values are the minimum nutrient requirements in their diet.
[1, 2] AAFCO (2014) Cat and dog food nutrient profile based on dry matter.
[3] AZA Small Carnivore TAG (2009) Otter target nutrient ranges based on dry matter.
[4] AZA Small Carnivore TAG (2011) Mongoose, meerkat, & fossa target nutrient ranges based on dry matter.
[5] AZA Small Carnivore TAG (2010) More omnivorous mustelids (skunk and tayra) target nutrient ranges based on dry matter.
[6] AZA Small Carnivore TAG (2010) More carnivorous mustelids (badger, ferret, fisher, wolverine) target nutrient ranges based on dry matter.
[7, 8] NRC (1982) Mink and Fox Nutrient Requirements based on dry matter.
[9] AZA Raptor TAG (2010) Andean condor target nutrient ranges based on dry matter.

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DISCUSSION

During the experiment, it was observed that male rats presented a faster growth rate and higher body mass, compared to females. For females, it took 13 weeks to reach the desired weight (approximately 320 g), which was twice the time needed for males (6 weeks). Most adult male mammals are usually larger and heavier than females. Maximum body weight in adult male and female Wistar rats has been reported to be 677.3 ± 9.2 g and 463.3 ± 8.6 g, respectively, and is attained by postnatal day 100 in males and slightly sooner in females (Ghasemi et al., 2021). These differences in weight between males and females have been attributed to being affected by sex hormones as testosterone enhances muscle growth and influences bone density, while estrogens inhibit female growth (Quirós et al., 2020). As reported by Tripton (2001), male rats have a bigger body mass and a faster growth rate due to the effects of testosterone on the regulation of body growth, while estrogens in females suppress growth rate and muscle hypertrophy.

The percentage of macronutrients in the rats of this experiment showed that males had an approximate protein: lipids: carbohydrates ratio of 69P:17L:2C while females had less crude protein and higher values of total fat, 51P:37L:2C (Table 1). It was found that testosterone also improved net muscle protein balance by stimulating muscle protein synthesis, decreasing muscle protein degradation, and improving the utilization of amino acids (Bhasin et al., 2003). Another factor that could be explained by the effect of hormones is the higher values of total fat observed for the female group. The accumulation of crude fat in females begins to increase at puberty and persists throughout adulthood, giving them a higher body fat percentage than males (O'Sullivan, 2009). A low testosterone level or testosterone deficiency deregulates lipid and glucose metabolism, resulting in increased adiposity in the liver and peripheral tissues (Baik et al., 2020). This means that the low concentrations of testosterone in females can be associated with decreased muscle mass and increased fat mass, which was in accordance with the findings of a study by Singh et al. (2003) on mice. Estrogen, as well as testosterone, affects adipocyte physiology. Due to estrogen's ability to reduce postprandial fatty acid oxidation, more fat is stored in the body, explaining the increased body fat in females (O'Sullivan, 2009). The effect of estrogen in males is not like that of females so estrogen is insufficient to promote body fat in males (Bell, 2018).

Regarding the mineral analysis, male and female rats differed significantly in terms of calcium percentage at 2.61% and 0.54%, respectively. Calcium is the most abundant mineral in the body, followed by phosphorus, and greater than 99% of it is stored in bone tissue in vertebrates (Institute of Medicine, 1997). The remainder is present in the blood, extracellular fluid, muscle, and other tissues, where it plays a role in mediating vascular contraction and vasodilation, muscle contraction, nerve transmission, and glandular secretion (Institute of Medicine, 1997). The only study that recorded calcium levels in rats was the one conducted by Sherman and MacLeod (1925). After they recorded the calcium content of the body in different ages of rats of both sexes, the findings indicated the fact that pubertal males have more calcium percentage than females. At 15 and 30 days of age, the females presented slightly higher values of calcium than males, but from 60 days onward, calcium content slowly increased in males, which was even above that of the female rats (Sherman and MacLeod, 1925). As concluded, the gross weight of calcium in the body is higher for males than females since the average weight of the males is greater, but more recent studies have shown other factors like the physiology of calcium absorption that may explain these differences.

Calcium is absorbed by active transport (dependent on Vitamin D) and passive diffusion across the intestinal mucosa (Institute of Medicine, 1997). Intestinal absorption is predicted to be halved in 2 months old females, compared with young males, mainly because vitamin D_3 levels are 50% lower in females (Granjon et al., 2016). While this will affect the amount of calcium absorption in the bones, it does not fully explain the reason females in this study had 4 times less calcium than males. Another factor could be the effect of sex hormones, which is similar to the effect they have on body mass, protein concentration, and fat gain. Testosterone is responsible for mediating bone mineral quantity in males by stimulating their body growth and thus increasing bone size and mass, while estrogens in females affect the development of the appendicular skeleton by suppressing their growth (Quirós et al., 2020). Zhang et al. (1999) studied the effects of gonadectomy on bone values in male and female rats and indicated that testosterone and growth hormone are growth-promoting in growing male rats, producing independent effects on bone size and mass. Moreover, they revealed that in growing female rats, estrogen was growth limiting at appendicular sites.

Phosphorus concentrations were higher in males (0.98%) than females (0.47%), which then showed higher levels of potassium (0.46%), compared to the values shown in males (0.25%). Mineral concentrations and the effects of gender on rats have not been widely studied. Variability in trace element composition can be caused due to several possible reasons, including the influence of different dietary trace mineral levels, gender-specific metabolism, varying accuracy of analytical techniques, and contamination of the samples (Dierenfeld et al., 2002). An old study performed by Sherman and Quinn (1926) determined phosphorus content in rats and no other recent studies were found in this area. They recorded that after 15 days of age phosphorus concentrations averaged higher in the males than the females because of their greater average body weights. The concentrations in males and females were the same by day 21 and then females showed higher percentages of phosphorus than males of the same age from day 28 onwards (Sherman and Quinn, 1926).

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This means that age could act as a factor that leads to a higher percentage of phosphorus in males since male rats in this study were 6 weeks younger than females.

CONCLUSION

Comparisons between the results of this study and the nutritional requirements of certain small carnivores showed that rats could provide the necessary nutrients, however, if given as a sole diet, they could not sufficiently supply the requirements these animals need in the long term. While male rats would be a better option for crude protein and calcium requirements of most carnivore animals, females fulfill best the target nutrient level of total fat. Differences found in growth rate, nutrient, and mineral composition can be attributed to the effect of sex hormones in males and females since testosterone and estrogens impact body growth, fat storage, and bone size and mass. The rat's gender should be taken into consideration since the level of nutrients an animal can obtain will differ if given a male or female rat as feed. Solid conclusions are difficult to make since many factors can affect the nutritional composition of a feed, such as age, diet, and storage time after death. Further studies are needed to be made to see the effect of those variations, as well as the exact nutritional requirements for each species of animal, desired to feed.

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Competing interests

The author declares that there are no competing interests.

Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and or falsification, double publication and or submission, and redundancy have been checked by the author.

REFERENCES

American Veterinary Medical Association (AVMA) (2020). AVMA guidelines for the euthanasia of animals. AVMA, Ilinois, United States, p. 40. Available at: https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf

Association of American Feed Control Officials (AAFCO) (2014). Methods for substantiating nutritional adequacy of dog and cat foods. AAFCO Org., Illinois, United States, pp. 4-14. Available at: https://www.aafco.org/Portals/0/SiteContent/Regulatory/Committees/Pet-Food/Reports/Pet_Food_Report_2013_Midyear-Proposed_Revisions_to_AAFCO_Nutrient_Profiles.pdf

Association of Official Analytical Chemists (AOAC) (2019). Official Methods of Analysis, 21st Edition. AOAC International, Maryland, United States. Available at: https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/

Association of Zoos and Aquariums (AZA) Raptor TAG (2010). Andean condor (Vultur gryphus) care manual. Association of Zoos and Aquariums, Silver Spring, Maryland, United States, p. 21. Available at: https://www.assets.speakcdn.com/assets/2332/andeancondorcaremanual20101.pdf

Association of Zoos and Aquariums (AZA) Small Carnivore TAG (2009). Otter (lutrinae) care manual. Association of Zoos and Aquariums, Silver Spring, Maryland, United States, p. 31. Available at: https://www.assets.speakcdn.com/assets/2332/otter_care_manual2.pdf

Association of Zoos and Aquariums (AZA) Small Carnivore TAG (2010). Mustelid (mustelidae) care manual. Association of Zoos and Aquariums, Silver Spring, Maryland, United States, p. 27. Available at: https://assets.speakcdn.com/assets/2332/mustelidcaremanual2010r.pdf

Association of Zoos and Aquariums (AZA) Small Carnivore TAG (2011). Mongoose, meerkat, and fossa (herpestidae/eupleridae) care manual. Association of Zoos and Aquariums, Maryland, United States, Silver Spring, p. 28. Available at: https://assets.speakcdn.com/assets/2332/mongoose_meerkat_and_fossa_acm_2011.pdf

To cite this paper: Delboy NMB (2022). Nutritional Content of Adult Norway Rats for Small Carnivores' Feeding. *World Vet. J.*, 12 (2): 141-150. DOI: https://dx.doi.org/10.54203/scil.2022.wvj18

Baik M, Jeong JY, Park SJ, Yoo SP, Lee JO, Lee JS, Haque MN, and Lee HJ (2020). Testosterone deficiency caused by castration increases adiposity in male rats in a tissue-specific and diet-dependent manner. BMC: Genes and Nutrition, 15: 14. DOI: https://www.doi.org/10.1186/s12263-020-00673-1

Ballard BM and Cheek R (2016). Exotic animal medicine for the veterinary technician 3rd Edition. Iowa, United States: John Wiley and Sons, pp. 152-153. Available at: https://orangebooks.ir/Books/View/9994/94031/Exotic-Animal-Medicine-for-the-Veterinary-Technician

Barnett SA (2002). The story of rats, their impact on us and our impact on them, 1st Edition. Sydney, Australia: Allen and Unwin, pp. 21-22. Available at: https://www.allenandunwin.com/browse/books/general-books/popular-science/The-Story-of-Rats-S-Anthony-Barnett-9781865085197

Bell MR (2018). Comparing postnatal development of gonadal hormones and associated social behaviors in rats, mice, and humans. Endocrine Society: Endocrinology, 159(7): 2596-2613. DOI: https://www.doi.org/10.1210/en.2018-00220

Bhasin S, Taylor WE, Singh R, Artaza J, Hikim IS, Jasuja R, Choi H, and Gonzalez NF (2003). The mechanisms of androgen effects on body composition: Mesenchymal pluripotent cell as the target of androgen action. The Journals of Gerontology, Series A, 58(12): 1103-1110. DOI: https://www.doi.org/10.1093/gerona/58.12.M1103

Cooper JE, and Williams DL (2014). The feeding of live foods to exotic pets: wellness and ethics issues. Journal of Exotic Pet Medicine, 23: 244-249. DOI: https://www.doi.org/10.1053/j.jepm.2014.06.003

Dierenfeld ES, Alcorn HL, and Jacobsen KL (2002). Nutrient composition of whole vertebrate prey (excluding fish) fed in zoos. Animal Welfare Information Center, pp. 79-94. Available at: https://feline-nutrition.org/pdfs/archive-nutrient-composition-of-whole-vertebrate-prey-excluding-fish-fed-in-zoos.pdf

Fernández S, Iglesias C, Marín C, Ruíz MJ, Delgado JV, and Navas FJ (2021). The winner takes it all: Risk factors and bayesian modelling of the probability of success in escaping from big cat predation. Animals, 12(1): 51. DOI: https://www.doi.org/10.3390/ani12010051

Flecknell P (2009). Laboratory animal anesthesia, 3rd Edition. Elsevier Inc., Newcastle University, United Kingdom, p. 74. Available at: http://www.bio.ufpr.br/portal/wp-content/uploads/2018/10/Laboratory-Animal-Anaesthesia-Third-Edition-2009.pdf

Ghasemi A, Jeddi S, and Kashfi K (2021). The laboratory rat: Age and body weight matter. EXCLI Journal, 20: 1431-1445. DOI: https://www.doi.org/10.17179/excli2021-4072

Granjon D, Bonny O, and Edwards A (2016). A model of calcium homeostasis in the rat. American Journal of Physiology: Renal Physiology, 311(5): 1047-1062. DOI: https://www.doi.org/10.1152/ajprenal.00230.2016

Gregg R (2016). Manual of procedures of the bromatology laboratory of the faculty of veterinary medicine and zootechnics. Unpublished bachelor's thesis. University of San Carlos of Guatemala, Guatemala, pp. 5-38.

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (1997). Dietary reference intakes for calcium, phosphorus, magnesium, Vitamin D, and fluoride. The National Academie Press, Washington DC, United States, pp. 71-72. Available at: https://pubmed.ncbi.nlm.nih.gov/23115811/

JoVE Science Education Database (2022). Lead analysis of soil using atomic absorption spectroscopy. Environmental Science: Cambridge, Maryland, United States. Available at: https://www.jove.com/v/10021/lead-analysis-of-soil-using-atomic-absorption-spectroscopy

Kleiman DG, Thompson KV, and Baer CK (2010). Wild mammals in captivity, 2nd Edition. University of Chicago Press, Chicago, United States, p. 95. Available at: https://press.uchicago.edu/ucp/books/book/chicago/W/bo8434953.html

Kohl KD, Coogans SC, and Raubenheimer D (2015). Do wild carnivores forage for prey or for nutrients? Bioessays, 37(6): 701-709. DOI: https://www.doi.org/10.1002/bies.201400171

Kulkarni Y, Warhade KK, and Bahekar SK (2014). Primary nutrients determination in the soil using UV spectroscopy. International Journal of Emerging Engineering Research and Technology, 2(2): 198-204. Available at: http://www.ijeert.org/pdf/v2-i2/33.pdf

Liesegang A, Baumgartner K, and Wehrle M (2008). Animal nutrition in zoos. Scientific Researchers Zoological Parks, 21: 214-222. DOI: https://www.doi.org/10.5167/uzh-4596

Lizcano F and Guzmán G (2014). Estrogen deficiency and the origin of obesity during menopause. BioMed Research International, 2014: e757461. DOI: https://www.doi.org/10.1155/2014/757461

Moraal M, Leenaars PP, Arnts H, Smeets K, Savenije BS, Curfs JH, and Ritskes-Hoitinga M (2012). The influence of food restriction versus *ad libitum* feeding of chow and purified diets on variation in body weight, growth, and physiology of female Wistar rats. Laboratory Animals, 46(2): 101-107. DOI: https://www.doi.org/10.1258/la.2011.011011

National Research Council (NRC) (1982). Nutrient requirements of mink and foxes, 2nd Edition. The National Academia Press, Washington DC, United States, pp. 33-34. Available at: https://nap.nationalacademies.org/catalog/1114/nutrient-requirements-of-mink-and-foxes-second-revised-edition-1982

O'Sullivan AJ (2009). Does oestrogen allow women to store fat more efficiently? A biological advantage for fertility and gestation. Obesity Reviews, 10(2): 168-177. DOI: https://www.doi.org/10.1111/j.1467-789x.2008.00539.x

Pierce R (2021). Confidence interval calculator. Math is fun v0.912. Accessed September 23, 2021. Available at: http://www.mathsisfun.com/data/confidence-interval-calculator.html

Quirós S, Reis WL, Silva M, Debarba LK, Mecawi AS, de Paula FJA, Rodrigues C, Elias LLK, and Antunes J (2020). Sex differences in body composition, metabolism-related hormones, and energy homeostasis during aging in Wistar rats. Physiological Reports, 8(20): e14597. DOI: https://www.doi.org/10.14814/phy2.14597

Schumacher BA (2002). Methods for the determination of total organic carbon (TOC) in soils and sediments. National exposure research laboratory, Las Vegas, United States, p. 8. Available at: http://www.bcodata.whoi.edu/LaurentianGreatLakes_Chemistry/bs116.pdf

To cite this paper: Delboy NMB (2022). Nutritional Content of Adult Norway Rats for Small Carnivores' Feeding. *World Vet. J.*, 12 (2): 141-150. DOI: https://dx.doi.org/10.54203/scil.2022.wvj18

Sherman HC and Quinn EJ (1926). The phosphorus content of the body in relation to age, growth, and food. Journal of Biological Chemistry, 67(3): 667-677. DOI: https://www.doi.org/10.1016/S0021-9258(18)84701-9

Sherman HC and MacLeod FL (1925). The calcium content of the body in relation to age, growth, and food. Journal of Biological Chemistry, 64(2): 429-459. DOI: https://www.doi.org/10.1016/S0021-9258(18)76492-2

Singh R, Artaza JN, Taylor WE, Gonzales NF, and Bhasin S (2003). Androgens stimulate myogenic differentiation and inhibit adipogenesis in c3h 10t1/2 pluripotent cells through an androgen receptor-mediated pathway. Endocrine Society: Endocrinology, 144(11): 5081-5088. DOI: https://www.doi.org/10.1210/en.2003-0741

Tipton, KD (2001). Gender differences in protein metabolism. Current Opinion in Clinical Nutrition and Metabolic Care, 4(6): 493-498. DOI: https://www.doi.org/10.1097/00075197-200111000-00005

Wilder SM, Couteur DG, and Simpson, SJ (2013). Diet mediates the relationship between longevity and reproduction in mammals. AGE, 35: 921-927. DOI: https://www.doi.org/10.1007/s11357-011-9380-8

Zhang XZ, Kalu DN, Erbas B, Hopper JL, and Seeman E (1999). The effects of gonadectomy on bone size, mass, and volumetric density in growing rats are gender-, site-, and growth hormone-specific. Journal of Bone and Mineral Research, 14(5): 802-809. DOI: https://www.doi.org/10.1359/jbmr.1999.14.5.802

Zolhavarieh SM, Nourian AR, and Sadeghi-nasab A (2011). A new method for on-farm euthanasia with animal welfare considerations. Iranian Journal of Veterinary Surgery, 6(2): 14-15. Available at http://www.ivsajournals.com/article_3134_8d0a5048c08bae713c74cb084a595e97.pdf

To cite this paper: Delboy NMB (2022). Nutritional Content of Adult Norway Rats for Small Carnivores' Feeding. *World Vet. J.*, 12 (2): 141-150. DOI: https://dx.doi.org/10.54203/scil.2022.wvj18

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Identification of Blood-sucking Flies of Bats in Lombok Island, Indonesia

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ABSTRACT

Bats and blood-sucking bat flies have an important role in transmitting several hemoparasites. Bat flies have been identified as vectors transmitting hemoparasites from wild bats. The purpose of the present study was to identify bats and their blood-sucking flies as vectors of hemoparasites in bat caves located at Lombok Island, Indonesia. In the course of the study, a survey was conducted on three bat caves from September to December 2018. The bats were captured by a net trap and the species of bats and bat flies were identified. A total of 66 captured bats were identified as *Hipposideros* species (n = 28), *Eonycteris spelaea* (n = 23), and *Taphozouss* species (n = 15). The blood-sucking flies were identified as *Eucampsipoda sundaica* on *Eonycteris spelaea*, and *Stylidia* cf. *euxesta*, *Brachytarsina* species, *Raymondia* species, and *Megastrebla nigriceps* on *Hipposideros* species. The results showed that five species of blood-sucking flies were present in captured bats. The bat and blood-sucking flies can influence the transmission of *Polychromophilus* species, *Babesia* species, *Plasmodium* species, and *Trypanosoma* species to humans and other hosts.

Keywords: Bats, Blood-sucking flies, Hemoparasites, Lombok, Vector

INTRODUCTION

Bats (Chiroptera) are unique mammals residing in large groups in caves. Bats and bat flies have an important role in transmitting several hemoparasites, including those which infect bats, such as *Babesia* species, *Plasmodium* species, and *Trypanosoma* species. Bat flies had been reported as vectors of hemoparasites, specially *Polychromophilus* species parasite (Obame-Nkoghe et al., 2016). Bats are the only mammals with the ability to fly and they are categorized into more than 1,100 different species (Calisher et al., 2006). Bats also have a high percentage of Lymphocytes and Monocytes as effectors of adaptive and innate immunity (Sa'diyah and Situmorang, 2020). Lombok Island has many caves where massive colonies of bats which are known hosts of blood-sucking ectoparasites live, however, little is known about bats and their blood-sucking flies can be potential vector transmission of hemoparasites in the island.

Species of bats, such as *Hipposederos bicolor*, *Eonycteris speleae*, and *Taphozous achates* have been recently identified from bat caves in Lombok Island (Agustin et al., 2019). Han et al. (2018) reported that 13.3% of 107 insectivorous bats were infected by *Babesia vesperuginis.* Several years before that, Schaer et al. (2013) documented that *Plasmodium* species. has infected insectivorous bats and fruit bats in Africa. The presence of *Rickettsia* species. and their ectoparasites (Diptera and Siphonaptera) on the bats have also been documented across South Africa and Swaziland (Dietrich et al., 2016). Also, 0.7% of *Trypanosoma* species. was recovered from 400 blood bat samples in Madagascar (Raharimanga et al., 2013). About 15 species of bat flies, *Nycteribia triangularis*, *Stylidia* cf. *euxesta*, *Stylidia* cf. *caudata*, *Basilia hispida*, *Archinycteribia octophalma*, *Eucampsipoda penthetoris*, *Eucampsipoda sundaica*, *Leptocyclopodia ferrari*, *Leptocylopodia brachytrinax*, *Leptocylopodia obliqua*, *Megastrebla gigantea*, *Megastrebla limbooliati*, *Megastrebla limbooliati*, and *Raymondia* species. have been documented from 24 species of bats in Malaysia (Azhar et al., 2015).

Cave-dwelling bats can transmit blood-sucking flies and hemoparasites among themselves and the human population due to their feeding habit, ability to travel long-distance, and aggregation behavior. The purpose of the present study was to identify bats and their blood-sucking flies as vectors of hemoparasites in bat caves in Lombok Island, Indonesia.

MATERIALS AND METHODS

Ethical consideration

The samples of bats and bat flies were collected by a qualified veterinarian from the Faculty of Veterinary Medicine, Universitas Pendidikan Mandalika, Mataram, Indonesia based on the sampling protocol of Azhar et al. (2015) and FAO (2011). The captured bats were immediately released after the flies were taken and examined for external morphology.

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Study design

The survey was conducted from September to December 2018. Bats and their bat flies were collected from three bat caves- Tanjung Ringgit Bat Cave (8.61471°S, 116.594420°E), Lembah Sempage Bat Cave (8.539900°S, 116.2776673°E), and Pujut Bat Cave (8.88693°S, 116.253830°E) in Lombok Island, Indonesia (Figure 1). Mist nets were used to collect bats in the sampling caves from 4 to 7 PM. The mist net was stretched between two fish sticks. The mist nets were monitored, and captured bats were collected by hands and placed in wire cages covered with a dark cloth. Captured bats were inspected for bat flies' presence. When found, bat flies from each bat were collected and placed in a labeled tube containing 70% ethanol.

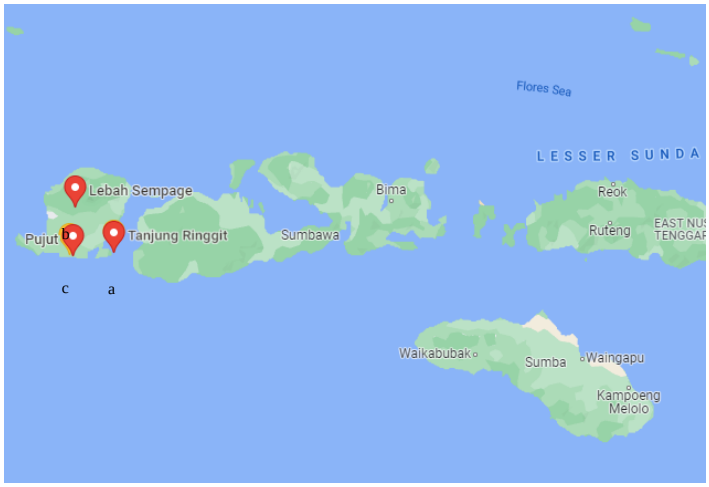

Figure 1. Bat cave locations in the study area (Google Map). a: Tanjung Ringgit Bat Cave, b: Lembah Sempage Bat Cave, c: Pujut Bat Cave

The captured bats were identified based on external morphology by measuring the length of the forearm (FA), third metacarpal (3MT), head body (HB), tibia (TIB), tail (TL), and hindfoot (HF), following the method used by Srinivasulu et al. (2010). The morphology of collected bat flies, including the head, thorax, abdomen, and wing, was identified under a microscope (Olympus, Japan, 40×) as reported by Azhar et al. (2015) and Alvarez et al. (2015).

RESULTS AND DISCUSSION

A total of 66 captured bats from three bat caves were identified as *Hipposideros* species, *Taphozous* species (insectivorous bats), and *Eonycteris spelaea* (Frugivorous bat) based on the external morphology. The details of external morphology measured are summarized in Table 1.

The FA length of *Hipposideros* species (41-50 mm) in the present study was similar to that of *Hipposideros galeritus* (FA: 45.0-51.3 mm) measured by Srinivasulu et al. (2010). The length of FA of *Eonycteris spelaea* (51-75 mm) was within the range of *Eonycteris spelaea* (FA = 66-78 mm) reported by the Srinivasulu et al. (2010). However, the length of *Taphozous* species (59-61 mm) in the present study was shorter than the key identification of *Taphozous perforatus* (FA = 59.2-63.8 mm) measured by Srinivasulu et al. (2010). The morphology of the cave-dwelling bats is shown in Figure 2.

The collected blood-sucking flies were morphologically identified as *Eucampsipoda sundaica*, *Stylidia* cf. *euxesta*, *Brachytarsina* species, *Raymondia* species, and *Megastrebla nigriceps* (Figure 3). The head morphology of *Eucampsipoda sundaica* observed in the present study was compressed and the thorax was pentagonal. The bat host of this fly was *Eonycteris spelaea*, which was previously described by Azhar et al. (2015). The morphology of *Stylidia* cf. *euxesta* was straight dark-pigmented claspers with long dorsal setae, while *Megastrebla nigriceps* had a long wing, the R1 vein had a weak bend near the base with the R2+3 vein apically curved as stated by Azhar et al. (2015). The *Brachytarsina* species recovered in the present study had a triangular and rounded head with non-prominent eyes, which was the same as the one described by Maa (1971). The head of *Raymondia* species was narrower than the distance between the two major humeral setae as previously described by Azhar et al. (2015). These species were collected from the fur area of the family Hipposideridae bat. The summary of the identification of 66 captured bats and their blood-sucking flies from bat caves in Lombok Island can be seen in Table 2.

The ecology and distribution of *Stylidia* cf. *euxesta*, *Brachytarsina* species, *Raymondia* species, and *Megastrebla nigriceps* in Indonesia are still unknown although some studies have been performed in Malaysia (Azhar et al., 2015) and Philippines (Alvarez et al., 2015). The present study could provide information on the identification and correlation between the number of bat and bat flies species found in Lombok Island.

Szentiványi et al. (2019) reported that 101 microparasites belonging to the bat fly families Nycteribiidae and Streblidae of 188 microparasites have been observed in bats. Bat flies such as *Brachytarsina* species, *Raymondia* species, and *Eucampsipoda* species in this study have been reported to be associated with blood parasites. *Polychromophilus melanipherus* Dionisi was mainly detected in *Nycteribia schmidlii scotti* Falcoz and less presence in *Eucampsipoda africana*, *Raymondia huberi* group and *Raymondia allaudi* bat flies.

The bats, including *Hipposideros* species, *Taphozous* species, and *Eonycteris spelaea*, as well as the blood-sucking flies including *Eucampsipoda sundaica*, *Megastrebla nigriceps*, *Brachytarsina* species, *Stylidia* cf. *euxesta*, and *Raymondia* species can be a potential vector of *Polychromophilus* species, *Babesia* species, *Plasmodium* species, and *Trypanosoma* species to other hosts. Nkoghe et al. (2016) reported the presence of *Polychromophilus* parasites in the bat fly fauna of Gabon. Lima et al. (2012) found *Trypanosoma erneyi* in African bats. Han et al. (2018) reported that insectivorous bats were infected by *Babesia vesperuginis*, also Schaer et al. (2013) indicated that *Plasmodium* species has infected insectivorous bats and fruit bats in Africa.

Table 1. Measurement of the external morphology of captured bats from three caves in Lombok Island, Indonesia

Species	FA (mm)	3MT (mm)	HB (mm)	TL (mm)	TIB (mm)	HF (mm)

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Hipposideros species	41-50	38-52	47-60	13- 23	22-23	14-15
Eonycteris spelaean	51-75	48-54	67-100	17-18	24-34	18-23
Taphozous species	59-61	50-54	70-74	25-25	23-24	13-14

FA: Forearm length, 3MT: Third metacarpal length, HB: Head body length, TL: Tail length, TIB: Tibia length, HF: Hind foot length

Table 2. Bats and their blood-sucking flies from bat caves in Lombok Island, Indonesia

Location	Number of bats	Species of bat	Feeding habits	Species of blood-sucking flies
Tanjung Ringgit Bat Cave (8.961°S,116.294°E)	23	*Eonycteris spelaea*	Fruits	*Eucampsipoda sundaica*
LembahSempage Bat Cave (8.5399°S,116.277°E)	28	*Hipposederos* spp	Insects	*Megastrebla nigriceps* *Brachytarsina* spp *Stylidia* cf. *euxesta*
Pujut Bat Cave (8.887°S,116.254°E)	15	*Taphozous* spp	Insects	*Raymondia* spp *Brachytarsina* spp
Total	66			

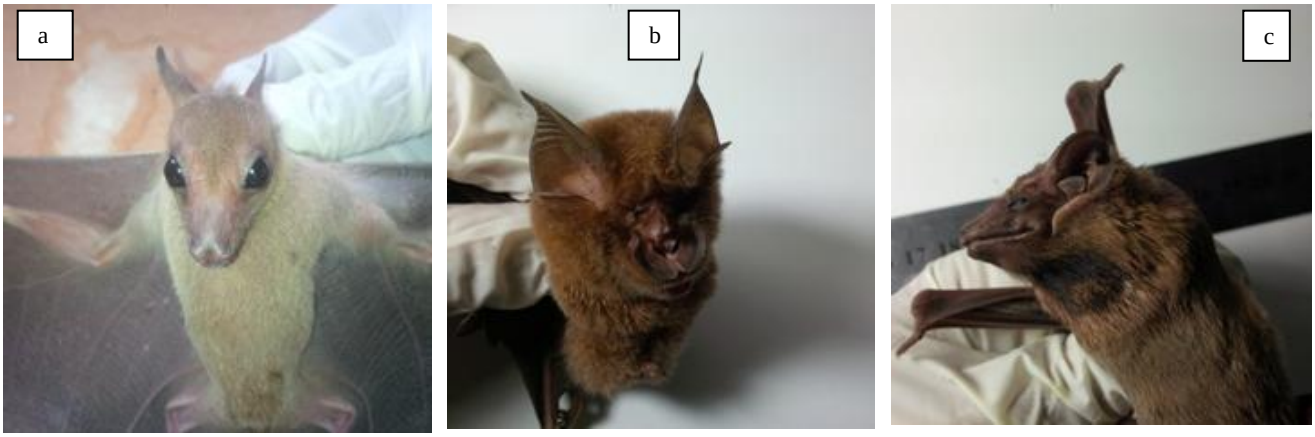

Figure 2. The morphology of the cave-dwelling bats. *Eonycteris spelaea* (a), *Hipposideros* species (b), *Taphozous* species (c)

Figure 3. Morpologhy of blood-sucking flies (40x magnification)**.** *Eucampsipoda sundaica* (a), *Stylidia* cf. *euxesta* (b), *Megastrebla nigriceps* (c), *Brachytarsina* species (d), *Raymondia* species (e), wing vein of *Megastrebla nigriceps* (f)

CONCLUSION

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The results of this study identified three species of bats, namely *Hipposideros* species, *Taphozous* species, and *Eonycteris spelaea*, in three caves on Lombok Island. The present study also documented five species of blood-sucking flies including *Eucampsipoda sundaica, Megastrebla nigriceps Brachytarsina* species, *Stylidia* cf. *euxesta*, and *Raymondia* species. Bat and bat flies can influence the transmission of *Polychromophilus species, Babesia* species, *Plasmodium* species, and *Trypanosoma* species to the other hosts in the study area and also spread the disease agent to different places.

DECLARATIONS

Authors' contribution

Kholik Kholik contributed to the design of research, data analysis, and the writing of the manuscript. Candra Dwi Atma, and Novarina SulsiaIsta'in Ningtyas collected the samples from the field and performed the laboratory analyses. All authors check the data of the present study and confirmed the final draft of the manuscript.

Competing interests

The authors declare that there are no competing interests.

Ethical consideration

The authors declare that this manuscript is original, has been checked by all the authors, and is not currently being considered for publication elsewhere.

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REFERENCES

Agustin ALD, Atma CD, Munawaroh M, Ningtyas NSI, Legowo AP, and Sukmanadi M (2019). Bacterial pathogens from cave-dwelling bats that are a risk to human, animal and environmental health on Lombok Island, Indonesia. EurAsian Journal of BioScinces, 13: 1509-1513. Available at: https://www.semanticscholar.org/paper/Bacterial-pathogens-from-cave-dwelling-bats-that-a-Kholik-Agustin/7df9d035adfb1a70d5237c670cb78ec949a06d9c

Alvarez JDV, Lit Jr IL, and Alviola PA (2015). Bat flies (Diptera: Nycteribiidae) from Mount Makiling, Luzon Island: New host and distribution records, with a checklist of species found in the Philippines. Check List, 11(1): 1-4. DOI: https://www.doi.org/10.15560/15.1.1509

Azhar I, Khan FAA, Ismail N and, and Abdullah MT (2015). Checklist of bat flies (Diptera: Nycteribiidae and Streblidae) and their associated bat hosts in Malaysia. Check List, 11(5): 1-12. DOI: https://www.doi.org/10.15560/11.5.1777

Calisher CH, Childs JE, Field HE, Holmes KV, and Schountz T (2006). Bats: Important reservoir hosts of emerging viruses. Clinical Microbiology Reviews, 19(3): 531-545. DOI: https://www.doi.org/10.1128/CMR.00017-06

Dietrich M, Tjale MA, Weyer J, Kearney T, Seamark ECJ, Nel LH, Monadjem A, and Markotter W (2016). Diversity of *Bartonella* and *Rickettsia* spp. in bats and their blood-feeding ectoparasites from South Africa and Swaziland. PLoS ONE, 11(3): e0152077. DOI: https://www.doi.org/10.1371/journal.pone.0152077

Food and Agriculture Organization of The United Nations (FAO) (2011). Investigating the role of bats in emerging zoonoses: Balancing ecology, conservation and public health interests. Edited by Newman, S.H., Field, H.E., De jong, C.E and Epstein, J.H.FAO Animal Production and Health Manual No. 12. Rome, pp. 49-51. Available at: https://www.fao.org/3/i2407e/i2407e00.htm

Han HJ, Liu JW, Wen HL, Qin XR, Zhao M, Wang LJ, Zhou CM, Qi R, Yu H, and Yu XJ (2018). *Babesia vesperuginis* in insectivorous bats from China. Parasites Vectors, 11: 317. DOI: https://wwwdoi.org/10.1186/s13071-018-2902-9

Lima L, Silva FM, Neves L, Attias M, Takata CS, Campaner M, de Souza W, Hamilton PB, and Teixeira MM (2012). Evolutionary insights from bat trypanosomes: morphological, developmental and phylogenetic evidence of a new species, *Trypanosoma* (*Schizotrypanum*) *erneyi* sp. nov., in African bats closely related to *Trypanosoma* (*Schizotrypanum*) *cruzi* and allied species. Protist, 163(6): 856-872. DOI: https://www.doi.org/10.1016/j.protis.2011.12.003

Maa TC (1971). Review of *Streblidae* (Diptera) parasitic on megachiropteran bats. Pacific Insect Monograph, 28: 213-243. Available at: http://hbs.bishopmuseum.org/pim/pdf/pim28-213.pdf

Obame-Nkoghe J, Rahola N, Bourgarel M, Yangari P, Prugnolle F, Maganga GD, Leroy EM, Fontenille D, Ayala D, and Paupy C (2016). Bat flies (Diptera*:* Nycteribiidae and Streblidae) infesting cave-dwelling bats in Gabon: Diversity, dynamics and potential role in *Polychromophilus melanipherus* transmission. Parasites Vectors, 9: 333. DOI: https://www.doi.org/10.1186/s13071-016-1625-z

Raharimanga V, Ariey F, Cardiff SG, Goodman SM, Tall A, Rousset D, and Robert V (2013). Hemoparasites of bats in Madagascar. Arch Inst Pasteur Madagascar, 69(12): 70-76. Available at: https://pubmed.ncbi.nlm.nih.gov/15678820/

Sa'diyah SN, and Situmorang FJIC (2020). The differential account of white blood cell as an image of immune system from flying fox (*Pteropus* spp) as a reservoir host. Journal of Physics: Conference Series, 1430: 012024. Available at: https://iopscience.iop.org/article/10.1088/1742-6596/1430/1/012024

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Schaer J, Perkins SL, Decher J, Leendertz FH, Fahr J, Weber N, and Matuschewski K (2013). High diversity of West African bat malaria parasites and a tight link with rodent Plasmodium taxa. Proceedings of the National Academy of Sciences, 110(43): 17415-17419. DOI: https://wwwdoi.org/10.1073/pnas.1311016110

Srinivasulu C, Racey PA, and Mistry S (2010). A key to the bats (Mammalia: Chiroptera) of South Asia. Journal of Threatened Taxa, 2(7): 1001-1076. DOI: https://www.doi.org/10.11609/JoTT.o2352.1001-76

Szentiványi T, Christe P, and Glaizot O (2019). Bat Flies and Their Microparasites: Current knowledge and distribution. Frontiers in Veterinary Science, 6: 115. DOI: https://www.doi.org/10.3389/fvets.2019.00115

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Effect of Labazyme on Growth Performance, Physiological Parameters, and Economic Efficiency of Broiler Chickens

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ABSTRACT

Enzymes have a significant positive effect on nutrient digestion, feed efficiency, and growth rate of poultry. The current experiment aimed to determine the optimal dosage levels of Labazyme as feed additives. A total of 240 one-day-old broiler chickens (Ross 308) were randomly assigned to four groups with three replicates. The feeding experiment was carried out from hatching to day 42 of age. Three experimental groups contained Labazyme at 0.5, 1, and 1.5 mg/kg of the total diet. The control group received a basal diet. Growth performance, European performance efficiency index (EPEI), production index (PI), biochemical and lipid profiles, as well as antioxidant parameters were then measured. The results showed that chickens fed Labazyme supplementation (1 and 1.5 mg/kg) had a higher growth performance than those in the control group. Nonetheless, there was a significant difference between the Labazyme and the control group in terms of feed intake. In addition, Labazyme groups had a significantly positive effect on broiler economic scores. The EPEI and PI of the Labazyme-fed chickens were both higher than the control. There was a non-significant difference in total protein, albumin, globulin, and uric acid. The serum glucose level of the chickens fed Labazyme (1 and 1.5 mg/kg) was lower, compared to the control group. In contrast, chickens that consumed a diet supplemented with Labazyme 1 and 1.5 mg/kg indicated lower serum cholesterol, triglyceride, low-density lipoprotein, and very-low-density lipoprotein levels in broilers, compared to the control group. Serum high-density lipoprotein levels were improved and more pronounced in chickens fed Labazyme, compared to the control group. In conclusion, the results of the current study indicated that supplementation of Labazyme could help the improvement of growth performance, lipid profile, and profitability of broiler chickens.

Keywords: Broiler, Labazyme, Lipid profiles, Production index

INTRODUCTION

Poultry nutrition and the need to improve feeding efficiency have accelerated the use of feed additives and decreased the use of antibiotics in poultry feeding for the past decades. In fact, the goal outlined by the researchers has been to increase production while maintaining poultry health (Hafez and Attia, 2020). Singh et al. (2018) indicated that the use of exogenous enzymes in poultry feeds as a growth promoter is beneficial for improving production parameters and improving the digestibility of nutrients in broiler chickens.

However, the additives are complexes of non-nutrient and nutrient compounds that help to improve feed efficiency and reduce the cost of feed (Abdurofi et al., 2017). Similar results showed that natural growth promoters, such as plant powders, plant extracts, enzymes, probiotics, and multi-enzyme, can be used in the poultry feed to improve production, the immune system, and the microbial population in the gut and reduced oxidative stress. Multi-enzymes with/without probiotics are usually included in the animal feeds to degrade the anti-nutritional factors, and consequently improve nutrient digestibility and the growth performance of poultry (Kiarie et al., 2013; Kiarie et al., 2014).

Several studies have indicated that such enzymes also reduce intestinal colonization by pathogens in poultry (Olukosi et al., 2015; Amerah et al., 2017). On another hand, feed additives, such as enzymes Kaczmarek et al. (2014), probiotics (Fesseha, 2019; Bonilla Carrero, 2021) or enzyme complexes can be attributed to changes in the gut tract that facilitate digestion and absorption of the nutrients as well as animal growth (Kiarie et al., 2013). According to Amerah et al. (2017), there is a need to improve the digestibility of different types of nutrients in a compound feed due to the complex nature of the feed. Feed cost is an important issue in developing countries, such as Iraq, as it can improve the economic efficacy of the poultry industry. Therefore, the present study aimed to investigate the effect of different levels of Labazyme as a feed additive on growth performance, blood parameters, and economical profitability of broiler chickens.

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MATERIALS AND METHODS

Ethical approval

The broiler chickens in the current study were handled according to guidelines passed by the institutional ethics committee for the care of animals and were approved by the Animal Ethics Committee of the Department of Animal Production, Tikrit University, Iraq.

Experimental design

A total of 240 one-day-old Ross-308 broiler chickens were purchased from a hatchery in Samarra, Iraq. The chickens were housed in a cage (2 cm^2) at the graduate department of the animal station, at the College of Agriculture, Tikrit University, Tikrit, Iraq. The temperature was 35°C in the first week and then gradually decreased to 25°C at the end of the third week. The chickens were providing a 24-hour photoperiod of light. Food and water were provided *ad libitum*. The chickens were also supervised daily in the morning and evening. The chickens were divided into four groups and each group contained three replicates (20 chickens each). The diets were designed according to the recommendations of the NRC's requirements for broiler chicks (NRC, 1994). Chickens in the control group received a basal diet with no supplementation, and the experimental groups of E_1, E_2, and E_3 received Labazyme supplemented in the basal diets at 0.5, 1.0, and 1.5 mg/kg, respectively.

Labazyme composition

The product was purchased from a local market in Baghdad, Iraq, and the composition for Multi-enzyme and Probiotics Labazyme^{100gm} (each 1 kg contains) was indicated in the company label as *Lactobacillus acidophilus* 2.75×10 CFU, *Streptococcus faecium* 8.25×10 CFU, *Bacillus subtilis* 1.1×10 CFU, protease 2.750 CSU, amylase 5.500 SLU, and cellulose 275 FPUI.

Growth performance

At the start of the experiment, body weight was measured, and it was repeated weekly. Body weight gain was calculated by subtracting the body weight at the start of each week from body weight at the end of the same week. Feed intake was calculated weekly for each group by subtracting the weight of food left at the end of the week from the weight of given food at the start of the same week. Every week, the feed conversion ratio was calculated using the Formula 1. The mortality of chickens was recorded until the end of the study.

Feed conversion ratio = feed ingested (g) / weight gain (g) Formula (1)

Blood collection and biochemical analysis

Blood samples were randomly collected from four chickens in each pen at the end of the experimental periods. Approximately 3-5 ml blood samples were collected from the wing vein using a 5 ml syringe with a 22-gauge needle and transferred into non-heparinized tubes. The clotted blood samples were centrifuged at 3000 rpm for 15 minutes and the clear serum was separated and stored in a -20°C freezer for the biochemical analysis. Serum glucose was determined by the glucose oxidase method prescribed by Trinder (1969). Total protein and albumin serum were determined using spectrophotometric methods (Biolabo, 2011).

Globulin concentration was calculated using the Formula 2:
Globulin = Total protein – albumin Formula (2)

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined using spectrophotometric methods (Biolabo, 2014). Cholesterol, triglyceride, and high-density lipoprotein (LDL) cholesterol were determined with a kit (SPIN800, Spain). Low-density lipoprotein and very-low-density lipoprotein (VLDL) were also calculated to fit this equation as LDL = cholesterol- (High-Density Lipoprotein [HDL] + VLDL) using the Formula 3 of Friedewald et al. (1972) as below:

VLDL = 5/triglyceride Formula (3)

Economic evaluation

The economic evaluation was determined by considering feed cost per chicken (USD), total income from the chicken (USD), and growth efficiency according to Gondwe and Wollny (2005). The total return (TR) was calculated according to Shehata et al. (2018). The European performance efficiency index (EPEI) was evaluated according to Panda et al. (2006). The production index (PI) was calculated during the experimental growing period.

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Table 1. Ingredients and chemical composition of the basal diet

Ingredients (%)	Starter (1-21 days)	Finisher (21-42 days)
Yellow maize	46.18	53.88
Wheat	9.92	9
Soybean 44%	36.9	29.5
Vegetable oil	2.8	4.2
Primix[1]	2.5	2.5
Di-calcium phosphate	1	0.2
Salt	0.3	0.5
Methionine	0.1	0.1
Lysine	0.3	0.12
Chemical Composition*	100	100
ME (Kcal/Kg)	3027.74	3202.42
Crude Protein (%)	23.02	20.02
Lysine (%)	1.48	1.12
Methionine (%)	0.57	0.54
Methionine+cysteine (%)	0.92	0.85
Calcium (%)	0.97	0.83
Phosphor (%)	0.61	0.45

* The Premix (1 kilogram of Premix): Vitamin E (500 IU), Vitamin B_{12} (0.06 mg), Vitamin B_1 (67 mg), Vitamin A (334000 IU), Vitamin D_3 (67000 IU), Vitamin B_2 (1000 mg), Vitamin B6 (0.66 mg), Folic acid (17 mg), Choline (17000 mg), N (1000 mg), Magnesium (3.334 mg), Zinc (334 mg), Iron (1.667 mg), Copper (10 mg), Iodine I (17 mg), Methionine (27000 mg), Phosphor (10.6%), Selenium (0.20 mg). * Starter, growth, and finisher diets were formulated according to the requirement for broiler chickens as outlined by National Research Council (NRC, 1994).

Statistical analysis

The data were subjected to analysis of variance for each parameter using the SAS statistical package (version 9, 2016). One-Way ANOVA followed by Duncan's multiple range test was used to compare the means of the groups (Duncan, 1955). The economic evaluation and biochemical parameters were subjected to the analysis of variance and graphing using GraphPad Prism (version. 8.0.1., Graph Pad Software, San Diego, California, USA). P-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Growth performance

The effect of Labazyme on broiler performance is presented in Table 2. On days 7, 14, 21, 28, 35, and 42 body weight varied between groups. Except for day 7, significant differences were observed during the experiment (42 days) among broiler fed the diet supplemented with 0.5, 1, and 1.5 mg/kg Labazyme, compared to the control groups ($p < 0.05$) in terms of body weight. In other words, body weight was higher in experimental chickens, compared to the control. Regarding body weight gain, there were significant differences between all groups on days 7, 14, 21, and 28, but there was no significant difference between the Labazyme and control groups on days 35 and 42. During days 1-42, the broiler chickens fed Labazyme recorded a higher weight, compared to the control group. Table 2 shows the results of feed intake and feed conversion ratio. The present result indicated that significant differences were observed between control and experimental groups on days 7, 14, 21, 28, 32, and 42. The Labazyme-fed broiler chickens had significantly the lowest feed and best feed conversion ratio, compared to the control ($p < 0.05$). Chickens in the experimental groups consumed significantly the least amount of food over the total period of 1-42 days. Meanwhile, the supplementation of Labazyme in broiler feed resulted in significant differences in the feed conversion ratio compared to the control on days 7, 14, 21, 28, and 42. The broilers fed Labazyme showed a better feed conversion ratio throughout the 1-42 days period.

The results showed improved growth performance in broiler chickens fed Labazyme due to the underlying improvement in digestibility of the feed ingredients. Labazyme has a synergistic effect since it contains probiotics (*Lactobacillus acidophilus*, *Streptococcus faecium*, and *Bacillus subtilis*) and a group of digestive enzymes (protease, amylase, and cellulase) capable of forming complexes with essential nutrients and the releasing these nutrients. This could improve nutrient utilization in the feed and have a positive impact on the growth and reduce the feed intake with a better feed conversion ratio. There are several mechanisms by which enzymes can remove alkylglycerone phosphate synthase and reduce the risks associated with their use which affects the endogenous enzyme in the broiler gut tract not being readily tapped, or perhaps, the Labazyme reduces the viscosity of the gut tract, which is a faster throughput speed. The results of the current experiment were supported by previous studies on the use of enzymes or multienzymes with or without probiotics to improve growth performance, reduce feed intake, and achieve the best value for feed conversion ratio in broiler feed supplementation with enzymes (Guo et al., 2014; Siadati et al., 2017). As a result, the inclusion of

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exogenous enzymes in poultry feed has become common practice to improve broiler production and performance (Allcorn, 2016). According to Siadati et al. (2017), chickens fed exogenous multienzyme complexes could benefit from improved performance, as well as enhanced fat and crude protein digestibility. Askelson (2013) reported that exogenous enzymes could increase the nutrients available to the broiler by hydrolyzing non-digestible feed components while reducing the anti-nutritional effects of plant-based feed components.

Table 2. Effect of dietary treatment of Labazyme on growth performance of broiler chickens

	Chickens age	Groups*				P-value
		Control	E_1 (0.5 g/km)	E_2 (1 g/km)	E_3 (1.25 g/km)	
Body weight (kg)	Day 1	43.00	43.00	43.00	43.00	-
	Day 7	140.13 ± 0.72^{ab}	139.11 ± 1.46^{ab}	138.23 ± 0.88^{b}	141.82 ± 0.89^{a}	0.118
	Day 14	327.56 ± 10.47^{b}	356.23 ± 5.49^{a}	349.56 ± 1.45^{ab}	356.88 ± 6.35^{a}	0.047
	Day 21	662.11 ± 5.84^{b}	723.72 ± 10.14^{a}	746.82 ± 8.70^{a}	739.49 ± 17.77^{a}	0.003
	Day 28	1092.92 ± 40.3^{b}	1250.13 ± 8.1^{a}	1248.85 ± 7.78^{a}	1257.27 ± 9.27^{a}	0.001
	Day 35	1553.06 ± 17.93^{c}	1693.99 ± 8.9^{b}	1710.81 ± 6.4^{a}	1751.64 ± 20.59^{a}	0.000
	Day 42	2252.42 ± 28.90^{b}	2447.48 ± 40.62^{a}	2453.96 ± 32.45^{a}	2463.54 ± 28.28^{a}	0.005
Body weight gain (g)	Day 7	96.13 ± 0.72^{ab}	96.11 ± 1.46^{ab}	95.23 ± 0.88^{b}	98.82 ± 0.89^{a}	0.118
	Day 14	186.43 ± 10.49^{b}	217.12 ± 4.08^{a}	211.33 ± 1.05^{a}	215.06 ± 5.83^{a}	0.030
	Day 21	334.55 ± 9.38^{b}	367.49 ± 5.90^{b}	397.26 ± 7.89^{a}	382.61 ± 9.33^{a}	0.039
	Day 28	430.80 ± 43.86^{b}	526.40 ± 15.32^{a}	502.02 ± 1.026^{a}	517.77 ± 8.54^{a}	0.074
	Day 35	460.14 ± 55.46	453.86 ± 16.98	461.96 ± 10.14	494.37 ± 29.38	0.748
	Day 42	699.35 ± 12.55	753.48 ± 31.94	743.15 ± 36.16	711.90 ± 43.07	0.632
	Total 7-42	2209.42 ± 8.90^{b}	2404.48 ± 40.62^{a}	2410.96 ± 32.45^{a}	2420.54 ± 28.28^{a}	0.0052
Feed intake (g/chicken)	Day 7	153.31 ± 3.43^{a}	127.12 ± 3.25^{b}	133.41 ± 2.24^{b}	132.78 ± 2.66^{b}	0.001
	Day 14	359.98 ± 5.90^{a}	320.01 ± 2.66^{b}	307.89 ± 4.15^{b}	305.43 ± 16.10^{b}	0.008
	Day 21	691.57 ± 22.28^{a}	593.58 ± 25.10^{b}	584.31 ± 20.16^{b}	547.82 ± 27.73^{b}	0.015
	Day 28	825.53 ± 8.02^{a}	732.50 ± 23.56^{b}	808.03 ± 6.95^{b}	667.38 ± 7.97^{c}	0.000
	Day 35	1000.80 ± 31.40^{a}	832.18 ± 8.34^{b}	859.44 ± 17.41^{b}	879.80 ± 20.44^{b}	0.002
	Day 42	1247.61 ± 17.27^{a}	1102.83 ± 30.39^{b}	1063.32 ± 13.80^{b}	1043.62 ± 36.21^{b}	0.002
	Total 7-42	4278.84 ± 59.23^{a}	3708.23 ± 67.52^{b}	3666.41 ± 34.75^{b}	3576.84 ± 85.10^{b}	0.000
Feed conversion ratio	Day 7	1.56 ± 0.04^{a}	1.32 ± 0.05^{b}	1.40 ± 0.03^{b}	1.34 ± 0.01^{b}	0.009
	Day 14	1.94 ± 0.09^{a}	1.47 ± 0.01^{b}	1.46 ± 0.02^{b}	1.42 ± 0.11^{b}	0.003
	Day 21	2.07 ± 0.11^{a}	1.61 ± 0.05^{b}	1.47 ± 0.03^{b}	1.43 ± 0.04^{b}	0.000
	Day 28	1.95 ± 0.19^{a}	1.39 ± 0.08^{b}	1.43 ± 0.01^{b}	1.29 ± 0.03^{b}	0.010
	Day 35	2.24 ± 0.27^{a}	1.88 ± 0.08^{ab}	1.86 ± 0.07^{ab}	1.79 ± 0.08^{ab}	0.236
	Day 42	1.78 ± 0.01^{a}	1.47 ± 0.08^{b}	1.43 ± 0.06^{b}	1.47 ± 0.03^{b}	0.007
	Total 7-42	1.93 ± 0.03^{a}	1.54 ± 0.03^{b}	1.52 ± 0.00^{b}	1.47 ± 0.01^{b}	0.000

$^{a\,b\,c}$ mean within the same row followed by different superscripts are significantly different ($p < 0.05$). *Groups: Control: 0.0, E1: 0.5 mg /kg Labazyme, E2:1 mg /kg Labazyme and E3:1.5 mg /kg Labazyme in broiler diet.

Biochemical levels and lipid profiles

The effect of Labazyme supplementation on glucose, total protein, albumin, globulin, and uric acid is shown in Table 3. The obtained results showed a significant difference between all groups in terms of glucose level ($p < 0.05$). Results revealed that chickens fed with different doses of Labazyme in E_2 and E_3 groups had the lowest level of glucose, compared to the control. However, there were non-significant differences between the groups regarding total protein, albumin, globulin, and uric acid ($p > 0.05$). There was a significant ($p < 0.05$) between-group effect in the lipid profile levels in the broiler chickens (Figure 1). On day 42, there was a significant decrease in serum cholesterol, triglycerides, LDL, and VLDL in all dietary supplemented groups, compared to the control group. There was an increase in serum high-density lipoprotein in the Labazyme supplemented groups, compared to the control group. Total protein, albumin, globulin, and uric acid concentrations were not significantly different among the groups. According to Siadati et al. (2017), feed additives should be effective and safe for broiler chickens. The results are consistent with the idea that the exogenous enzymes can be safely used in poultry nutrition without affecting the function of vital organs. The effect of Labazyme on concentrations of lipid profiles is consistent with previous studies indicating that multienzymes decrease lipid profile properties due to the changes in the composition of intestinal bacterial flora, through which probiotics ferment to reduce short-chain fatty acids in the gut and then reduce the systemic blood lipids and cholesterol (Zhao and Yang, 2005). However, some probiotic bacteria can interfere with cholesterol absorption in the gut by de-conjugating bile salts or assimilating cholesterol directly (Kumar et al., 2012).

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Table 3. Effect of dietary treatment of Labazyme on biochemical parameters of broiler chickens

Variable	Groups *				P value
	Control	E_1 (0.5 g/km)	E_2 (1 g/km)	E_3 (1.25 g/km)	
Glucose (mg/dL)	233.43 ± 12.87[a]	204.59 ± 6.50[ab]	195.69 ± 4.26[b]	190.34 ± 5.35[b]	0.010
Total protein (g/dL)	2.97 ± 0.34	2.80 ± 0.07	3.29 ± 0.35	3.10 ± 0.29	0.690
Albumin (g/dL)	1.56 ± 0.13	1.82 ± 0.17	1.99 ± 0.08	1.76 ± 0.09	0.168
Globulin (g/dL)	1.41 ± 0.38[a]	0.98 ± 0.15[ab]	1.29 ± 0.28[a]	1.34 ± 0.38[a]	0.790
Uric acid (mg/dL)	2.59 ± 0.62[a]	1.73 ± 0.21[ab]	2.23 ± 0.19[a]	1.53 ± 0.22[ab]	0.202

[a and b] mean within the same row followed by different superscripts are significantly different ($p < 0.05$). *Groups: Control: 0.0, E1: 0.5 mg /kg Labazyme, E2:1 mg /kg Labazyme and E3:1.5 mg /kg Labazyme in broiler diet.

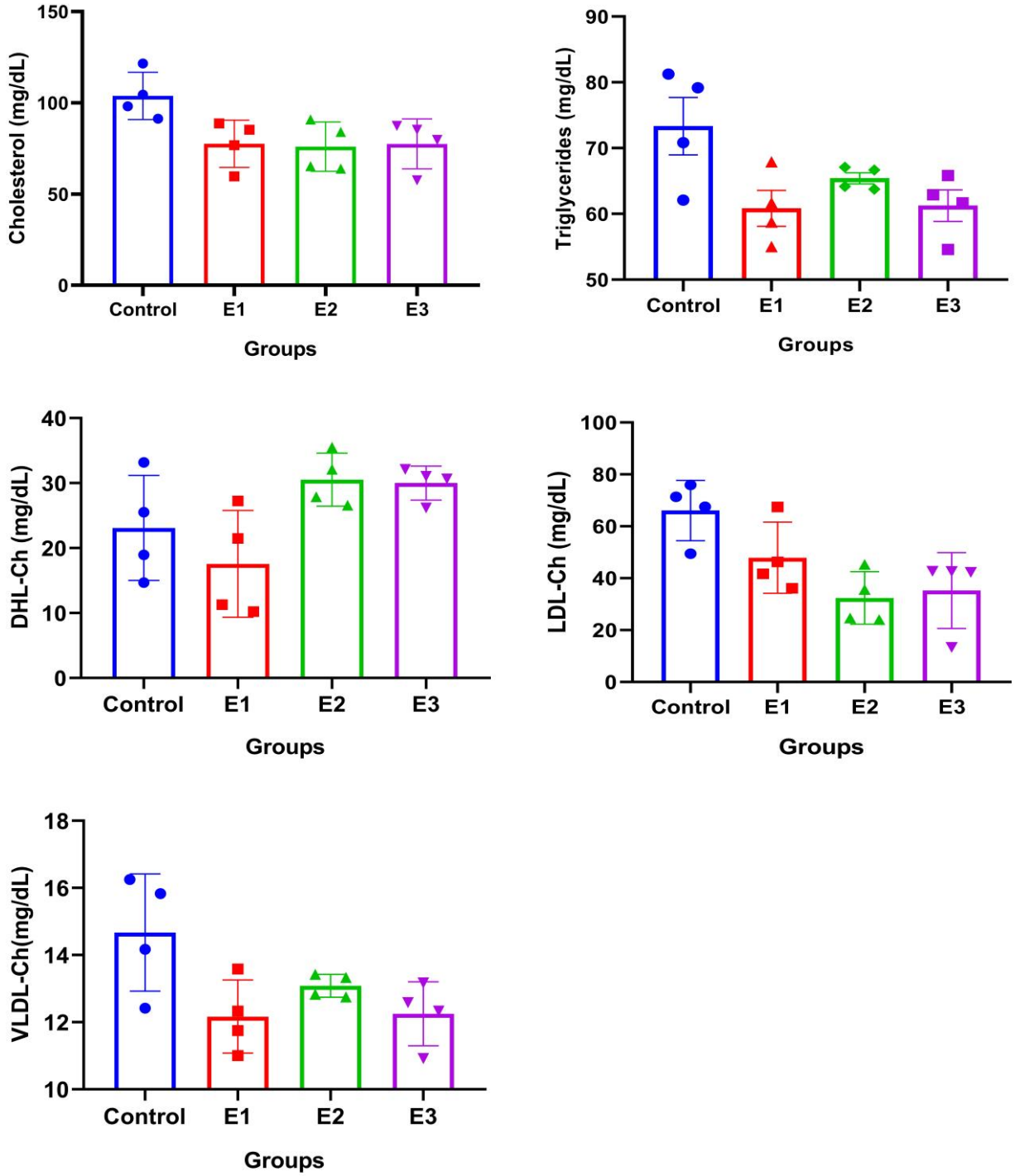

Figure 1. Serum lipid profile of broiler chickens fed Labazyme. Groups: Control: 0.0, E1: 0.5 mg /kg Labazyme, E2:1 mg /kg Labazyme and E3:1.5 mg /kg Labazyme in broiler diet. The Plot: Mean with standard error mean , $p < 0.05$

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Economic evaluation

The economic impact of using Labazyme as a feed additive for broiler chickens is shown in Figure 2. Regarding feeding cost (FC), there was a highly significant difference between Labazyme groups and the control group ($p < 0.05$, Figure 2A), meaning that the cost of one ton of feed was reduced in groups E_2 and E_3 Labazyme, saving 2.18 and 2.18/ton, compared to 2.50/ton for the control group. Further, similar results found in TI and TR indicated a highly significant difference between the Labazyme groups and the control group ($p < 0.05$, Figures 2B and 2C). The highest values of 5.72 and 5.75 TI and 1.54 and 1.60 TR were respectively recorded for the E_2 and E_3 groups compared to the lowest value of the control as 5.25 TI and 0.75 TR. The economic evaluation, EPEI, and PI were highly significant in broiler chickens fed a Labazyme supplement, compared to control (Figure 3). The highest TR and the highest profitability from the sale of broiler chickens were achieved with Labazyme supplemented, as there was a significant increase in the final body weight ($p < 0.05$). These results are consistent with those of Shehata et al. (2018) indicating that feed costs decreased and enzyme levels increased (Haque et al., 2017).

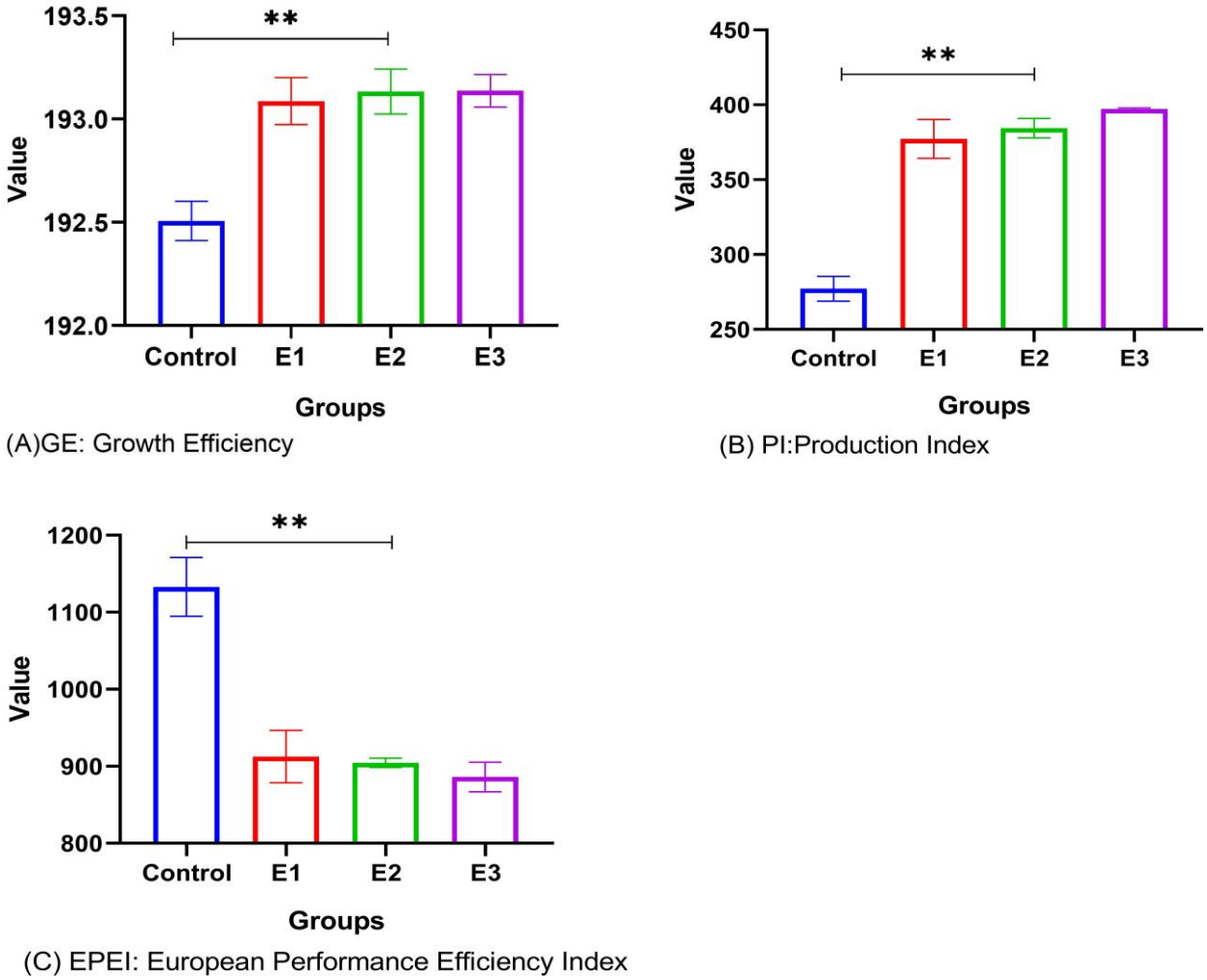

Figure 3. Growth efficiency, European performance, efficiency index and production index of broiler chickens fed Labazyme. Control: 0.0, E1:0.5, E2:1 and E3:1.5 mg /kg Labazyme in broiler diet. The Plot: Mean with standard error mean , $p < 0.05$.

CONCLUSION

Under the current study conditions, due to the content of Labazyme, including *Lactobacillus acidophilus*, *Streptococcus*, *Bacillus subtilis*, protease, amylase, and cellulase, the synergistic effect of Labazyme appeared to be beneficial in the experimental groups, compared to the control group. Labazyme could optimize digestion in a way that improves feed conversion ratio, production, and high-density lipoprotein, as well as reducing cholesterol and triglycerides. Present results from this study showed that supplementing broiler feed with Labazyme is economically helpful. It can be concluded that using a Labazyme in broiler diets as a feed additive can be of great help.

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DECLARATIONS

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Authors' contribution

Arkan Mohammed and Tariq Aljumaily was the coordinator of the research and analyzed and interpreted the data. Arkan Mohammed, Ammar Abdulwahab, and Samah Raouf in the study were the supervisor of data collection and wrote draft manuscripts, Ammar, Tariq and Samah were assistants in the collection of data. All authors read and approved the final manuscript.

Competing interests

The authors have not declared any conflict of interest.

Ethical consideration

All authors have checked the ethical issue such as plagiarism, consent to publish, data fabrication and falsification, and redundancy.

REFERENCES

AbdelRaheem S, AbdAllah S, and Hassanein K (2012). The effects of prebiotic, probiotic, and synbiotic supplementation on intestinal microbial ecology and histomorphology of broiler chickens. International Journal for Agro Veterinary and Medical Sciences, 6(4): 277. Available at: https://www.citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.301.8603&rep=rep1&type=pdf

Abdurofi I, Ismail MM, Kamal HAW, and Gabdo BH (2017). Economic analysis of broiler production in Peninsular Malaysia. International Food Research Journal, 24(4): 1387-1392. Available at: http://www.ifrj.upm.edu.my/24%20(04)%202017/(6).pdf

Allcorn TG (2016). Evaluation of exogenous enzyme combinations on broiler performance in reduced energy diets. Thesis, p. 61. Available at: http://oaktrust.library.tamu.edu/bitstream/handle/1969.1/158603/ALLCORN-THESIS-2016.pdf?sequence=1

Amerah AM, Romero LF, Awati A, and Ravindran V (2017). Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/soy diets. Poultry Science, 96(4): 807-816. DOI: https://www.doi.org/10.3382/ps/pew297

Askelson TE (2013). Effect of phytate-degrading probiotics on broiler performance. Thesis. p. 63. Available at: https://oaktrust.library.tamu.edu/bitstream/handle/1969.1/151632/ASKELSON-THESIS-2013.pdf?sequence=1&isAllowed=y

Biolabo (2011). Total protein Biuret method. Manufacturer: BIOLABO SAS. Available at: http://www.biolabo.fr/biolabo/pdfs/noticesE/biochimieE/AT-80016.pdf

Biolabo (2014). ALT GPT (IFCC) Single vial. Manufacturer: BIOLABO SAS. Available at: http://www.biolabo.fr/biolabo/pdfs/noticesE/biochimieE/AT-80027.pdf

Bonilla Carrero PI (2021). The Effects of tryptophan and probiotic treatment on behavior and production parameters of laying hens. Thesis. p. 95. Available at: https://drum.lib.umd.edu/bitstream/handle/1903/27702/BonillaCarrero_umd_0117N_21650.pdf?sequence=2&isAllowed=y

Duncan DB (1955). Multiple range and multiple F test. Biometrics, 11:1-42. Available at: https://www.jstor.org/stable/3001478

Fesseha H (2019). Probiotics and its potential role in poultry production: A review. Veterinary Medicine-Open Journal, 4(2): 69-76. DOI: https://www.doi.org/10.17140/VMOJ-4-138

Friedewald W, Levy R, and Fredrichson D (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clinical Chemistry, 226: 499-502. PMID: 4337382. Available at: https://pubmed.ncbi.nlm.nih.gov/4337382

Gondwe TN and Wollny CBA (2005). Evaluation of the growth potential of local chickens in Malawi. International Journal of Poultry Science, 4(2): 64-70. Available at: https://www.hdl.handle.net/10568/3935

Guo S, Liu D, Zhao X, and Li Cand Guo Y (2014). Xylanase supplementation of a wheat-based diet improved nutrient digestion and mRNA expression of intestinal nutrient transporters in broiler chickens infected with Clostridium perfringens. Poultry Science, 93(1): 94-103. DOI: https://www.doi.org/10.3382/ps.2013-03188

Hafez HM and Attia YA (2020). Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. Frontiers in Veterinary Science, 7: 516. DOI: https://www.doi.org/10.3389/fvets.2020.00516

Haque MI and Ahmad Nand Miah MA (2017). Comparative analysis of body weight and serum biochemistry in broilers supplemented with some selected probiotics and antibiotic growth promoters. Journal of Advanced Veterinary and Animal Research, 4(3): 288-294. DOI: http://www.doi.org/10.5455/javar.2017.d226

Kaczmarek SA, Rogiewicz A, Mogielnicka M, Rutkowski A, Jones RO, and Slominski BA (2014). The effect of protease, amylase, and nonstarch polysaccharide-degrading enzyme supplementation on nutrient utilization and growth performance of broiler chickens fed corn-soybean meal-based diets. Poultry Science, 93(7): 1745-1753. DOI: https://www.doi.org/10.3382/ps.2013-03739

Kiarie E, Romero LF, and Nyachoti CM (2013). The role of added feed enzymes in promoting gut health in swine and poultry. Nutrition Research Reviews, 26(1): 71-88. DOI: https://www.doi.org/10.1017/S0954422413000048

Kiarie E, Romero LF, and Ravindran V (2014). Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. Poultry Science, 93(5): 1186-1196. DOI: https://www.doi.org/10.3382/ps.2013-03715

Kumar M, Nagpal R, and Kumar Rand Jain S (2012). Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Experimental Diabetes Research. DOI: https://www.doi.org/10.1155/2012/902917

National Research Council (NRC) (1994). Nutrient Requirements of Poultry. (N. A. Press, Ed.), 4th edition, Washington: National Academy Press. DOI: https://www.doi.org/10.17226/2114

Olukosi OA, Beeson LA, Englyst K, and Romero LF (2015). Effects of exogenous proteases without or with carbohydrases on nutrient digestibility and disappearance of non-starch polysaccharides in broiler chickens. Poultry Science, 94(11): 2662-2669. DOI: https://www.doi.org/10.3382/ps/pev260

Panda AK, Rao SVR, Raju MVLN, and Sharma SR (2006). Dietary supplementation of Lactobacillus sporogenes on performance and serum biochemico-lipid profile of broiler chickens. The Journal of Poultry Science, 43(3): 235-240. Available at: https://www.jstage.jst.go.jp/article/jpsa/43/3/43_3_235/_pdf

Shehata S, Kamel E, Abo-Salem M, and Atallah S (2018). Effect of Some Dietary Supplementation on Economic efficiency of growing Japanese Quails. Benha Veterinary Medical Journal, 34(1): 219-231. Available at: https://www.bvmj.journals.ekb.eg/article_54237_968a7ab952766ea3ea75538cf514606b.pdf

Siadati SA, Ebrahimnezhad Y, Salehi Jouzani G, and Shayegh J (2017). Evaluation of probiotic potential of some native lactobacillus strains on the growth performance and serum biochemical parameters of Japanese quails (Coturnix Coturnix Japonica) during rearing period. Revista Brasileira de Ciencia Avicola, 19(3): 399-408. DOI: https://www.doi.org/10.1590/1806-9061-2016-0393

Singh AK (2018). Effects of Multi-Enzymes on Growth Performance, and Effects of Multi-Enzymes and Probiotics on Nutrient Utilization in Broilers Fed Different Level of Fibers. University of Hawai‘i at Mānoa. Thesis, p. 58. Available at: https://scholarspace.manoa.hawaii.edu/server/api/core/bitstreams/b5489428-2264-4e4f-907b-4f1ef3425795/content

Trinder P (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry, 6(1): 24-27. Available at: https://www.journals.sagepub.com/doi/pdf/10.1177/000456326900600108

Zhao J-R and Yang H (2005). Progress in the effect of probiotics on cholesterol and its mechanism. Wei Sheng Wu Xue Bao= Acta Microbiologica Sinica, 45(2): 315-319. Available at: https://pubmed.ncbi.nlm.nih.gov/15989285/

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Molecular Diversity and Histopathological Findings of Novel Bovine Viral Diarrhea Virus Strains Isolated from Bull Semen

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ORIGINAL ARTICLE
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ABSTRACT

Bovine viral diarrhea virus (BVDV) is one of the most common viral pathogens affecting the cattle industry worldwide. The present study aimed to molecularly characterize BVDV isolates that are currently circulating in breeding bulls farmed with cattle suffering from reproductive disorders, and also to assess the consequences of BVDV infection on bulls' semen quality and conception, and its pathological effects on the structure of testicular tissue and spermatozoa. For this purpose, semen, serum, and testicular samples were collected from four breeding bulls in four private dairy farms in the governorates of Kafr-El Sheik, Beni-Suef, Giza, and Assuit, in Egypt from April 2019 to May 2020. An evaluation of sperm abnormalities was carried out by assessing the integrity of the plasma and acrosomal membranes where severe damage and abnormalities were found. Ultrastructure analysis of the spermatozoa by transmission electron microscopy revealed the presence of a swollen plasma membrane with segmented outer acrosomal membrane of spermatozoa and vacuolar degenerated mitochondria. Histopathological examination of testicular and epididymal tissues indicated moderate to severe degenerative effects of virus infection on seminiferous tubules with hypospermatogenesis. By detection of virus antigen in the serum samples using ELISA, bulls were identified as persistently infected with BVDV. Virus isolation revealed four noncytopathic (NCP-BVDV) strains that were confirmed by fluorescent antibody technique *(*FAT) and amplification of the 5′ untranslated genomic region (5'UTR) and molecularly typed by amplification of the E^{rns} glycoprotein region. Isolates' Phylogenetic analysis revealed two subgenotypes: BVDV-1b (Genbank accession numbers; LC634512, LC634513, LC634515) and BVDV-1d (LC634516). According to the knowledge of the authors of the present study, the circulation of the BVDV-1d subgenotype is not reported in Egypt. Therefore, it would be of great importance to track circulating strains in specific countries for successful vaccination programs or accurate diagnostic tests, and this necessitates regular updates.

Keywords: BVDV, Isolation, Spermatozoa ultrastructure, Sperm abnormalities, Testicular histopathology

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is one of the most important viruses affecting cattle worldwide, which has been recorded for more than six decades, however, it remains a crucial hazard in the cattle industry (Hou et al., 2019; Al-Kubati et al., 2021).

Bovine viral diarrhea virus is classified as a Pestivirus within the Flaviviridae family (Simmonds et al., 2017). Its genomic RNA is non-polyadenylated and single-stranded (ss RNA) with positive polarity and an average of 12.5 Kb in length. It has a long open reading frame (ORF) flanked at the 5' and 3' termini with a highly conserved untranslated region (UTR) for translation and replication controls, respectively. The ORF encodes polyproteins that are cleaved into four structural (C, E^{rns}, E1, and E2) and eight nonstructural (Npro, p7, NS2-3, NS4A, NS4B, NS5A, and NS5B) proteins by viral and cellular proteases during and after translation (Neill, 2013; Tautz et al., 2015). More than two decades ago, BVDV isolates were classified as BVDV-1 or BVDV-2, based on phylogenetic analysis of incomplete sequences (Ridpath et al., 1994; Simmonds et al., 2017). Later investigations revealed an expanding number of BVDV-1 and

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BVDV-2 subgenotypes, and it is now widely understood that pestiviruses are genetically highly variable, even within single subgenotypes (Yeşilbağ et al., 2017).

Bovine viral diarrhea virus affects the immune, respiratory, reproductive, and enteric systems. Reproductive problems caused by BVDV include early embryonic death, fetal mummification, abortions, and the birth of deformed calves (Stott et al., 2012; Scharnböck et al., 2018). In bulls, BVDV can produce persistent localized testicular infection (Givens et al., 2003; Newcomer et al., 2014) with testicular hypoplasia, degeneration of seminiferous tubules, and specific dissemination of BVDV antigen throughout the male genital tract.

The seroprevalence of infection with BVDV in non-vaccinated cattle herds ranges from 28% to 66% depending on geographical areas, and from 40% to 90% in individual cattle. The persistently infected (PI) cattle percentage is between 0.5% and 2.5% in most countries without any control program being in place against the virus (Velasova et al., 2017; Scharnböck et al., 2018; Oguejiofor et al., 2019). Vaccination can be used to prevent infection but, without the elimination of PI animals, it is impossible to eradicate the virus in a susceptible population. Diagnostics and vaccines that act well against analogous strains may be less effective against genetically different viruses. Therefore, considering the genetic variants described for BVDV-1 and BVDV-2 may play a role in disease prevention (Vilcek et al., 2001; Bauermann et al., 2013).

In Egypt, the prevalence of BVDV infection in cattle is about 40-47.4%, and the percentage of PI animals ranges from 1.6% to 18% based on the following factors; the variance observed in the regions where populations were investigated, the management system in the analyzed herd, and the diagnostic tests used in each study **(**El-Bagoury et al., 2014; Selim et al., 2018; Atwa et al., 2019). Only a few studies have been conducted on the characterization and sub-genotyping of the circulating virus (El-Kholy et al., 2005; Abdel-Latif et al., 2013; Soltan et al., 2015b). Thus, the primary goals of this study were to molecularly characterize BVDV isolates that are currently circulating in breeding bulls. Moreover, the study sought to assess the effect of BVDV infection on bulls' semen quality and conception as well as its pathological effects on the structure of testicular tissue and spermatozoa.

MATERIALS AND METHODS

Ethical approval

All procedures for samples collection and animals' manipulation were performed following the protocol approved by the Ethical Research Committee of the Animal Reproduction Research Institute, Agriculture Research Center ARC with Code No.2 11429.

Investigation of the reproductive problem and data collection

A history of poor conception rates in dairy cows in four private farms in the governorates of Kafr-El Sheik, Beni-Suef, Giza, and Assuit, in Egypt, was reported from April 2019 to May 2020. Data records showed 125 cases of abortion in cows between the third and fifth month of gestation, 17 cases of early fetal deaths and reabsorption, and 74 normal parturitions with some calves displaying congenital malformations. These herds had received only the official vaccines (foot-and-mouth disease and Rift Valley fever vaccines). For laboratory investigation conducted in the present study, semen and serum samples from four local hybrid Holstein breeding bulls, with the age range from 4 to 6 years, and body weights range from 600 to 700 Kg (one per farm) were collected for semen evaluation and detection of pathogenic agents. Serum samples were taken again 3 weeks after sampling. In this regard, one bull was slaughtered (Beni-Suef farm), the testes were subjected to gross examination and were then processed for virus isolation and histopathological examination.

Sample collection and preparation

Two ejaculates were obtained in the early morning (average volume 4 to 6 ml) from each bull with a pre-warmed artificial vagina (40-42°C). The ejaculates were tested for sperm abnormalities as soon as they were collected. Then, 1 ml of each semen sample was transferred to the laboratory on ice for virus isolation. From testis, 1 g was homogenized in 5 ml of phosphate-buffered saline (PBS) for virus isolation, and the other part was immersed and fixed in formal saline solution (10% formalin in 0.9% NaCl) in "1:10 proportion" for histopathological examination. The blood samples were taken twice, 14 days apart, from the coccygeal vein of a properly secured bull and centrifuged (Beckman, USA) at 3000 rpm for 15 minutes in clean sterilized tubes. No medication was used before sampling; Serum samples were collected and preserved at -20°C for serological examination.

Sperm abnormalities assessment

Plasma membrane integrity

The plasma membrane integrity of bull spermatozoa was evaluated using the hypo-osmotic swelling test, as described in Revell and Mrode (1994).

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Acrosomal defect

Acrosome integrity was examined using silver nitrate staining based on the procedure described in Chinoy et al. (1992).

Histopathological examination of testicular tissue and ultrastructure analysis of spermatozoa

Formalin-fixed tissue specimens (testicular and epididymal, after gross examination) were processed in an automated tissue processor. The tissue sections were stained with hematoxylin and eosin and examined microscopically (Bancroft and Gamble, 2018). The ultrastructural changes in spermatozoa were examined and evaluated using transmission electron microscopy (TEM, JEOL-EM-100 S at 80 Kv Tokyo, Japan) at the Electron Microscopy Unit, Faculty of Agriculture, Cairo University, Giza, Egypt) and photographed for further analysis as mentioned in Boonkusol et al. (2010).

Detection of the causative agent

After exclusion of bacterial and parasitic infection, viral diagnosis was performed with a focus on BVDV infection.

Detection of bovine viral diarrhea virus antigen by ELISA

Four Serum samples were used for the detection of BVDV infection by anti-p80/p125 monoclonal antibody-coated double sandwich ELISA plate (INGEZIM BVD DAS) kits (Ingensasa, Madrid, SPAIN) following the manufacturer's instructions.

Virus isolation

A reference strain National Animal Diseases Laboratory (NADL) supplied from Ames Iowa, USA was used as a positive control during the isolation and identification of the virus. Semen and testicular samples were processed and diluted as 1.0 ml^{-1} in PBS (with antibiotic/antimycotic at the recommended concentration) 100 IU Penicillin, 100 µg dihydrostreptomycin) as mentioned in OIE (2018). The supernatant of each sample was inoculated three successive passages on mycoplasma and BVDV-free MDBK monolayer cell culture (6 days each) Supplied by the national animal disease center, Ames Iowa, USA. Daily microscopic examinations with an inverted microscope (Zeiss, USA) were performed to monitor the cytopathic effects (CPE).

Virus identification using fluorescent antibody techniques

On coverslips, monolayer MDBK cells were inoculated with the second passage and after 24 hours of the third passage, the inoculated cells were air-dried and fixed with chilled acetone. Viral antigens were captured and detected by direct fluorescence isothiocyanate (FITC) conjugated anti-BVDV as recommended manufacturer dilution (CJ-F-BVD; VMRD, Pullman, WA 99163 USA). The mounted coverslips were examined using an inverted epifluorescence trinuclear Nikon microscope (Nikon ECLIPSE-TS 100, Japan with 10× plan achromatic lens and a digital camera DS-US and it is NIS elements software, Lens 10X).

Viral genome detection

RNA isolation

Total RNA was extracted from the tissue culture suspension of the third passage after three successive freezing and thawing cycles for each sample using QIAzol Lysis Reagent (QIAGEN, Germany) according to the manufacturer's instructions, and it was kept at -80°C. Complementary DNA (cDNA) was synthesized with a RevertAid First Strand cDNA Synthesis kit (Thermoscientific, Germany) following the manufacturer's instructions, and was stored at -20°C. The positive (BVDV-NADL strain inoculated cell culture) and negative (BVDV-free cell culture) controls were included in all the assay steps, from RNA extraction to amplification.

5' untranslated genomic region amplification

Amplification of a 208 bp fragment within the 5' UTR of the viral genome was based on a primer set outlined in Hoffmann et al. (2006) and reported in OIE (2018). The reaction mixture (20 µl) consisted of DreamTaq™ Green PCR Master Mix (2X) (Thermo Scientific, Lithuania), 20 pmol of each forward (190-F) and reverse (V326) primers and 4 µl of cDNA. The reaction was performed in a thermocycler (Nexus Gradient Eppendorf, Germany). The primer sequences, thermal conditions, and expected PCR products were outlined in Table 1.

Virus genotyping by nested multiplex RT-PCR within the E^{rns} region

As mentioned by Sullivan and Akkina (1995) and shown in Table 1, the first round of the cDNA amplification was carried out in a single tube reaction using P1 and P2 primers. Then the nested amplification was performed using the type-specific primers (TS1 for BDV, TS2 for BVDV-2, and TS3 for BVDV-1) and P2 on amplified products within the first round. The amplified products were analyzed using 1.5% agarose gel electrophoresis on a UV transilluminator.

Partial sequencing and analysis of the E^{rns} region

The genotype E^{rns}-specific PCR products were subjected to purification by Gene JET Gel Extraction Kit (ThermoScientific K0691, Germany) following the manufacturer's instructions. The purified PCR products were subjected to sequencing in an automated ABI 3730×l DNA sequencer (Applied Biosystems, USA) using forward and

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reverse primers. Sequencing was performed only by combining the traditional Sanger technology with the new 454 technology. The CLUSTAL W was used to align the obtained nucleotide and amino acid sequences with each other and with sample sequences from Genbank. Then, a phylogenetic tree was generated using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993); the evolutionary analyses were carried out in MEGA X (Kumar et al., 2018).

Statistical analysis

The semen evaluation and a number of service/conception values were expressed as means ± standard error for all variants using one-way ANOVA followed by Bonferroni multiple comparison tests to determine the significance of differences, which was set at p value ≤ 0.05

Table 1. The oligonucleotide primers and amplification conditions used in bovine viral diarrhea virus detection and typing

Primer name	Primer sequence	PCR Conditions	Amplicon size (bp)	Reference
190-F V326	5'-GRAGTCGTCARTGGTTCGAC-3' 5'-TCAACTCCATGTGCCATGTAC-3'	95°C for 5 min and 35 cycles of 45 sec at 95°C, 45 sec at 58°C, 45 sec at 72°C and final extension for 10 min at 72°C	208	Hoffmann et al. (2006)
P1-F P2-R	5'-AACAAACATGGTTGGTGCAACTGGT-3' 5'- CTTACACAGACATATTTGCCTAGGTTCCA-3'	(First round) 94°C for 5 min and 30 cycles of 60 sec at 94°C, 60 sec at 55°C, 60 sec at 72°C and final extension for 10 min at 72°C	826	Sullivan and Akkina (1995)
TS1-F TS2-F TS3-F P2-R	5'-TATATTATTTGGAGACAGTGAATGTAGTAGCT-3' 5'- TGGTTAGGGAAGCAATTAGG-3' 5'- GGGGGTCACTTGTCGGAGG-3' 5'- CTTACACAGACATATTTGCCTAGGTTCCA-3'	(Nested amplification) 95°C for 5 min and 35 cycles of 60 sec at 94°C, 45 sec at 55°C, 45 sec at 72°C and final extension for 10 min at 72°C	(TS1-F, P2) 566 (TS2-F, P2) 448 (TS3-F, P2) 223	

RESULTS

Services/conception and conception rates of *bovine viral diarrhea virus* infected farms

Based on the collected data, cows fertilized with semen from BVDV-infected bulls showed a significantly ($p \leq 0.05$) lower conception rate with a mean percentage of 24.42% ± 0.98% (53/216) and 6.12 ± 0.53 service per conception, compared to those bred with BVDV-free semen, for which the respective values were 60.74% ± 2.04% (95/158) and 2.67 ± 0.75.

Assessment of sperm abnormalities

Microscopical examination of the stained spermatozoa revealed a highly damaged plasma membrane (53-59.67%) and elevated acrosomal defect (27.66-36%). The mean and standard deviation for acrosomal defect and plasma membrane integrity for each bull were calculated and are shown in Table 2.

Histopathological findings and ultrastructure of spermatozoa

Grossly, the examined testes and epididymis were slightly congested and had a small size (atrophy). The pathological changes observed in the testicular sections were characteristic of chronic orchitis with moderate to severe degeneration, Orchitis represented by moderate infiltration of mononuclear inflammatory cells (mostly lymphocytes and/or plasma cells and few histiocytes) in the interstitial tissues along with oedema and congestion of blood vessels (Figure 1 A). Also, many giant multinucleated cells of eosinophilic cytoplasm were seen in seminiferous tubules (Figure 1 B), while those observed in the epididymal sections were characteristic of chronic epididymitis with mild to moderate degeneration, some ductal tubules showing homogenous eosinophilic masses of lysis epididymal spermatozoa in lumen and absence of spermatozoa in the lumen of other tubules. Moreover, hyperplasia of the lining epithelial cells of the tubules was seen in more than one columnar cell layer as shown in Figure 2. Transmission electron microscopy images of the sperm's sagittal sections indicated the presence of swollen plasma membranes with segmented outer acrosomal membranes. The nucleus content was not homogenous in terms of electron density. Transverse sections of the neck region showed vacuolar degenerated mitochondria with electron-translucent spaces and absence of mitochondrial cristae (Figures 3 A and B).

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Table 2. Evaluation of sperm abnormalities in bulls infected with Bovine viral diarrhea virus

Bull number (Governorate and Year)	Acrosomal defect (%)	Plasma membrane integrity (%)
1 (Kafr-El Sheik, 2019)	36.00 ± 3.61	54.00 ± 4.36
2 (Beni-Suef, 2019)	31.67 ± 3.53	59.67 ± 5.61
3 (Giza, 2019)	27.66 ± 4.41	53.00 ± 6.09
4 (Assuit, 2020)	32.00 ± 1.73	54.67 ± 5.51

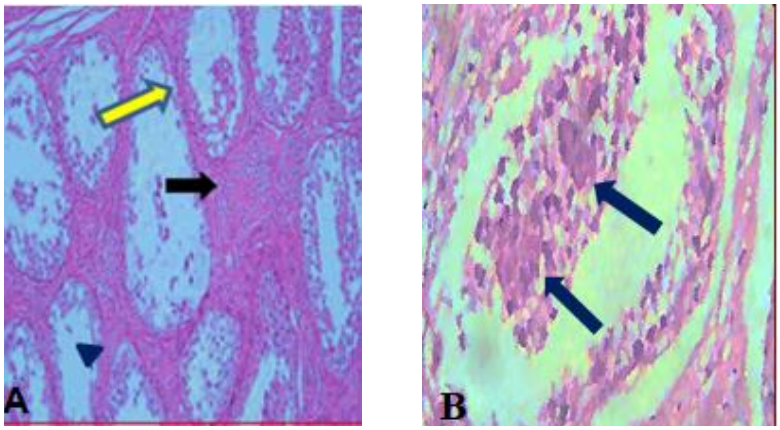

Figure 1. Histopathological alterations associated with Bovine viral diarrhea virus infection in bull testis. **A:** Severe degeneration with thickened basement membranes of seminiferous tubules (yellow arrow) and hypospermatogenesis (blue triangle). Also, mononuclear inflammatory cells infiltration (mainly lymphocyte and plasma cells) with congested blood vessels and oedema (black arrow) in interstitial tissues were seen. (H&E, X100). **B:** Giant cells of multinucleated eosinophilic cytoplasm in seminiferous tubules, indicated by the arrows (H&E, X400).

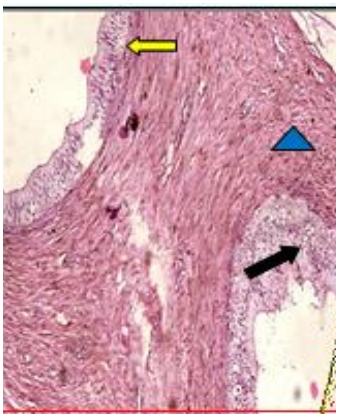

Figure 2. Histopathological alterations associated with Bovine viral diarrhea virus infection in bull epididymis. The epididymis affected by Bovine viral diarrhea virus infection showing moderate vacuolar degenerated tubules (yellow arrow) with cellular debris and reduced sperm in lumen, and epithelial cell hyperplasia in many tubules (black arrow). Lymphocytic inflammatory infiltration (blue triangle) in interstitial tissues is also visible (H&E, X100).

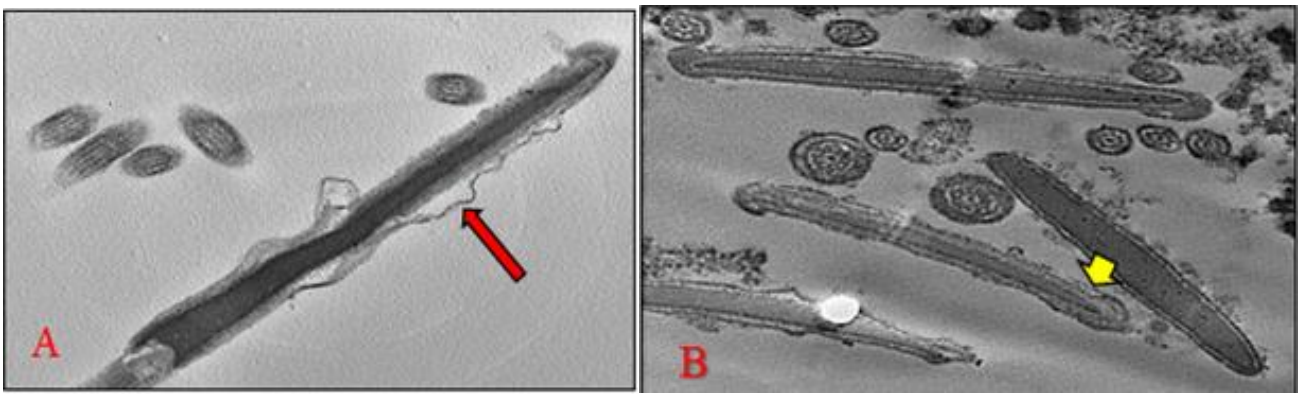

Figure 3. The sagittal section of the sperm head from bull semen under transmission electron microscopy **A:** sperm head sagittal section shows swollen plasma membrane (red arrow). **B:** The segmented outer acrosomal membrane (yellow arrowhead); the nucleus content is not homogenous in terms of electron density. The transverse sections of the neck region show vacuolar degenerated mitochondria with electron-translucent spaces and the absence of mitochondrial cristae (X15000 and X12000).

Bovine viral diarrhea virus detection

ELISA

Using a double-sandwich ELISA, all serum samples (at the time of collection and three weeks later) tested positive for the presence of BVDV antigen. The optical density obtained was 15% greater than the cut-off value considered positive according to the kits used.

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Virus isolation

After three successive serial passages, no specific CPE was detected for BVDV on MDBK cells inoculated with previously prepared semen samples and testicular tissues.

Fluorescent antibody techniques

Perinuclear and intracytoplasmic fluorescence granules were detected in the MDBK cells inoculated with the four semen samples and testicular one after the third passage (Figure 4).

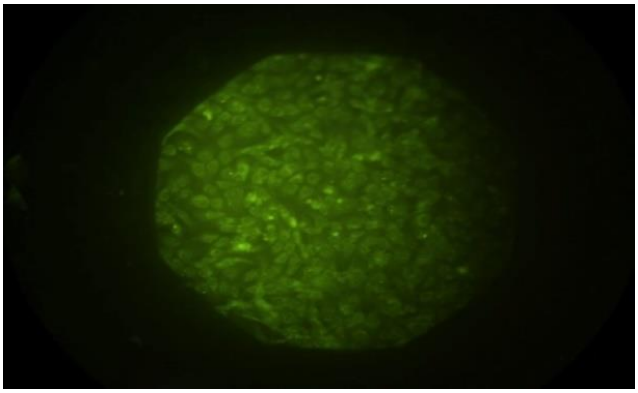

Figure 4. Identification of Bovine viral diarrhea virus isolated from bull semen using FAT. Bovine viral diarrhea virus-free MDBK cells inoculated with the third cell culture passage of the samples, Perinuclear intracytoplasmic fluorescence granules were observed (X100).

Detection of bovine viral diarrhea virus genome

5' untranslated genomic region amplification

As an additional confirmation of the isolated virus, amplicons with the size of 208 bp were clearly detected as a result of the 5'UTR amplification of the four isolates (Figure 5).

Nested multiplex Reverse Transcription -PCR within the E^{rns} glycoprotein region

The nested PCR products showed specific size for BVDV-1 at 223 bp. Specific fragments for genotype BVDV-2 and BDV were not detected with a predicted size of 448 and 566 bp, respectively (Figure 6).

Partial E^{rns} glycoprotein region sequencing and phylogenetic analysis for virus subgenotyping

The sequences of the E^{rns}-amplified fragments of the four isolates (B2, C2, F2, and G2), as well as the reference one (E2), were submitted to Genbank with the following accession numbers: LC634512 (strain ID Sakha-ARRI-eg2019), LC634513 (strain ID Suef-ARRI-eg2019), LC634515 (strain ID Giza-ARRI-eg2019, LC634516 (strain ID Assuit-ARRI-eg2020), and LC634514 (NADL reference strain). Nucleotide sequences were aligned with each other to determine sequence identity among the isolated strains (figures 7 and 8), and with the corresponding sequences of BVDV-1 strains in Genbank. The identity percentage among the Sakha-ARRI-eg2019, Suef-ARRI-eg2019, and Giza-ARRI-eg2019 strains was 100% in the E^{rns} glycoprotein region, and the phylogenetic relationship revealed clustering of these strains with BVDV-1b (showing the highest sequence identity of 98%) and noncytopathic BVDV-1 strains originally isolated from alpaca, in the USA in 2015 (accession nos. JX297515.1, JX297512.1, JX297520.1, JX306012.1, JX297521.1, and JX297519.1). The Assuit-ARRI-eg2020 strain was found to be different from the other isolates with an identity percentage of 90% and phylogenetic analysis confirmed the clustering of this strain with the BVDV-1d subgenotype. It shared the highest homology of 98% with the BVDV-1d LV01-2018 strain (MG923683.1) originally isolated from cattle in Brazil, and with three other BVDV-1d strains (MF166858.1, KT951841.1, and KT943518.1) isolated from yak and cow in China (Figure 9).

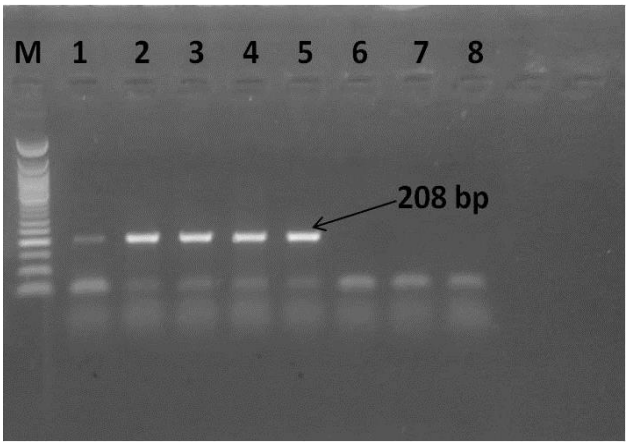

Figure 5. Reverse transcription-PCR within the 5' UTR for detection of Bovine viral diarrhea virus in bull semen. Agarose gel (1.5%) analysis of RT-PCR products for genomic detection within 5' UTR. Lane M represents the 50 bp DNA ladder, lane 1 is a positive control (NADL strain), lanes 2–5 show the amplified products of the novel isolates (208 bp), lanes 6 and 7 are negative controls, and lane 8 is a non-target control.

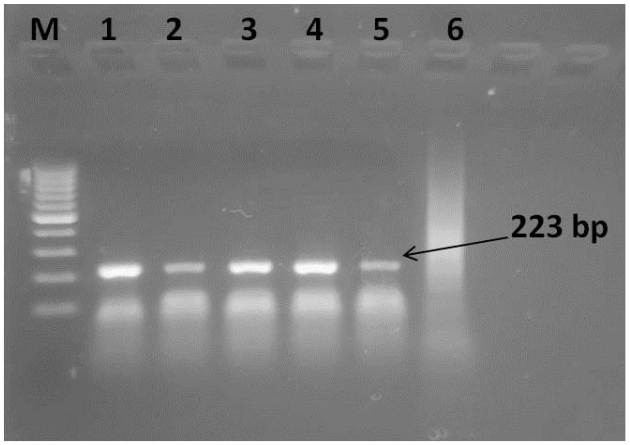

Figure 6. Nested multiplex RT-PCR within the E^{rns} region for typing of BVDV isolated from bull semen. Agarose gel (1.5%) analysis of nRT-PCR products with type-specific primers. Lane M represents the 100 bp DNA ladder, lane 1 is a positive control (NADL strain as genotype 1), lanes 2–5 are the amplified products of the novel isolates (223 bp; genotype 1), and lane 6 is a negative control.

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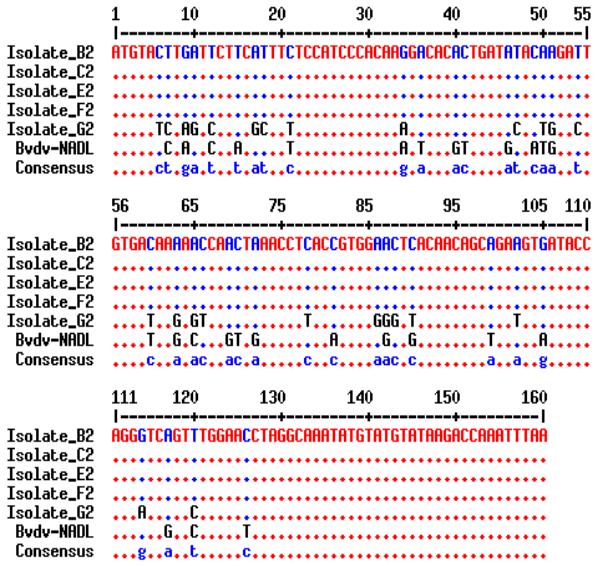

Figure 7. Multiple sequence alignment of nucleotide sequences of polyproteins from Bovine viral diarrhea virus isolates and NADL strains.

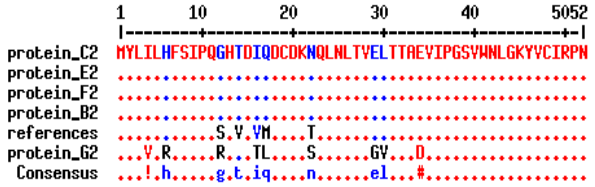

Figure 8. Multiple sequence alignment of predicted amino acid sequences of polyproteins from Bovine viral diarrhea virus isolates and NADL strains.

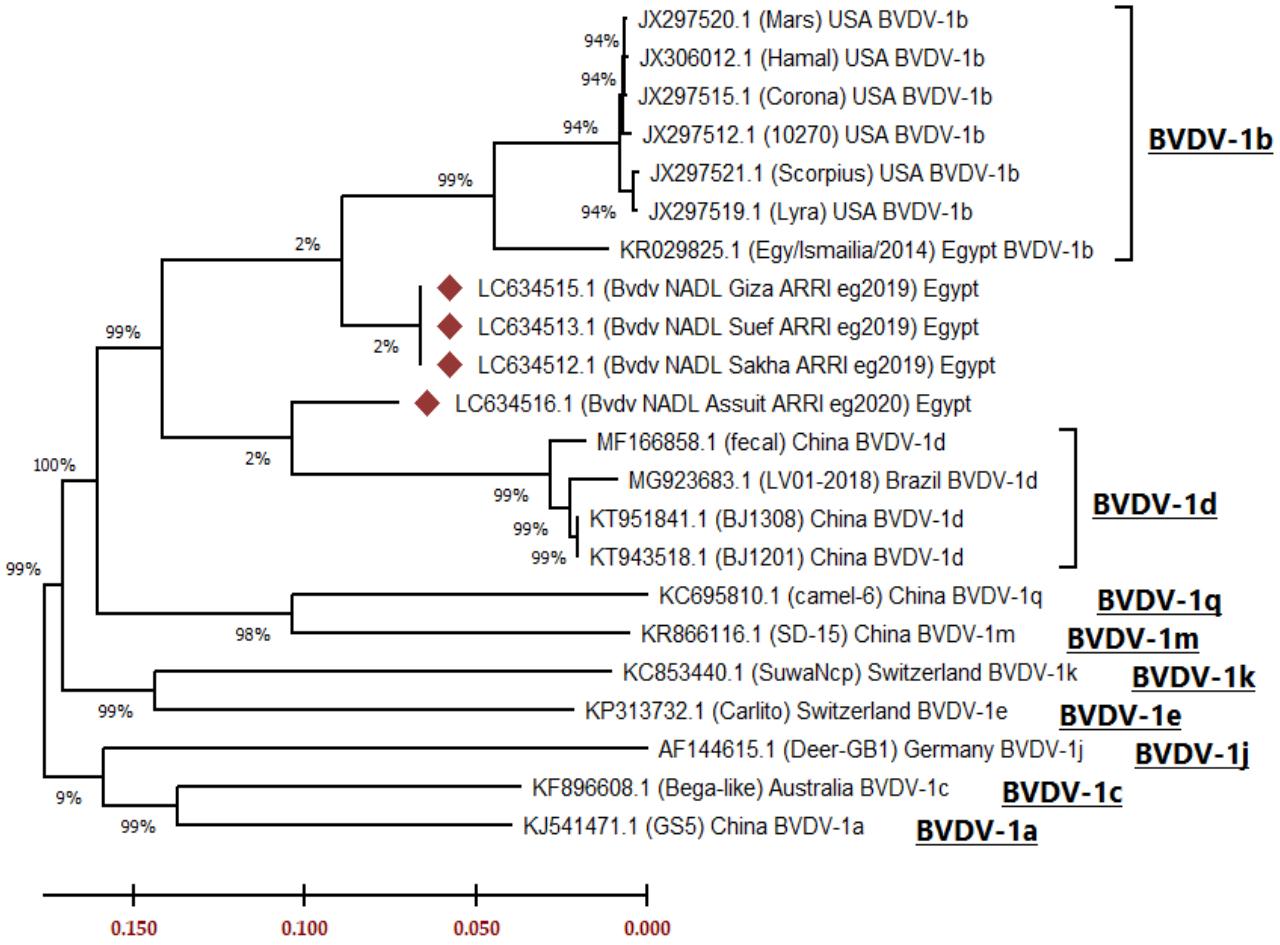

Figure 9. Phylogenetic analysis of Bovine viral diarrhea virus strains isolated from bull semen Phylogenetic tree was constructed using the Maximum Likelihood method and Tamura-Nei model with 1000 bootstrap replicates in MEGA X. Sequencing was conducted on the second step nested multiplex PCR positive samples to confirm the Bovine viral diarrhea virus genotype based on the partial E^{rns} gene. The accession number, isolate name, and country are listed at the beginning of each isolate. The horizontal bar indicates the substitutions per site, and the vertical bars and letters indicate different BVDV-1 subgenotypes. Isolates identified in this study are marked by the red diamond symbol.

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DISCUSSION

It is widely known that maintaining animal health is crucial for improving livestock performance. Among a variety of diseases, BVDV is a difficult challenge for any cattle producer. Understanding the pathogenesis of this virus represents a powerful weapon against the threat of infection.

Persistently infected animals are usually virus carriers for the rest of their lives, shedding substantial amounts of the virus through most of their body excretions and secretions (Alenius et al., 1997; Garoussi et al., 2019). In particular, PI bulls were shown to regularly shed BVDV-contaminated semen of significantly reduced quality (El-Mohamady et al., 2020). In the present study, the assessment of semen collected from breeding bulls, either by direct microscopic examination or TEM, revealed damage of the sperm plasma membrane with high acrosomal defect (Table 2), which was consequently reflected in low conception rates. As a result, BVDV infection has the potential to affect testicular function as well as producing sperm abnormalities. This could have an impact on cows' conception rates and fertility after natural breeding or artificial insemination. Moreover, the semen of these infected bulls could be a source of infection for receptive cows (Oguejiofor et al., 2019; Montoya-Monsalve et al., 2021).

A consistent finding in PI bulls is the very high titer of the virus in the semen, usually at least 100 times higher than in the blood, which may lead to replication of the virus within the male genital tract, for example, in the prostatic and vesicular glands, and epididymides (Selim et al., 2018). In previous studies, the BVDV antigen was detected in the media of arterial walls in the male genital organs. The BVDV antigen was also identified in the testicular Sertoli cells and to a limited extent, in the intact spermatogonia, but not in Leydig cells (Barlow et al., 1986; Brodersen, 2004; Borel et al., 2007). Moreover, Givens et al. (2003) reported that BVDV antigen was found within the seminiferous tubules near the basement membrane. The above-mentioned studies reported reproductive tract lesions, such as testicular hypoplasia and (to a greater or lesser extent) degeneration of seminiferous tubules. This came in accordance with findings of the current study in the testicular and epididymal tissues which presented different degrees of degenerative changes.

Novel pestivirus isolation and characterization will have far-reaching implications for epidemiology and vaccine development research. Since various genotypes of pestiviruses can be identified in different hosts, diagnostic tests that can discriminate between known pestivirus genotypes are required (Booth et al., 2013). Differences among BVDV strains can be assessed using a variety of approaches, including monoclonal antibody reactions, cross-neutralization assays, and nucleotide sequence comparisons (Yeşilbağ et al., 2017). In the present study, the bulls were identified as being infected with BVDV by the anti-p80/p125 monoclonal antibody-coated double sandwich (DAS) ELISA in serum samples. The BVDV p80 antibody is a nonstructural protein (NS3) that can be identified 2 to 3 weeks after natural infection (Sayers et al., 2015). Antibodies against the NS2-3 BVDV were detected in dairy cattle that had a history of reproductive problems and did not receive the BVDV vaccine (Curti and Jaeger, 2013). This indicated that dairy cattle were exposed to the circulating BVDV because they had not been vaccinated (Wuryastuti et al., 2016).

The virus isolated from semen samples was identified by FAT (Figure 4) and RT-PCR amplification within the 5' UTR (Figure 5). Furthermore, using primers that are homologous to the conserved E^{rns} part of the pestivirus genomic sequences ensured that all strains were amplified (Sullivan and Akinna, 1995). The E^{rns} genomic region nested multiplex PCR is able to differentiate the BVDV-1, BVDV-2, and BDV genotypes during the initial screening of the BVDV-infected samples.

The specificity of this assay is given by the capability of the type-specific primers (TS1, TS2, and TS3) to identify genomic sequences specific to each corresponding pestivirus genotype within the first PCR product amplified by the P1 and P2 primers (Sullivan and Akkina, 1995) In the present study, no cross-reactivity between type-specific primers and heterogeneous pestivirus genotypes was found, only one amplified product was produced. Here, BVDV-1 was the detected genotype in the tested field samples (Figure 7). Based on this result and previous research findings, it is concluded that the worldwide spread of BVDV-1 isolates (88.2%) is substantially wider than the distribution of BVDV-2 isolates (11.8%, Soltan et al., 2015a; Yeşilbağ et al., 2017).

Phylogenetic analysis of partial and whole genomic sequences provides more precise information than antibody-based investigations and allows the quick detection and differentiation of BVDV-1 and BVDV-2 subgenotypes, in addition to the recognition of new subgenotypes. Different genomic areas have been used to genotype and classify BVDV and other pestiviruses. The 5'UTR is a highly conserved region of the viral genome, equipped with an internal ribosome entry site that is primarily involved in the translation of viral polyproteins. Partial 5'UTR sequences have been commonly employed for phylogenetic studies and genotyping of BVDV isolates. However, their limited sequence length and lack of diversity are regarded as the primary drawbacks of their use for phylogenetic analysis (Yeşilbağ et al., 2017). The E^{rns} polyprotein region presents intracellular ribonuclease activity that prevents the production of type I interferons and facilitates the establishment of persistent infection (Shirato et al., 2004; Li et al., 2008). The phylogenetic analysis conducted in the present study, which was based on the E^{rns} glycoprotein region, segregated the isolated BVDV strains into two subgenotypes. Three isolates (Sakha-ARRI-eg2019, Suef-ARRI-eg2019, and Giza-ARRI-eg 2019), with 100% identity, were identified as belonging to the BVDV-1b subgenotype, suggesting the spread of an identical strain in the

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corresponding governorates (Kafr el Sheikh, Beni-Suef, and Giza). The other isolate (Assuit-ARRI-eg2020) was identified as BVDV-1d subgenotype, and this was the first study to report the existence of this subgenotype in Egypt. Previously, the BVDV-1a strain had been detected in Egyptian cattle (El-Kholy et al., 2005) and BVDV-1b in Egyptian goats (Abdel-Latif et al., 2013), dairy cows, and buffalo farms, and these showed 89.04% homology with the BVDV-1b isolate identified in this study (Soltan et al., 2015a).

According to previously published data, BVDV-1b is the most common subgenotype in the world, followed by BVDV-1a and -1c. At the individual continent level, BVDV-1b is the most common subgenotype in the Americas, Asia, and Europe (Yeşilbağ et al., 2017). In comparison, almost all the Australian field isolates have been identified as BVDV-1c. In Africa, the overall number of the studied viral isolates is comparatively low and does not represent the entire continent, however, BVDV-1a is considered the most frequently detected subgenotype in South Africa, and BVDV-1b is the predominant subgenotype circulating in Egypt (Abdel-Latif et al., 2013; Soltan et al., 2015b; Yeşilbağ et al., 2017).

Currently, at least 21 BVDV-1 subgenotypes are either widely accepted or have recently been proposed. It is increasingly probable that further subgenotypes will be reported due to the very diverse structure of pestivirus genomes (Yeşilbağ et al., 2017). Therefore, the tracking of strains circulating in a particular country is a useful indicator for the planning of successful vaccination programs or accurate diagnostic tests, and it necessitates regular updates.

CONCLUSION

In conclusion, the molecular investigation verified the circulation of BVDV-1b, and BVDV-1d in breeding bulls in Egypt for the first time. The isolated BVDV strains had negative effects on semen quality and conception rate and produced pathological changes in the bulls' reproductive organs. Thus, regular screening and vaccine matching with the currently prevalent BVDV strains are necessary to ensure accurate management programs.

DECLARATIONS

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Authors' contribution

Yasser Gamil Mahmoud Abd El-Hafeiz designed the plan of the study and participated in the manuscript writing. Rania Salah El-Mohamady performed virus isolation and identification, and participated in data analysis and manuscript writing. Tahani Salama Behour performed the molecular work and participated in data analysis and manuscript writing. Ahmed Mohamed Saber Mahmoud Azab performed the histopathological examination. Mohamed Ahmed Assi performed the ultrastructure finding of the sperm. Magdy Ramadan Badr evaluated semen abnormalities. Ragab Abdel Monem Dohreig carried out samples and data collection. Inas Mohamed Gamal and Hany Mohamed Hassan performed the ELISA work. All authors have read and approved the data and final draft of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

Consent to publish

The authors agreed to publish the article.

REFERENCES

Abdel-Latif AO, Goyal SM, Chander Y, Abdel-Moneim AS, Tamam SM, and Madbouly HM (2013). Isolation and molecular characterisation of a pestivirus from goats in Egypt. Acta Veterinaria Hungarica, 61(2): 270-280. DOI: https://www.doi.org/10.1556/AVet.2013.007

Alenius S, Lindberg A, and Larsson B (1997). A national approach to the control of bovine viral diarrhoea virus. In: Edwards S, Paton, Wensvoort G (Editors). Proceeding of the 3rd ESVV symposium on the control of pestivirus infections. Lelystad, The Netherlands, 19- 20 September 1996. DLO Institute for Animal Science and Health, pp. 162-169.

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Al-Kubati AAG, Hussen J, Kandeel M, Al-Mubarak AIA, and Hemida MG (2021). Recent advances on the bovine viral diarrhea virus molecular pathogenesis, immune response, and vaccines development. Frontiers in Veterinary Science, 8: 665128. DOI: https://www.doi.org/10.3389/fvets.2021.665128

Atwa SM, Emad YE, Rizk MA, El-Beskawy M, and Zeidan SM (2019). An epidemiological survey of bovine viral diarrhea infection in calves in Egypt with identification of high prevalence of persistent infected animals. Comparative Clinical Pathology, 28: 447-453. DOI: https://www.doi.org/10.1007/s00580-018-2867-2

Bancroft JD, and Gamble M (2018). Theory and practice of histological techniques. 8th Edition, Churchill Livingstone London, UK, pp. 125-138 and pp. 328-329. Available at: https://www.us.elsevierhealth.com/bancrofts-theory-and-practice-of-histological-techniques-9780702068645.html

Barlow RM, Nettleton PF, Gardiner AC, Greig A, Campbell JR, and Bonn JM (1986). Persistent bovine virus diarrhoea infection in a bull. Veterinary Record, 118(12): 321-324. DOI: https://www.doi.org/10.1136/vr.118.12.321

Bauermann FV, Ridpath JF, Weiblen R, and Flores EF (2013). HoBi-like viruses: An emerging group of pestiviruses. Journal of Veterinary Diagnostic Investigation, 25(1): 6-15. DOI: https://www.doi.org/10.1177/1040638712473103

Boonkusol D, Saikhun K, and Ratanaphumma P (2010). Effect of extender and storage time on motility and ultrastructure of cooled-preserved boar spermatozoa. Kasetsart Journal Natural Science, 44: 582-589. Available at: https://www.li01.tci-thaijo.org/index.php/anres/article/view/244965/167454

Booth RE, Thomas CJ, El-Attar LM, Gunn G, and Brownlie J (2013). A phylogenetic analysis of Bovine Viral Diarrhoea Virus (BVDV) isolates from six different regions of the UK and links to animal movement data. Veterinary Research, 44(1): 43. DOI: https://www.doi.org/10.1186/1297-9716-44-43

Borel N, Janett F, Teankum K, Zlinszky K, Iteny C, and Hilbe M (2007). Testicular hypoplasia in a bull persistently infected with bovine diarrhoea virus. Journal of Comparative Pathology, 137: 169-173. DOI: https://www.doi.org/10.1016/j.jcpa.2007.06.002

Brodersen BW (2004). Immunohistochemistry used as screening method for persistent bovine viral diarrhoea virus infection. Veterinary Clinics of North America: Food and Animal Practice, 20(1): 85-93. DOI: https://www.doi.org/10.1016/j.cvfa.2003.11.007

Chinoy N, Ranga G, Highland H, D'Souza K, and Sequeira E (1992). A modified method for the differential staining of spermatozoa using alcoholic acidic silver nitrate. International Journal of Fertility, 37(4): 232-236. Available at: https://www.pubmed.ncbi.nlm.nih.gov/1379996

Curti E, and Jaeger J (2013). Residues Arg283, Arg285, and Ile287 in the nucleotide binding pocket of bovine viral diarrhea virus NS5B RNA polymerase affect catalysis and fidelity. Journal of Virology, 87(1): 199-207. DOI: https://www.doi.org/10.1128/JVI.06968-11

El-Bagoury GF, El-Nahas EM, El-Deen SSS, and Salem SAH (2014). Detection and genotyping of bovine viral diarrhea virus in cattle sera. Benha Veterinary Medical Journal, 27: 348-353. Available at: https://www.bvmj.bu.edu.eg/issues/27-2/33.pdf

El-Kholy AA, Vilcek S, and Daoud AM (2005). Phylogenetic characterization of some bovine viral diarrhea viruses in Egypt. Egyptian Journal of Virology, 1: 421-435. Available at: https://scialert.net/abstract/?doi=ijv.2005.41.41

El-Mohamady RS, Behour TS, and Rawash ZM (2020). Concurrent detection of bovine viral diarrhoea virus and bovine herpesvirus-1 in bulls' semen and their effect on semen quality. Interntional Journal of Veterinary Science and Medicine, 8(1): 106-114. DOI: https://www.doi.org/10.1080/23144599.2020.1850197

Garoussi MT, Mehrzad J, and Nejati A (2019). Investigation of persistent infection of bovine viral diarrhoea virus (BVDV) in Holstein dairy cows. Tropical Animal Health and Production, 51(4): 853-858. DOI: https://www.doi.org/10.1007/s11250-018-1765-6

Givens MD, Heath AM, Carson RL, Brock KV, Edens MSD, Wenzel JGW, and Stringfellow DA (2003). Analytical sensitivity of assays used for detection of bovine viral diarrhoea virus in semen samples from the Southeastern States. Veterinary Microbiology, 96(2):145-55. DOI: https://www.doi.org/10.1016/s0378-1135(03)00213-x

Hoffmann B, Depner K, Schirrmeier H, and Beer M (2006). A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. Journal of Virological Methods, 136: 200-209. DOI: https://www.doi.org/10.1016/j.jviromet.2006.05.020

Hou P, Zhao G, Wang H, and He H (2019). Prevalence of bovine viral diarrhea virus in dairy cattle herds in eastern China. Tropical Animal Health Production, 51(4): 791-798. DOI: https://www.doi.org/10.1007/s11250-018-1751-z

Kumar S, Stecher G, Li M, Knyaz C, and Tamura K (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35: 1547-1549. Available at: https://pubmed.ncbi.nlm.nih.gov/29722887/

Li L, Lok SM, Yu IM, Zhang Y, Kuhn RJ, Chen J, and Rossmann MG (2008). The flavivirus precursor membrane-envelope protein complex: Structure and maturation. Science, 319(5871): 1830-1834. DOI: https://www.doi.org/10.1126/science.1153263

Montoya-Monsalve G, Sánchez-Calabuig MJ, Blanco-Murcia J, Elvira L, Gutiérrez-Adán A, and Ramos-Ibeas P (2021). Impact of overuse and sexually transmitted infections on seminal parameters of extensively managed bulls. Animals (Basel), 11(3): 827. DOI: https://www.doi.org/10.3390/ani11030827

Neill JD (2013). Molecular biology of bovine viral diarrhea virus. Biologicals, 41(1): 2-7. DOI: https://www.doi.org/10.1016/j.biologicals.2012.07.002

Newcomer BW, Toohey-Kurth K, Zhang Y, Brodersen BW, Marley MS, Joiner KS, Zhang Y, Galik PK, Riddell KP, and Givens MD (2014). Laboratory diagnosis and transmissibility of bovine viral diarrhea virus from a bull with a persistent testicular infection. Veterinary Microbiology, 170: 246-257. DOI: https://www.doi.org/10.1016/j.vetmic.2014.02.028

Oguejiofor CF, Thomas C, Cheng Z, and Wathes DC (2019). Mechanisms linking bovine viral diarrhea virus (BVDV) infection with infertility in cattle. Animal Health Research Reviews, 20(1): 72-85. DOI: https://www.doi.org/10.1017/S1466252319000057

Revell S, and Mrode R (1994). An osmotic resistance test for bovine semen. Animal Reproduction Science, 36: 77-86. DOI: https://www.doi.org/10.1016/0378-4320(94)90055-8

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Ridpath JF, Bolin SR, and Dubovi EJ (1994). Segregation of bovine viral diarrhea virus into genotypes. Virology, 205(1): 66-74. DOI: https://www.doi.org/10.1006/viro.1994.1620

Sayers RG, Sayers GP, Graham DA, and Arkins S (2015). Impact of three inactivated bovine viral diarrhoea virus vaccines on bulk milk p80 (NS3) ELISA test results in dairy herds. Veterinary Journal, 205(1): 56-61. DOI: https://www.doi.org/10.1016/j.tvjl.2015.03.025

Scharnböck B, Roch FF, Richter V, Funke C, Firth CL, Obritzhauser W, Baumgartner W, Käsbohrer A, aund Pinior B (2018). A meta-analysis of bovine viral diarrhoea virus (BVDV) prevalences in the global cattle population. Scientific Reports, 8(1): 14420. DOI: https://www.doi.org/10.1038/s41598-018-32831-2

Selim AM, Elhaig MM, Moawed SA, and El-Nahas E (2018). Modeling the potential risk factors of bovine viral diarrhea prevalence in Egypt using univariable and multivariable logistic regression analyses. Veterinary World, 11(3): 259-267. DOI: https://www.doi.org/10.14202/vetworld.2018.259-267

Shirato K, Miyoshi H, Goto A, Ako Y, Ueki T, Kariwa H, and Takashima I (2004). Viral envelope protein glycosylation is a molecular determinant of the neuroinvasiveness of the New York strain of West Nile virus. Journal of General Virology, 85(Pt12): 3637-3645. DOI: https://www.doi.org/10.1099/vir.0.80247-0

Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, Muerhoff S, Pletnev A, Rico-Hesse R, and Smith DB (2017). ICTV virus taxonomy profile: Flaviviridae. Journal of General Virology, 98(1): 2-3. DOI: https://www.doi.org/10.1099/jgv.0.000672

Soltan MA, Wilkes RP, Elsheery MN, Elhaig MM, Riley MC, and Kennedy MA (2015a). Complete genome sequence of bovine viral diarrhea Virus-1 strain Egy/Ismailia/2014, Subtype 1b. Genome Announcements, 3(6): e01518-15. DOI: https://www.doi.org/10.1128/genomeA.01518-15

Soltan MA, Wilkes RP, Elsheery MN, Elhaig MM, Riley MC, and Kennedy MA (2015b). Circulation of bovine viral diarrhea virus--1 (BVDV-1) in dairy cattle and buffalo farms in Ismailia Province, Egypt. Journal of Infection in Developing Countries, 9(12): 1331-1337. DOI: https://www.doi.org/10.3855/jidc.7259

Stott AW, Humphry RW, Gunn GJ, Higgins I, Hennessy T, O'Flaherty J, and Graham DA (2012). Predicted costs and benefits of eradicating BVDV from Ireland. Irish Veterinary Journal, 65(1): 12. DOI: https://www.doi.org/10.1186/2046-0481-65-12

Sullivan DG, and Akkina RK (1995). A nested polymerase chain reaction assay to differentiate pestiviruses. Virus Research, 38: 231-239. DOI: https://www.doi.org/10.1016/0168-1702(95)00065-x

Tamura K, and Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution, 10: 512-526. DOI: https://www.doi.org/10.1093/oxfordjournals.molbev.a040023

Tautz N, Tews BA, and Meyers G (2015). The molecular biology of pestiviruses. Advances in Virus Research, 93: 47-160. DOI: https://www.doi.org/10.1016/bs.aivir.2015.03.002

Velasova M, Damaso A, Prakashbabu BC, Gibbons J, Wheelhouse N, Longbottom D, Van Winden S, Green M, and Guitian J (2017). Herd-level prevalence of selected endemic infectious diseases of dairy cows in Great Britain. Journal Dairy Science, 100(11): 9215-9233. DOI: https://www.doi.org/10.3168/jds.2016-11863

Vilcek S, Paton DJ, Durkovic B, Strojny L, Ibata G, Moussa A, Loitsch A, Rossmanith W, Vega S, Scicluna MT et al. (2001). Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. Archives of Virology, 146(1): 99-115. DOI: https://www.doi.org/10.1007/s007050170194

World Organization for Animal Health (Office International des Epizooties: OIE) (2018). Manual of diagnostic tests and vaccines for terrestrial animals. 8th edition. chapter 3.4.11. Paris: OIE, pp. 1139-1157. Available at: https://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/3.04.07_BVD.pdf

Wuryastuti H, Putro PP, and Wasito R (2016). Prevalence of antibody to bovine viral diarrhea field virus in dairy cattle in central Java, Indonesia. Proceeding of 19th FAVA Congress. Ho Chi Minh City, Vietnam, pp. 194-195. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6702556/pdf/Vetworld-12-1108.pdf

Yeşilbağ K, Alpay G, and Becher P (2017). Variability and global distribution of subgenotypes of bovine viral diarrhea virus. Viruses, 9(6): 128. DOI: https://www.doi.org/10.3390/v9060128

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Growth, Laying, and Survival Rates of the *Galba truncatula* Snails Infected with *Fasciola hepatica*

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ABSTRACT

Fasciolosis is one of the most important parasitic diseases in ruminants in Algeria, of which the intermediate host is *Galba truncatula* (*G. truncatula*) snail. The current study aimed to investigate the prevalence of *Fasciola* sp. in naturally infected *G. truncatula* snails using multiplex PCR. Secondly, it was targeted toward examining the rate of growth, survival, and laying of the snails in experimental conditions during 6 weeks of rearing in three tanks. This study was conducted in two different regions of Algeria, namely El Tarf and Constantine. The investigated tanks 1, 2, and 3 consisted of 12 (size 3-4 mm), 30 (size 5-6 mm), and 30 (size 7-8 mm) snails, respectively. *Fasciola* sp. DNA was detected in 33.33% of *G. truncatula* snails (25% in Constantine and 42.85% in El Tarf). The total survival rates in the first, second, and third tanks were 50%, 43.3%, and 40%, respectively. The obtained results indicated that the growth rate of the snail depended on its initial size (the smaller the initial size, the higher the weekly growth rate). The total growth rates were 3, 1.7, and 1.1 mm in tanks 1, 2, and 3, respectively. The use of multiplex PCR indicated a relatively high level of infestation of the snails by *Fasciola* sp. Snails larger than 7 mm had the highest lay rate. Further studies are needed to investigate other snails that may be infested with *Fasciola* sp.

Keywords: *Fasciola*, *Galba truncatula*, PCR multiplex, Snail

INTRODUCTION

Fasciola hepatica (*F. hepatica*, Linné 1758) is a zoonotic parasitic disease that affects mainly ruminants (Esteban et al., 2003). It has a complex life cycle with snails (Lymnaeidae) as intermediate hosts and ungulate mammals as final hosts (Špakulová et al., 2003). Fascioliasis represents a major economic problem by decreasing the productivity performance of animals due to the condemnation of affected organs (Reinaldo Gonzalez et al., 2002; Chauvin et al., 2007).

In Algeria, fasciolosis is one of the most important helminthic parasites of ruminants (Ouchene-Khelifi et al., 2018). In a study conducted in El Tarf, northeastern Algeria, the prevalence of fascioliasis infection was revealed at 26.7 ± 2.5%, 6.5 ± 0.4%, and 2.5 ± 0.2% in cattle, sheep, and goats, respectively (Ouchene-Khelifi et al., 2018). In Jijel, northern Algeria, the prevalence of fasciolosis was reported at 27.0% and 18.2% in cattle and sheep, respectively (Mekroud et al., 2004). The economic losses related to liver condemnation are very high and estimated at 8.2 million euros in Belgium (Charlier et al., 2009), 52 million euros in Switzerland (Schweizer et al., 2005), and 10000-60000 euros in Algeria (Mekroud et al., 2004; Ouchene-Khelifi et al., 2018). *Galba truncatula* (*G. truncatula)* is a lymnaeid snail intermediate host of *F. hepatica* (Torgerson and Claxton, 1999; Mekroud et al., 2002; Righi et al., 2016). It is a widespread species found mainly in periodically inundated sandy-muddy habitats as well as in running water (Vignoles et al., 2010; Rondelaud et al, 2014; Vignoles et al., 2017).

Detection of *Fasciola* sp. in snails is possible by microscopy or molecular techniques (Caron et al., 2014). Microscopic methods are fundamentally based on crushing, dissection of the snail, and/or cercarial excretion. Molecular detection can be efficient in screening naturally infected snails. Molecular analysis is done by using an internal control (multiplex PCR) to exclude the possibility of false-negative results (Caron et al., 2011; Caron et al., 2014).

In Algeria, there is a dearth of published data on the molecular prevalence of *Fasciola* sp. in *G. truncatula* snails. The aims of the present study were first to investigate the molecular prevalence of *Fasciola* sp. in naturally infected *G. truncatula* snails using multiplex PCR, and secondary, to study snails' growth and laying survival rates in El Tarf and Constantine, Algeria.

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MATERIALS AND METHODS

Ethical approval

The study was conducted based on the ethical rules of the Institute of Veterinary Sciences of Constantine University, Algeria.

Study region

The study was conducted in Algeria in two different regions of El Tarf and Constantine. El Tarf is characterized by clayey soil with moderate permeability, a humid and warm Mediterranean climate, and an average temperature of 19.6°C varying generally from 7°C to 32°C. The annual average rainfall is 550 mm. Constantine is characterized by a warm temperate climate. The average temperature is 15.6°C (ranges from 2°C to 34°C) and the average annual rainfall is 469 mm.

Molecular detection of *Fasciola hepatica* in *Galba truncatula* snails using multiplex PCR

A total of 32 snails were collected from typical biotopes on a perimeter of 1 m^2 in each biotope according to the recommendations of Mekoud et al. (2002). The snails were rinsed with water and then transported to the laboratory for molecular analysis. The size of all collected snails was measured before being analyzed by multiplex PCR. Of the 32 snails, 12 (mean size 4.3 mm) were collected from Constantine, and 20 (mean size 5.73 mm) were collected from El Tarf.

DNA extraction

Chelex® method was used for DNA extraction (Caron et al., 2011). Initially, the snails were disrupted by a pellet mixer (Trefflab) in 100 µl of Chelex® 5% (Biorad, Nazareth Eke, Belgium), then incubated for 1 hour at 56°C and 30 minutes at 95°C in a Peltier Thermal Cycler. The mixture was centrifuged (OHAUS Europe GmbH, Switzerland) at 13000 × g for 7 minutes. A spectrophotometer (Thermo Scientific, NanoDrop 1000) was used to measure DNA concentration and purity. The supernatant was collected and stored at -20°C until further analyses.

Multiplex PCR

The multiplex PCR was used according to a study by Caron et al. (2011) to amplify a highly repeated 124 bp sequence specific for *Fasciola* sp. (Kaplan et al., 1995) and ITS-2 rDNA sequence specific for lymnaeids (500–600 bp) (Bargues et al., 2001). For the amplification of *Fasciola* sp. sequences, the used primers were Fsh1 5′-GAT-CAA-TTC-ACC-CAT-TTC-CGT-TAG-TC C-TAC-3′ and Fsh2 5′-AAA-CTG-GGC-TTA-AAC-G GC-GTC-CTA-CGG-GCA-3′ and for lymnaeids, ITS-2 amplification were News2 5′-TGT-GTC-GAT-GAA-GA A-CGC-AG-3′ and Its2Rixo 5′-TTC-TAT-GCT-TAAATT-CAG-GGG-3′ (Almeyda-Artigas et al., 2000; Bargues et al., 2001).

The sequences were amplified using a commercial kit (Taq PCR Master Mix, Qiagen) in a total volume of 25 µl in a Peltier Thermal Cycler with an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles, each comprising denaturation at 95°C for 1 minute, annealing at 56°C for 1 minute, extension at 72°C for 1 minute and a final extension step at 72°C for 10 minutes. After amplification, the electrophoresis was performed in agarose gel at 2% prepared in TAE buffer with ethidium bromide (myGel InstaView™, USA).

Collection of snails to study their growth, laying, and survival rates

The snails were collected from typical biotopes on a perimeter of 1 m^2 according to the recommendations of Mekroud et al. (2002). The snails were transported to the laboratory in special snail tanks at room temperature within 30 minutes. The allotment of the snails was done based on their size, on three rearing tanks. Tank 1 consisted of 12 snails (size 3-4 mm), tank 2 entailed 30 snails (size 5-6 mm), and tank 3 was filled with 30 snails (size 7-8 mm).

The rearing conditions were artificially ensured (water, oxygenation, lighting, and food) during a 6-week-follow-up period according to standardized *in vitro* rearing procedures (Rondelaud et al., 2002). The rearing tanks were checked daily and cleaned weekly. Food was distributed as needed (macerated salad), and the water level at the temperature of 22-25°C in the tanks was systematically checked (2 cm). The tanks were covered to avoid water evaporation and laying eggs of dipteran flies. Oxygenation of the water was ensured by a bubbler.

The snails were kept in culture for 6 weeks to study their survival rate, the growth rate of the shell, and some reproduction parameters of *G. truncatula*, including average number of embryos/ laying, average number of laying/limnea, and average number of embryos/limnea. The studied parameters and their mode of calculations are presented in Table 1.

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Table 1. Calculation method of the biological parameters studied in snails

Parameters	Expression
Weekly survival rate (relative) of snails in rearing tanks for 6 weeks (%)	(Number of live snails in the tank at the end of the week / Number of live snails at the beginning of the week) × 100 (%)
The total (absolute) survival rate of snails in rearing tanks for 6 weeks (%)	(Number of live slugs in the tank after 6 weeks/ Initial number of slugs put in the tank) x100 (%)
Average weekly growth rate (relative) of snails for 6 weeks	Average weekly snail size - Average snail size the previous week
Average growth rate (absolute) of the snails for 6 weeks	Average snail size in the last week - Average snail size in the first week
Total number of eggs for 6 weeks	Total number of eggs collected in the tank during the whole study period
Total number of embryos for 6 weeks	Total number of embryos counted in all clutches during the study period
Average number of eggs per snail	Average number of eggs collected/number of snails in the tank
Average number of embryos/ laying	Total number of embryos counted / Number of eggs harvested in the tank
Average number of embryos/ snail	Number of embryos counted/number of snails in the tank

RESULTS

Molecular study

The 32 collected snails were identified morphologically as *G. truncatula* and 15 of them were confirmed using multiplex PCR. Of the total snails, 17 (53.12%) were eliminated from the study due to PCR inhibition. The DNA of *Fasciola* sp. was detected in 5/15 snails (33.33%), of which 2/8 snails in Constantine and 3/7 snails in El Tarf, resulting in a prevalence of 25% and 42.85%, respectively.

Survival, growth, and laying rates of snails

The snails were identified morphologically as *G. truncatula*. The survival rates of snails were 61.9-100%, 70-100%, and 70.5-100% in tanks 2, 1, and 3, respectively. At the end of the experiment, the total survival rates were 40%, 43.3%, and 50% in tanks 3, 2, and 1, respectively (Table 2). The hebdomadal growth of the snails during 6 weeks in tanks 1, 2, and 3 were measured as 0-1.3 mm, 0-1.1 mm, and 0-0.6 mm, respectively (Table 3). The total growth of the snails during the study was 3 mm, 1.7 mm, and 1.1 mm in tanks 1, 2, and 3, respectively (Table 3). Regarding the laying rates, tank 1 included 29 eggs with 360 embryos (an average number of 30 embryos per snail). Tank 2 had 100 layings with 1851 embryos (61.7 embryos/snail), and tank 3 entailed 136 eggs with 2707 embryos with an average number of 90.23 embryos per snail (Table 4).

Table 2. Weekly and absolute survival rates of snails in the three tanks

	Tank 1		Tank 2		Tank 3	
Breeding weeks	**Initial number (mortality)**	**Survival rate**	**Initial number (mortality)**	**Survival rate**	**Initial number (mortality)**	**Survival rate**
First week	12 (00)	100%	30 (00)	100%	30 (00)	100%
Second week	12 (02)	83.3%	30 (06)	80%	30 (03)	90%
Third week	10 (03)	70%	24 (03)	87%	27 (05)	81.4%
Fourth week	07 (01)	82.7%	21 (00)	100%	22 (01)	95.4%
Fifth week	06 (00)	100%	21 (08)	61.9%	21 (04)	80.9%
Sixth week	06 (00)	100%	13 (00)	100%	17 (05)	70.5%
Total survival rate	06/12	50%	13/30	43.33%	12/30	40%

Table 3. Weekly and absolute growth of snails (mm/week) in the three tanks

Breeding weeks	Tank 1 (12 Snails)	Tank 2 (30 Snails)	Tank 3 (30 Snails)
First week	3.5 mm (--)	5.5 mm (--)	7.5 mm (--)
Second week	4.8 mm (1.3 mm)	6.6 mm (1.1 mm)	7.5 mm (00)
Third week	5.3 mm (0.5 mm)	6.9 mm (0.3 mm)	8.1 mm (0.6 mm)
Fourth week	6.3 mm (1 mm)	7.1 mm (0.2 mm)	8.2 mm (0.1 mm)
Fifth week	6.3 mm (00)	7.1 mm (00)	8.5 mm (0.3 mm)
Sixth week	6.5 mm (0.2 mm)	7.2 mm (0.1 mm)	8.6 mm (0.1 mm)
Total growth rate	3 mm	1.7 mm	1.1 mm

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Table 4. Characteristics of snail laying in the three tanks during 6 weeks of rearing

Parameters	Tank 1 (12 Snails)	Tank 2 (30 Snails)	Tank 3 (30 Snails)
Number of snails	12	30	30
Total number of laying's	29	100	136
Total number of embryos	360	1851	2707
Average number of laying per snail	2.42	3.33	4.53
Average number of embryos per laying	12.41	18.51	19.90
Average number of embryos per snail	30	61.7	90.23

DISCUSSION

Microscopy or molecular techniques are methods used for the detection of *F. hepatica* in snails (Caron et al., 2008). Molecular tools must be efficient enough to detect naturally infected snails (Caron et al., 2014). Molecular techniques have been used in several studies to investigate the prevalence of *F. hepatica* in experimentally infested snails, but a few studies have tested naturally infected snails (Relf et al., 2009; Kozak and Wedrychowicz, 2010; Martinez-Ibeas et al., 2013). In the current investigation, molecular investigation of *F. hepatica* DNA was performed in naturally infested *G. truncatula* snails.

In the present study, DNA extraction was employed using the Chelex® technique. This technique presents many advantages, including the ability to obtain amplifiable DNA in a rapid time, at a minimal cost, and without using toxic solvents and multiple tube transfers. The multiplex PCR was adapted to eliminate eventual PCR inhibitions (Gonzalez et al., 2004; Caron et al., 2008). However, in the present study, 17 samples of snails showed PCR inhibition. Using the internal control and the apparition of the ITS2 band, the snail parasite was deduced in contrast to other molecular techniques where false negatives cannot be excluded (Gonzalez et al., 2004; Caron et al., 2008).

In the current study, the proportion of PCR inhibition (53.12%) was relatively higher than the findings of Righi et al. (2016, 3.32%) and Caron et al. (2014, 7.89%). The eliminated snails can be contaminated by some PCR inhibitors, such as complex polysaccharides, humic acid, and proteinase (Caron et al., 2014). In the present study *Fasciola* sp. DNA was isolated by multiplex PCR from 33.33% of *G. truncatula* snails. The prevalence of *Fasciola* sp. in the current research was more than the reported prevalence in animals with liver lesions in a slaughterhouse in Algeria (Ouchene-Khelifi et al., 2018). The prevalence rates of animals in the mentioned study were 26.7 ± 2.5%, 6.5 ± 0.4%, and 2.5 ± 0.2% in cattle, sheep, and goats, respectively. Therefore, the infestation level of ruminants at the slaughterhouses did not correspond to the infestation level of snails with *Fasciola* sp. in the pasture.

In Constantine, the molecular prevalence rate of *Fasciola* sp. in snails was 25%, compared to 26.2% in Tunisia (Hammami and Ayadi, 2000; Hammami et al., 2007) and 26.6% in Poland (Kozak and Wedrychowicz, 2010). However, in El Tarf, the prevalence was very high (42.85%). This could be explained firstly by the humid climate of this region which eases the development of snails and facilitates their contamination by *F. Hepatica*. Secondly, the size of the snails in this area which is the largest in the region of El Tarf supports the high prevalence in this region (Rondelaud et al, 2014).

In the present investigation, the survival rates of snails in the three tanks for 6 weeks were relatively high, compared to the results of Righi et al. (2016) in Algeria (average rates vary from 28 to 30%). The differences could be explained by the fact that the collected snails can be contaminated by larvae of some flies that can kill them (Muniz-Pareja and Iturbe-Espinoza, 2018). Moreover, the findings indicated that the survival rate in tank 3 (adult snails) was the lowest (40%), which was in agreement with some other studies where the mortality rate was higher in adult snails than in younger ones (Rondelaud et al., 2009; Righi et al., 2016).

The growth rate of snails depends largely on the environment in which they live, meaning that they grow better in calcareous or siliceous soil (Vignoles et al., 2010; Rondelaud et al, 2014; Vignoles et al., 2017). However, in experimental conditions like the present study, the growth rate was largely dependent on the initial size of the snail. Thus, the smallest snails (3 to 4 mm), recorded the greatest growth (3mm). On the other hand, the larger snails (7-8 mm) recorded a low growth rate (1.1 mm). It seems clear that the smaller snails are growing faster.

The largest number of laying was recorded in tank 3 (136), while in tank 1, only 29 were recorded. This was due to the fact that the snails in tank 3 (over 7 mm) were sexually mature and laid more eggs, which naturally affected all other fertility parameters.

CONCLUSION

The use of multiplex PCR clearly indicated a relatively high level of infestation of the snails by *Fasciola* sp. The survival rate of the snails under the experimental conditions varied between 40 and 50%. The growth rate of the snail

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depended on the initial size of the snail. Snails larger than 7 mm seemed to be the most mature ones and had the highest lay rate. Further studies with a higher number of snails and in other regions of Algeria could be carried out in order to have more information and to investigate other snails that could be infested by *Fasciola* sp.

DECLARATIONS

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Authors' contribution

Fayçal Aimeur, Meriem Mekroud, and Amal Titi conceived and designed the research. Nassim Ouchene and Abdeslam Mekroud analyzed the data, Nadjet Amina Ouchene-Khelifi and Abdeslam Mekroud wrote the manuscript. All authors checked and approved the final version of the manuscript.

Competing interests

There is no conflict of interest.

Consent to publish

All authors approved the final version and agreed to publish the article in the present journal.

Ethical consideration

The authors checked for ethical issues including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

REFERENCES

Almeyda-Artigas RJ, Bargues MD, and Mas-Coma S (2000). ITS-2 rDNA sequencing of *Gnathostoma* species (Nematoda) and elucidation of the species causing human gnathostomiasis in the Americas. Journal of Parasitology, 86(3): 537-544. DOI: https://www.doi.org/10.1645/0022-3395(2000)086[0537:irsogs]2.0.co;2

Bargues MD, Vigo M, Horak P, Dvorak J, Patzner RA, Pointier JP, Jackiewicz M, Meier-Brook C, and Mas-Coma S (2001). European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA ITS-2 sequences. Infection, Genetics, and Evolution, 1(2): 85-107. DOI: https://www.doi.org/10.1016/s1567-1348(01)00019-3

Caron Y, Martens K, Lempereur L, Saegerman C, and Losson B (2014). New insight in lymnaeid snails (Mollusca, Gasteropoda) an intermediate host of *Fasciola hepatica* (Trématoda, digenea) in Belgium and Luxembourg. Parasites and Vectors, 13: 7-66. Available at: https://www.doi.org/10.1186/1756-3305-7-66

Caron Y, Righi S, Lempereur L, Saegerman C, and Losson B (2011). An optimized DNA extraction and multiplex PCR for the detection of *Fasciola* sp. in lymnaeid snails. Veterinary Parasitology, 178: 93-99. DOI: https://www.doi.org/10.1016/j.vetpar.2010.12.020

Caron Y, Rondelaud D, and Losson B (2008). The detection and quantification of a digenean infection in the snail host with special emphasis on *Fasciola* sp. Parasitology Research, 103(4): 735-744. DOI: https://www.doi.org/10.1007/s00436-008-1086-1

Charlier J, De Cat A, Forbes A, and Vercruysse J (2009). Measurement of antibodies to gastrointestinal nematodes and liver fluke in meat juice of beef cattle and associations with carcass parameters. Veterinary Parasitology, 166: 235-240. DOI: https://www.doi.org/10.1016/j.vetpar.2009.09.040

Chauvin A, Zhang W, and Moreau E (2007). Ruminant fasciolosis: immunity, immunomodulation and prevention strategy. Bulletin of the Veterinary Academy of France - Tome 160 - N°2, pp. 85-92. DOI: https://www.doi.org/10.4267/2042/47871

Esteban JG, Gonzalez C, Curtale F, MunozAntoli C, Valero MA, Bargues MD, ElSayed M, El-Wakeel AA, Abdel-Wahab Y, and Montresor A et al. (2003). Hyperendemic fascioliasis associated with schistosomiasis in villages in the Nile Delta of Egypt. American Journal of Tropical Medicine and Hygiene, 69(4): 429-437. DOI: https://www.doi.org/10.4269/ajtmh.2003.69.429

Gonzalez LG, Tapia JR, Lazo PS, Ramos S, and Nieto CS (2004). DNA extraction using chelex resin for oncogenic amplification analysis in head and neck tumours. Acta Otorrinolaringológica Española, 55: 139-144. DOI: https://www.doi.org/10.1016/s0001-6519(04)78497-6

Hammami H, and Ayadi A (2000). Study of the natural infestation of *Lymnaea truncatula* Müller by *Fasciola* hepatica in the oases of Tozeur (southwest Tunisia). Tropical Medicine, 60 (2): 159-162. Available at: https://www.pubmed.ncbi.nlm.nih.gov/11100443/

Hammami H, Hamed N, and Ayadi A (2007). Epidemiological studies on *Fasciola hepatica* in Gafsa oases (South West of Tunisia). Parasite, 14: 261-264. DOI: https://www.doi.org/10.1051/parasite/2007143261

Kaplan RM, Dame JB, Reddy GR, and Courtney CH (1995). A repetitive DNA probe for the sensitive detection of *Fasciola hepatica* infected snails. International Journal for Parasitology, 25(5): 601-610. DOI: https://www.doi.org/10.1016/0020-7519(94)00159-l

Kozak M and Wedrychowicz H (2010). The performance of a PCR assay for field studies on the prevalence of *Fasciola hepatica* infection in *Galba truncatula* intermediate host snails. Veterinary Parasitology, 168: 25-30. DOI: https://www.doi.org/10.1016/j.vetpar.2009.10.014

Martinez-Ibeas AM, Gonzalez-Warleta M, Martinez-Valladares M, CastroHermida JA, Gonzalez-Lanza C, Minambres B, Ferreras C, Mezo M, and MangaGonzalez MY (2013). Development and validation of a mtDNA multiplex PCR for identification and discrimination of *Calicophoron daubneyi* and *Fasciola hepatica* in the *Galba truncatula* snail. Veterinary Parasitology, 195: 57-64. DOI: https://www.doi.org/10.1016/j.vetpar.2012.12.048

Mekroud A, Benakhla A, Vignoles P, Rondelaud D, and Dreyfuss G (2004). Preliminary studies on the prevalences of natural fasciolosis in cattle, sheep, and the host snail (*Galba truncatula*) in north-eastern Algeria. Parasitology Research, 92(6): 502-505. DOI: https://www.doi.org/10.1007/s00436-004-1072-1

Mekroud A, Benakhla A, Benlatrache C, Rondelaud D, and Dreyfuss G (2002). First studies on the habitas of *Galba truncatula* (mollusca Gastropoda Lymnaeidae), the snail host of *Fasciola hepatica*, and the dynamics of population in northeastern Algeria. Revue de Médecine Vétérinaire, 153: 181-188. Available at : https://www.agris.fao.org/agris-search/search.do?recordID=FR2002001446

Muniz-Pareja FC, and Iturbe-Espinoza PA (2018). Effectiveness of *Chaetogaster limnaei* as a controller of *Fasciola hepatica* in experimental infections of *Galba truncatula*.Tropical Parasitology, 8(2): 88-93. DOI: https://www.doi.org/10.4103/tp.tp_24_15

Ouchene-Khelifi NA, Ouchene N, Dahmani H, Dahmani A, Sadi M, and Douifi M (2018). Fasciolosis due to *Fasciola hepatica* in ruminants in abattoirs and its economic impact in two regions in Algeria. Tropical Biomedicine, 35(1): 181-187. Available at: https://www.pubmed.ncbi.nlm.nih.gov/33601790/

Reinaldo Gonzalezn L, Perez Ruano M, and Brito S (2002). Bovine fasciolosis in Cuba. Retrospective study at slaughter and analysis of losses by liver condemnation. Journal of Animal Husbandry and Veterinary Medicine of Tropical Countries, 55(1): 31-34. DOI: http://www.doi.org/10.19182/remvt.9842

Relf V, Good B, McCarthy E, and de Waal T (2009). Evidence of *Fasciola hepatica* infection in *Radix peregra* and a mollusc of the family Succineidae in Ireland. Veterinary Parasitology, 163: 152-155. DOI: https://www.doi.org/10.1016/j.vetpar.2009.04.003

Righi S, Benakhla A, Mekroud A, Ouchene N, and Sedraoui S (2016). Prevalence of *Fasciola hepatica* in *Galba truncatula* detected by multiplex PCR in the province of El Tarf (Algeria). Tropical Biomedicine, 33(1): 149-158. Available at: https://pubmed.ncbi.nlm.nih.gov/33579152/

Rondelaud D, Novobilský A, Höglund J, Kasný M, Pankrac J, Vignoles P, and Dreyfuss G (2014). Growth rate of the intermediate snail host *Galba truncatula* influences redial development of the trematode *Fascioloides magna*. Journal of Helminthology. 88(4): 427-33. DOI: https://www.doi.org/10.1017/S0022149X13000370

Rondelaud D, Abrous M, and Dreyfuss G (2002). The influence of different food sources on cercarial production in *Lymnaea truncatula* experimentally infected with Digenea. Veterinary Research, 33(1): 95-99. DOI: https://www.doi.org/10.1051/vetres:2001009

Rondelaud D, Belfaiza M, Vignoles P, Moncef M, and Dreyfuss G (2009). Redial generations of *Fasciola hepatica*: A review. Journalof Helminthology, 83(3): 245-54. DOI: https://www.doi.org/10.1017/s0022149x09222528

Schweizer G, Braun U, Deplazes P, and Torgerson PR (2005). Estimating the financial losses due to bovine fasciolosis in Switzerland. Veterinary Record, 157(7): 188-193. DOI: https://www.doi.org/10.1136/vr.157.7.188

Špakulová M, Rajský D, Sokol J, and Vodňanský M (2003). Giant liver fluke (*Fascioloides magna*), an important liver parasite of ruminants. Bratislava, Slovak Republic, PaRPress, p. 61. Available at; https://www.gettextbooks.com/isbn/9788088789963/

Torgerson P and Claxton J (1999). Epidemiology and control. In Fasciolosis. Edited by Dalton JP. Wallingford, Oxon, U.K: CABI Publishing, pp. 113-149.

Vignoles P, Rondelaud D, and Dsreyfuss G (2017). Determination of zones at risk for fasciolosis in the department of Haute-Vienne, central France: A retrospective study on natural infections detected in 108,481 *Galba truncatula* for 37 years. Parasite, 24: 55. DOI: https://www.doi.org/10.1051/parasite/2017055

Vignoles P, Aimeur F, Titi A, Rondelaud D, Mekroud A, and Dreyfuss G (2010). Total cercarial output in two populations of *Galba truncatula* experimentally infected with *Fasciola hepatica*. Journal of Helminthology, 84(1): 77-80. DOI: https://www.doi.org/10.1017/s0022149x09990253

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Parascaris equorum in Horses of Payakumbuh City, West Sumatra, Indonesia

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ABSTRACT

Parascaris equorum is a species of the *Ascarididae* family which belongs to the phylum nematoda (roundworms) and is a type of parasite that affects equine health, performance, and production. The current study was carried out from April to August 2021 to determine the prevalence of equine *Parascaris equorum* in horses residing in Payakumbuh City, Indonesia. A total number of 128 fecal samples from horses were examined in the current study. Coprological examination was performed for the detection of *Parascaris equorum* eggs inside the amassed samples. The overall occurrence of *Parascaris equorum* was 14.06% (18 out of 128). The prevalence rates of sub-districts differed with the highest in East at 18.18% (8/44), followed by North, South, and West at 16.66% (6/36), 14.06% (2/22), and 7.7% (2/26), respectively. The obtained results indicated a significant difference in the prevalence rate of *Parascaris equorum* between males and females as well as those horses aged < 5 years (26.22%) and > 5 years (3%). Therefore, it is critical to not only enhance horse health management, maintenance, and health but also to provide anti-parasitic medications on a regular basis.

Keywords: Gastrointestinal nematode, Horse, *Parascaris equorum*, Prevalence

INTRODUCTION

Horse (*Equus caballus*) is a type of non-ruminant intelligent herbivorous animal that has a good learning ability to recognize an object. Horses can act as pets, sports animals, or as a means of transportation since they are easy to manage and control, and are friendly to humans. At first, horses were only used as human food. Over time, humans use horses as a means of transportation, a means of war, sports, and recreation (Parakkasi, 1999). Accordingly, the system of care and maintenance of horses ave an impact on their health. The center for horse breeding in West Sumatra Province, Indonesia, is Payakumbuh City which has a higher population, compared to other regions. In addition, many diseases, such as diarrhea, can decrease body weight, growth, and endurance in horses leading to gastrointestinal parasitic infections (Andrianty, 2015). In livestock health, efforts to prevent infection caused by worms must be carried out before infection. One way to diagnose the presence of parasitic worms in livestock is by examining fresh feces to look for parasitic worm eggs. This can be performed for the early detection of parasitic worm infections, especially digestive parasites quickly, easily, and effectively (Nezar et al., 2014).

Parascaris equorum is a large horse roundworm that is the most pathogenic common disease in foals worldwide. *Parascaris equorum* infection can cause nasal discharge, coughing, stunted growth, and even death due to the obstruction of the small intestine (Boyle and Houston, 2006; Cribb et al., 2006). *Parascaris equorum* is a common nematode found in horses that can migrate from its host to the size of an adult worm (female 18-37 cm, male 15-28 cm). In older horses, infection occurs subclinically due to the presence of immunity in these nematodes. *Parascaris equorum* infection is acquired through infective eggs from environments, such as pastures or the environment around cages (Larsen et al., 2011). Studies conducted on horses in the Palestine West Bank at various ages found a prevalence rate of 15.6% for *Parascaris equorum* (Othman and Alzuheir, 2019). In addition, other studies reported the *Parascaris equorum* prevalence rates of 2%, 4%, and 1.6% for horses in East Lombok, Sumbawa, and Madura of Indonesia, respectively (Setiawan et al., 2014; Prawira et al., 2017; Apriliawati et al., 2019). The aim of the present study was to identify the *Parascaris equorum* horses in Payakumbuh city, West Sumatra, Indonesia. In this regard, *Parascaris equorum* is a type of parasite that affects equine health, performance, and production. The result of the present study could have an important impact on improving equine health and performance.

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MATERIALS AND METHODS

Ethical approval

All experimental protocols and procedures were approved by the Institutional Animal Care of Indonesia between April and August 2021.

Study design

Fecal samples were collected from 128 horses on a farm in Payakumbuh City 100°35" - 100°45" BT 00°10" - 00^{0}17"LS. Sampling was carried out at 07.00-10.00 AM in each horse stable with gloved arms, and the samples were packed, labeled with the name, age, sex of the animal, and date of collection, and then transported to the Laboratory of Animal Disease and Health Agricultural State Polytechnic of Payakumbuh. It took 1-2 hours to transport samples from the collection location to the laboratory. During the transportation, samples were kept in a media cooler box equipped with an ice pack as a cooling material to keep the sample temperature below 5°C.

Direct smear approach

A small amount of overwhelmed fecal pattern (about 3 g) become blended with 10 ml of water in a beaker, and stirring was done constantly, and then a drop of this solution was put on an easy glass slide, then covered with a cowl slip. The examination was carried out under a low-energy compound microscope (Olympus, Japan) with 10× and 40× magnifications to determine the presence of any parasitic eggs or larvae in the stool samples (Yadav et al., 2014).

Flotation method

Nematode and cestode were detected using Sheather's sugar-modified flotation method (Sloss and Kemp, 1997). Therefore, 3 grams of feces were mixed with 30 ml of water until homogeneous and filtered through a tea filter. After filtering, 1-2 ml of the suspension was taken and then put into a 15 ml centrifuge tube, then Sheather's sugar was added and homogenized again by centrifugation at 1500 rpm for 4 minutes. After being centrifuged, they were lifted vertically and stored on a tube rack, and then tested for the presence of eggs.

Statistical analysis

Statistical analysis was performed using Chi-square testing statistical package for the social science (SPSS, 16.0 version, SPSS Inc., Chicago, IL). The differences in parasite prevalence were considered significant at $p < 0.05$, the assessment of risk factors to determine the association between the presence of parasites and the following variables sub-district, sex (male and female), and age (adult and colt).

RESULTS AND DISCUSSION

The results showed that the prevalence rate in each sub-district was different, the highest prevalence was in the Eastern region at 18.18% (8/44), followed by the North, South, and West at 16.66% (6/36), 14.06% (2/22), 7.70% (2/26), respectively. As presented in Table 1, the prevalence of *Parascaris equorum* in horses in Payakumbuh city was 14.06% (18/128). Based on research that has been done related to intestinal *Parascaris equorum* infestation in horses of Payakumbuh city, the impact of gastrointestinal nematode worm infection was very large, including decreased work productivity because it can cause weakness, weight loss, colic, loss of appetite, diarrhea, and even death (Hillyer, 2004). The results of Hillyer's study indicated that *Parascaris equorum* was found in horses of various ages and sexes in all sub-districts in Payakumbuh City. The highest infection rate was detected in East and North sub-districts with horse breeding centers in Payakumbuh City. These regions have the highest horse population, compared to other sub-districts.

The prevalence of *Parascaris equorum* in horses in Payakumbuh city, Indonesia, was lower than in Palestine at 15.6% (Othman and Alzuheir, 2019), Italy at 35.8% (Scala et al., 2021), Finland at 47% (Aromaa et al., 2018), the UK at 50% (Relf et al., 2013), and Kentucky at 86% (Lyons et al., 2006). Several studies conducted in other developing countries have indicated a higher prevalence of infection. For instance, the prevalence of *Parascaris equorum* was 50% in Ethiopia, (Ayele et al., 2006), 47.5% in India (Yadav et al. 2014), and 18.48% in Cameroon (Lem et al., 2012). The results of other studies from different countries indicated a wide range of prevalence rates was 16.7% in some regions of Germany (Hinney et al., 2011), and the prevalence rate was 5% in Australia and Brazil (Bucknell et al., 1995; Pereira and Vianna, 2006). Variations in prevalence are related to the number of horses in an area as well as maintenance management, feed management, health management which differ from country to country. In addition, the prevalence rate can also be influenced by climatic conditions, temperature, and humidity (Singh et al., 2012).

The prevalence rate in men was 12.50% (6/48), while the sample prevalence was 15% (12/80) in females. As can be seen in Table 2, the prevalence rate of *Parascaris equorum* infection differed significantly in male and female horses in Payakumbuh city ($p < 0.05$). Sex had a significant ($p < 0.05$) effect on the prevalence of *Parascaris equorum* in horses

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which was different from that report of Othman and Alzuheir who conducted a study in West Bank Palestine and with the result that there was no significant difference in the prevalence rate of *Parascaris equorum* between male and female (Othman and Alzuheir 2019). The results of this study were in accordance with other studies in different areas; In India, the prevalence of *Parascaris equorum* in horses was higher in females (60.97%), compared to males (58.51%, Yadav et al., 2014). In Palestine, the prevalence rate was 15% in males and 16.5% in female horses (Singh et al., 2012; Othman and Alzuheir, 2019). There is no significant difference in prevalence rates between male and female horses in seven provinces of southern Poland which can be caused by the way of rearing, nutrition, and maintenance management ($p > 0.05$, Kornas et al., 2010). The mare can re-infect a nursing foal. Heavy infections of *Parascaris equorum* cause impaction and perforation leading to fatal peritonitis (Tayer, 2008).

Furthermore, the obtained results showed that there was a significant difference ($p < 0.05$) in the prevalence rate of *Parascaris equorum* among horses younger than 5 years (26.22%) and in horses older than 5 years (3%, Table 3). The results of this study were similar to those reported by Chemeda et al. (2016) on horses in Around Ambo Town, Central Ethiopia indicating a significant variation with the prevalence of young horses at 60%, compared to 25.9% in older horses. The high prevalence of *Parascaris equorum* in young horses is due to the fact that immunity has not been formed completely so as the horses grow older, the level of immunity increases (Mahfooz et al., 2008; Tayer, 2008). Infection with worm parasites from different groups can result in a high level of morbidity and mortality in horses (Hodgkinson, 2006).

Table 1. The prevalence rate of *Parascaris equorum* in Payakumbuh city, West Sumatra, Indonesia

Sub-district	Sample analyzed (N)	Positive sample (N)	Prevalence rate (%)
West	26	2	7.7
East	44	8	18.18
North	36	6	16.66
South	22	2	9.1
Total	128	18	14.06

Table 2. The prevalence rate of *Parascaris equorum* in native horses based on sex in Payakumbuh city, West Sumatra, Indonesia

Sex	Sample analyzed (N)	Positive sample (N)	Prevalence rate (%)
Male	48	6	12.5[a]
Female	80	12	15[b]
Total	120	18	14.06

[a-b]: Different superscript letters mean significance level at $p < 0.05$.

Table 3. The prevalence rates of *Parascaris equorum* in native horses based on the age in Payakumbuh city, West Sumatra, Indonesia

Age	Sample analyzed (N)	Positive sample (N)	Prevalence rate (%)
<5 years	61	16	26.22[a]
>5 years	67	2	3[b]
Total	128	18	14.06

[a-b]: Different superscript letters mean significance level at $p < 0.05$.

CONCLUSION

The helminthiasis in the gastrointestinal tract of horses in Payakumbuh city, West Sumatra, Indonesia is influenced by sex and age factors. Therefore, the findings of this study necessitate improvement in health management, maintenance, and health condition of horses leading to enhanced health and welfare status. Further studies are needed to show other effective risk factors related to *Parascaris equorum* infestation in horses.

DECLARATIONS

Acknowledgments

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Authors' contribution

Engki Zelpina, Sujatmiko, Prima Silvia Noor, and Delli Lefiana designed this research. Collection and analysis of laboratory samples were done by Sujatmiko, Prima Silvia Noor, and Delli Lefiana. Engki Zelpina performed the statistical analysis and wrote the draft of the manuscript. The authors reviewed and approved the final draft of the manuscript prior to submission to the present Journal.

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Competing interests

The authors have not declared any competing interests.

Ethical considerations

Authors have been checked ethical issues associated with plagiarism, approval to publish, error in fabrication, double publication, and submission.

REFERENCES

Andrianty V (2015). The incidence of gastrointestinal nematodiasis in Balinese calves in Marioriwawo sub-district, Soppeng district. Hasanuddin University, Thesis. Available at: https://core.ac.uk/display/77620753

Apriliawati E, Mufasirin, Wurlina, Hastutiek P, Suwanti LT, and Tehupuring BC (2019). Prevalence and nematode infection level on gastrointestinal tract at horse (Equus caballus) in Bangkalan Madura. Journal of Parasite Science, 3(2): 83-88. DOI: http://www.dx.doi.org/10.20473/jops.v3i2.16523

Aromaa M, Hautala K, Oksanen A, Sukura A, and Näreaho A (2018). Parasite infections and the risk factors in foals and young horses in Finland. Veterinary Parasitology: Regional Studies and Reports, 12: 35-38. DOI: http://www.dx.doi.org/10.1016/j.vprsr.2018.01.006

Ayele G, Feseha G, Bojia E, and Joe A (2006). Prevalence of gastro-intestinal parasites of donkeys in Dugda Bora District, Ethiopia. Livestock Research for Rural Development, 18(10): 1-11. Available at: https://www.researchgate.net/publication/289841216_Prevalence_of_gastro-intestinal_parasites_of_donkeys_in_Dugda_Bora_District_Ethiopia

Boyle AG, and Houston R (2006). Parasitic pneumonitis and treatment in horses. Clinical Techniques in Equine Practice, 5: 225-232. DOI: http://www.dx.doi.org/10.1053%2Fj.ctep.2006.03.018

Bucknell DG and Gasser RB (1995). The prevalence and epidemiology of gastrointestinal parasite of horses in victoria, Australia. International Journal for Parasitology, 25(6): 711–724. DOI: http://www.dx.doi.org/10.1016/0020-7519(94)00214-9

Chemeda R, Mekonnen N, Muktar Y, and Terfa W (2016). Study on prevalence of internal parasites of horses in and around Ambo Town, Central Ethiopia. American-Eurasian Journal of Agricultural and Environmental Sciences, 16(6): 1051-1057. DOI: http://www.dx.doi.org/10.5829/idosi.aejaes.2016.16.6.10366

Cribb NC, Cote NM, Boure LP, and Peregrine AS (2006). Acute small intestinal obstruction associated with *Parascaris equorum* infection in young horses: 25 cases (1985-2004). New Zealand Veterinary Journal, 54: 338–343. DOI: https://www.doi.org/10.1080/00480169.2006.36721

Hillyer M (2004). A Practical Approach to Diarrhea in The Adult Horse. Practice, 26(1): 2-11. DOI: http://www.dx.doi.org/10.1136/inpract.26.1.2

Hinney B, Wirtherle NC, Kyule M, Miethe N, Zessin KH, Clausen PH (2011). Prevalence of helminths in horses in the state of Brandenburg, Germany. Parasitolo Res, 108:1083–1091. DOI: https://doi.org/10.1007/s00436-011-2362-z

Hodgkinson JE (2006). Molecular diagnosis and equine parasitology. Veterinary Parasitology, 136(2): 109-116. DOI: http://www.dx.doi.org/10.1016/j.vetpar.2005.12.006

Kornas S, Cabaret J, Skalska M, and Nowosad B (2010). Horse infection with intestinal helminths in relation to age, sex, access to grass and farm system. Veterinary Parasitology, 174: 285-291. DOI: http://www.dx.doi.org/10.1016 /j.vetpar.2010.09.007

Larsen ML, Ritz C, Petersen SL, and Nielsen MK (2010). Determination of ivermectin efficacy against cyathostomins and *Parascaris equorum* on horse farms using selective therapy. Veterinary Journal, 188: 44-47. DOI: http://www.dx.doi.org/10.1016/j.tvjl.2010.03.009

Lem MF, Vincent KP, Pone JW, and Joseph T (2012). Prevalence and intensity of gastro-intestinal helminths in horses in the Sudano-Guinean climatic zone of Cameroon. Tropical Parasitology, 2: 45. DOI: http://www.dx.doi.org/10.4103/2229-5070.97239

Lyons ET, Tolliver SC, and Collins SS (2006). Prevalence of large endoparasites at necropsy in horses infected with population B small strongyles in a herd established in Kentucky in 1966. Parasitology Research, 99(2): 114-118. DOI: http://www.dx.doi.org/10.1007/s00436-005-0116-5

Mahfooz A, Masood MZ, Yousaf A, Akhtar N, and Zafar MA (2008). Prevalence and anthelmintic efficacy of Abamectin against gastrointestinal parasites in horses. Pakistan Veterinary Journal, 28(2): 76-78. DOI: http://www.pvj.com.pk/pdf-files/28_2/76-78.pdf

Nezar MR, Susanti R, and Setiati N (2014). Types of worms in cow feces at the Jatibarang Final Disposal Site and the Sidomulyo Livestock Farmer Group, Nongkosawit Village, Semarang. Journal of Life Science, 3(2): 93-102. Available at: https://journal.unnes.ac.id/sju/index.php/UnnesJLifeSci/article/view/4565

Othman R and Alzuheir I (2019). Prevalence of *Parascaris equorum* in native horses in West Bank Palestine. Iraqi Journal of Veterinary Sciences, 33(2): 433-436. DOI: http://www.dx.doi.org/10.33899/ijvs.2019.163192

Parakkasi A (1999). Nutrition Science and Ruminant Animal Feed. Jakarta. University of Indonesia Press. p, 23-37. Available at: https://uipublishing.ui.ac.id/cari.html

Pereira J, and Vianna S (2006). Gastrointestinal parasitic worms in equines in the Paraíba Valley, State of São Paulo, Brazil. Veterinary Parasitology, 140: 289-295. DOI: https://www.doi.org/10.1016/j.vetpar.2006.03.036

Prawira SY, Apsari IAP, and Widyastuti SK (2017). Prevalence and identification of local horse (Equus caballus) digestive nematodes channel in Moyo Hilir Subdistrict Sumbawa. Indonesia Medicus Veterinus, 6(3): 206-212. Available at: https://ojs.unud.ac.id/index.php/imv/article/view/34962

Relf VE, Morgan ER, Hodgkinson JE, and Matthews JB (2013). Helminth egg excretion with regard to age, gender and management practices on UK Thoroughbred studs. Parasitology, 140: 641-652. DOI: http://www.dx.doi.org/10.1017/S0031182012001941

Setiawan DK, Dwinata IM, and Oka IBM (2014). Identification of nematode worm on gastrointestinal tract of Cidomo Workhorse In District Selong, East Lombok. Indonesia Medicus Veterinus, 3(5): 351-358. Available at: https://ojs.unud.ac.id/index.php/imv/article/view/14111

Singh G, Soodan J, Singla L, and Khajuria J (2012). Epidemiological studies on gastrointestinal helminths in horses and mules. Veterinary Practice, 13: 23-27. Available at: https://www.cabdirect.org/cabdirect/abstract/20133045674

Sloss MW and Kemp RL (1997). Veterinary Clinical Parasitology. 6th Edition. International Book Distributing Co., Lucknow, p, 198. Available at: https://download.e-bookshelf.de/download/0000/8070/16/L-G-0000807016-0002366454.pdf

Tayer M (2008). Survey on GIT helminthes of equine in around Asella, DVM Thesis, Addis Ababa University. Faculty of Veterinary Medicine, Debrezeit, Ethiopia. Available at: https://addisababa.academia.edu/Departments/College_of_Veterinary_Medicine_and_Agriculture

Yadav KS, Shukla PC, Gupta DK, and Mishra A (2014). Prevalence of gastrointestinal nematodes in horses of Jabalpur region. Research Journal for Veterinary Practitioners, 2: 44-48. DOI: http://www.dx.doi.org/10.14737/journal.rjvp/2014/2.3.44.48

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Antimicrobial Resistance and Virulence Genes of *Campylobacter jejuni* Isolates from Diarrheic Sheep

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ABSTRACT

One of the important agents causing gastroenteritis worldwide is *Campylobacter jejuni (C. jejuni)*. The current study aimed to detect five virulence genes (flaA, virB11, ciaB, iam, and dnaJ) and two antibiotic resistance genes (gyrA and tetO) in *C. jejuni* obtained from sheep stool. The virulence genes were detected by PCR in 64 *C. jejuni* strains. The phenotypic resistance to five selected antibiotics (Ciprofloxacin, Erythromycin, Gentamycin, Streptomycin, and Tetracycline) was screened with the microdilution method. The isolates with antibiograms were tested for detection of *gyrA and tetO* genes via PCR using specific primers. The virulence genes *flaA* (32%) and *dnaJ* (29%) had the highest prevalence. The tested isolates of *C. jejuni* revealed high resistance to both quinolone (68.3%) and tetracycline groups (48.4%) with an increased prevalence of antibiotic resistance of gyrA and tetO genes. Gentamycin and erythromycin offered better alternative drugs for the treatment of campylobacteriosis. To generalize the findings, extensive profiling that involves more virulence genes is required in several strains of *Campylobacter*.

Keywords: Antibiotic resistance, *Campylobacter jejuni*, Sheep, Virulence genes

INTRODUCTION

Campylobacter is a zoonotic infection that causes foodborne diarrhea in people all over the world (Sheppard and Maiden, 2015; Babazadeh and Ranjbar, 2022). The most common *Campylobacter* species that cause different infections in sheep, including enteritis, colitis, and reproductive disorders, are *Campylobacter jejuni* (*C. jejuni*), *Campylobacter fetus* subspecies *fetus*, and *Campylobacter coli* (*C. coli*, İlhan et al., 2021). Sheep breeding represents a large and essential part of animal husbandry in Egypt, and consequently, diarrhea caused by *Campylobacter* species can influence the production characteristics in Egypt.

Campylobacter species are globally found in soil, water, and food, so they can be in the gall bladder and the intestine without any clinical signs as a result of contact with contaminated sources, such as animal stool, genital excretions, and aborted tissues (Indykiewicz et al., 2021). Wild and domestic animals have been identified as potential carriers of this bacteria (Rukambile et al., 2019). *Campylobacter* species are present in the gut of many animal species with interspecies transmission risk. *Campylobacter* species, such as *C. jejuni* and *C. coli*, can be isolated from different farm animals, including cattle, sheep, and goats. Comparing sheep and goat breeding farms, sheep are more potential carrier of *C. jejuni* as a contaminant (Pao, et al., 2014).

Healthy sheep act as reservoirs where the bacteria are intermittently excreted in their feces, particularly in stressful situations (birth, weaning, and changes in feeding systems) although their activity is rapidly inactivated on pastureland at high temperatures. It is worth noting that the presence of wild birds in sheep farms raises the potentiality of lamb infection, especially at very young ages (Sproston et al., 2010).

The gene expression for motility, colonization, invasion, and excretion of toxins is believed to be an essential cause of disease progression (Dasti et al., 2010). Bacterial cell movement involving the coordination of many genes (such as *flaA*) is responsible for the bacteria passage through the gastrointestinal environment (Park, 2002) where *Campylobacter* delivers many cell surface proteins encoded by several genes (such as *virB11, ciaB,* and *iam*) that support adhesion and invasion of enterocytes (Dasti, et al., 2010). Furthermore, *C. jejuni* can produce defense factors such as cytokines and enzymes like superoxide dismutase to get rid of superoxide radicals as a defense mechanism against oxidative damage.

Campylobacteriosis is considered a self-limiting disease. In acute cases, macrolides Macrolides, fluoroquinolones, and aminoglycosides are classified as critically important antimicrobials, while tetracycline is considered a highly important antimicrobial (World Health Organisation, 2018). Unfortunately, nowadays there is a growing trend of antibiotic resistance among *Campylobacter* species mainly due to misuse of antibiotics (Wieczorek et al., 2017). Therefore, the present study was designed to find the prevalence of virulence and antimicrobial resistance genes of *C. jejuni* isolated from sheep suffering from diarrhea.

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MATERIALS AND METHODS

Ethical approval

Ethical approval was not necessary for this study; however, samples were collected as per standard sample collection procedure and consent was taken from the animal owners with their signature using a prescribed consent form (License No. AHRI 42102017), according to local Egyptian laws.

Collection of *Campylobacter jejuni* isolates

A total of 262 fecal samples were collected from sheep aged 2-3 years (Barki sheep ewes and Rams) suffering from different levels of watery diarrhea. The clinical signs included diarrhea, decreased appetite, and vomiting with or without fever. The feces were usually watery or bile streaked with mucus and sometimes blood. The animals were obtained from different sheep herds on the Northwest coast of Egypt. All collected samples were transferred in sterile plastic bags and refrigerated up to the time of investigation (within 24 hours after collection). *Campylobacter* isolation was performed by the culture method following a study by Hagos et al. (2021). The strains were grown on blood-based agar (BD BBLTM, United States) with 5% defibrinated sheep blood and incubated at 42°C for 48 hours under microaerobic conditions (85% nitrogen, 10% carbon dioxide, and 5% oxygen). The strains were confirmed as *C. jejuni* or *C. coli* using Lior's biotyping scheme (Lior, 1984) and the PCR technique based on the highly conserved gene glyA (serine hydroxymethyltransferase, Quino et al., 2022).

DNA extraction and PCR

All *Campylobacter* isolates were subjected to DNA extraction following the instructions of QIAamp DNA Mini kit (Qiagen, Germany, Catalogue no.51504) with slight modifications. Briefly, 10 µl of proteinase K and 200 µl of lysis buffer were added to 200 µl of the sample DNA and incubated at 56°C for 10 minutes. Then, 200 µl of absolute ethyl alcohol was added to the lysate. The sample was washed and centrifuged. Nucleic acid was obtained in 100 µl of elution buffer. Then, the PCR technique was carried out for thermophilic *Campylobacter* species (*C. jejuni* and *C. coli*, Iraola et al., 2012).

Investigation of virulence genes

Campylobacter isolates were examined for the virulence genes of *flaA* (responsible for motility) and *virB11* (for adhesion and colonization). In Addition, the gene markers of *ciaB* and *iam* (for the *Campylobacter* invasiveness) were also amplified. The primer sets targeting the 23S rRNA gene of *Campylobacter* species, and the virulence genes of *flaA, dnaJ, virBII, iam,* and *ciaB* were used and specific amplified products were detected at 217, 177, 494, 518, and 527 bp, respectively. The amplicons were detected using capillary electrophoresis. Primer sequences, target genes, amplicon sizes, and cycling conditions are illustrated in Table 1. PCR conditions and techniques for all the above genes were based on a study by Datta et al. (2003).

Table 1. PCR primers used for *Campylobacter* detection and antimicrobial resistance genes

Target genes	Primers sequences	Amplified segment	Reference
23S Rrna	TATACCGGTAAGGAGTGCTGGAG ATCAATTAACCTTCGAGCACCG	650	Wang et al. (2008)
FlaA	TCCAAATCGGCGCAAGTTCA TCAGCCAAAGCTCCAAGTCC	217	Zheng et al. (2006)
DnaJ	ATTGATTTTGCTGCGGGTAG ATCCGCAAAAGCTTCAAAAA	177	Chansiripornchai and Sasipreeyajan (2009)
virB11	TCTTGTGAGTTGCCTTACCCCTTTT CCTGCGTGTCCTGTGTTATTTACCC	494	Datta et al. (2003)
Iam	GCGCAAATATTATCACCC TTCACGACTACTACTATGCGG	518	Wieczorek, (2011)
ciaB	TGC GAG ATT TTT CGA GAA TG TGC CCG CCT TAG AAC TTA CA	527	Zheng et al. (2006)
tetO	GGCGTTTTGTTTATGTGCG ATGGACAACCCGACAGAAGC	559	Gibreel et al. (2004)
gyrA	GATGGTTTAAAGCCTGTTCAT CGCCATACCTACAGCTATACC	423	Lindmark et al. (2004)

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Antimicrobial resistance

Campylobacter jejuni isolates were evaluated for the resistance to selected antimicrobial agents with the microdilution method using microtitration plates. The five tested antimicrobial drugs are the most common ones used in the treatment of *Campylobacter* infections. Solutions of each tested antibiotic included streptomycin, ciprofloxacin, tetracycline, erythromycin, and gentamicin solutions (Table 2). Mueller Hinton broth with 2.5% lysed horse blood was prepared. The inoculated plates were incubated at 37°C for 48 hours in a microaerophilic atmosphere (the same conditions as above). The parameters for individual antibiotics, including interpretation criteria, were based on recommendations issued by the CLSI guidelines (McDermott et al., 2005). Quality control was done using a reference strain of *C. jejuni* (ATCC 33560). The detailed parameters for testing are shown in Table 2.

Investigation of antibiotic resistance genes

Once the identification was performed, the isolates were screened for the existence of resistance genes to quinolone, the Thr-86-lle mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* gene in *C. jejuni* and the tetracycline resistance gene (*tetO)* were amplified and identified using PCR with two specific primer sets for amplification at 423 and 559 bp.

Sequence

PCR products were purified using QIAquick PCR Product extraction kit (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction which was then purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 Genetic Analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) was initially performed to establish sequence identity to GenBank accessions (Altschul et al., 1990). The phylogenetic tree was created by the MegAlign module of LasergeneDNAStar version 12.1 (Thompson et al., 1994), and Phylogenetic analyses were done using maximum likelihood, neighbor-joining, and maximum parsimony in MEGA6 (Tamura et al., 2013).

RESULTS

Investigation of *Campylobacter jejuni* spacemen

A total of 262 samples from sheep feces (235 diarrhea and 27 non-diarrhea) yielded 64 *C. jejuni* strains (24.4%). Two *C. jejuni* strains were found in stool samples that appeared to be non-diarrheal, and 62 *C. jejuni* strains were found in clearly diarrheal stool samples.

Detection of antimicrobial susceptibility of *Campylobacter jejuni* isolates

Of the 5 antibiotics tested, the highest phenotypic resistance exhibited by *C. jejuni* from stool samples was against ciprofloxacin (68.3%), followed by tetracycline, streptomycin, gentamycin, and erythromycin as 48%, 4%, 27.5%, 6.3%, and 4.5%, respectively while complete susceptibility (100%) was detected against gentamycin (Table 2). The highest prevalence of resistant strains was against type 4 of antibiotics (24%) while resistance to type 5 was the least (5%, Figure 1).

Investigation of antibiotic resistance genes in *Campylobacter jejuni*

Following the detection of phenotypic resistance to the chosen antibiotics, the resistance of *gyrA and tetO* genes was detected using PCR. The *tetO* gene which is liable for tetracycline-resistant was detected at 595 bp while the *gyrA* gene which is responsible for the quinolone resistance was detected at 423 bp (Figure 2). A comparison of the presence of selected antibiotic resistance genes with the phenotypic resistance is shown in Table 3. As can be seen, the resistance in the evaluated genes is more common in isolates that showed phenotypical resistance.

Detection of virulence genes in *Campylobacter jejuni* isolates

A total of 24.4% of examined stool samples contained *C. jejuni*. The proportion of the virulence genes in *C. jejuni* isolates is displayed in Figure 3. The results revealed that the *flaA* gene (93%), which encodes the motility, is the most prevalent virulence gene in *C. jejuni* isolates followed by *dnaJ* encodes heat shock protein (ATPase activity), and *ciaB*; encodes invasion in 88% and 42%, respectively. Two genes of *virBII* and *iam* recorded the minimal frequency as 7.3% and 6.8%, respectively.

Investigation of phylogenetic relationship

Based on the analysis of 23S rRNA, dendrograms help to determine simmilarites and difference between the isolates when compared to the reference strains (Figure 5). The graphs illustrate that *C. jejuni* isolates differ significantly. According to present dendrogram, the 5 analyzed isolates (3, 4, 6, 7, and 11) are characterized by genetic variation (Figure 6). Strains 3, 4, and 6 are closely linked with a smaller degree of relatedness to strain 11, however, strain 7 has a lot of genetic diversity, compared to the other strains. OK095294, OK095295, OK095296, OK095297, and OK095298 are the accession numbers of isolates 3, 4, 6, 7, and 11, respectively. However, all strains were similar to *C. jejuni* strains present in gene bank by 98.3-100% (Figure 6).

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Table 2. Profiles of antimicrobial-resistant *Campylobacter jejuni* for different antibiotics

Antibiotic	Antibiotic dilution (mg/L)	*C.jejuni* dilution (mg/L)	Resistance in fecal isolates (%)
Ciprofloxacin	0.03-64	0.5	68.3
Tetracycline	0.125-256	1	48.4
Streptomycin	0.25-512	4	27.5
Gentamycin	0.125-256	2	6.3
Erythromycin	0.25-512	4	4.5

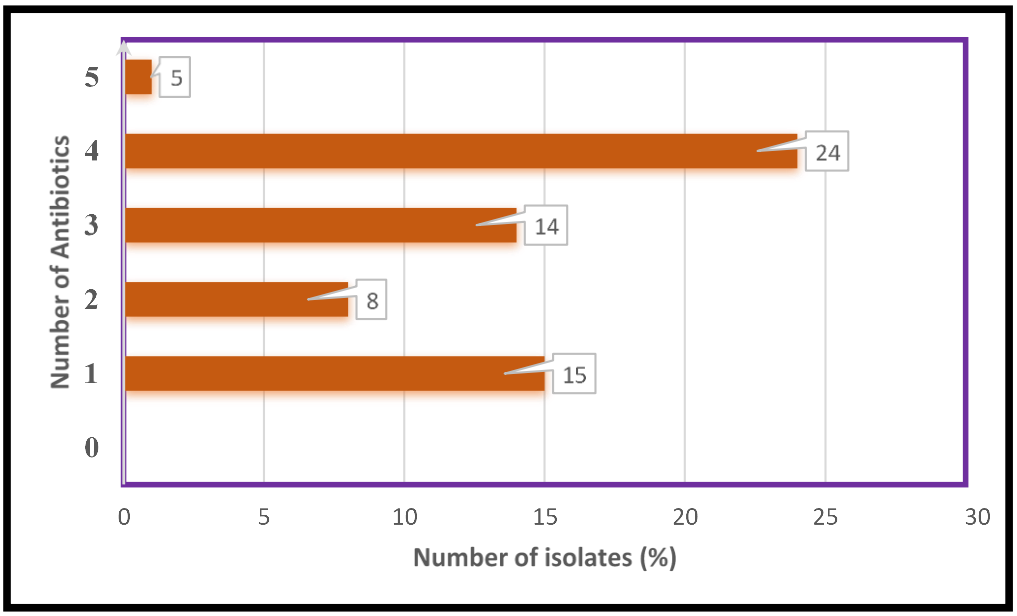


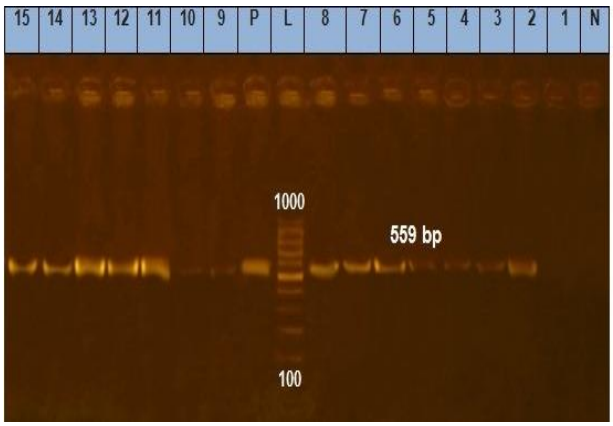
Figure 1. *Campylobacter jejuni* with antimicrobial resistance

Table 3. Relationship between genotypic and phenotypic resistance to ciprofloxacin and tetracycline in *Campylobacter jejuni*

Detection of the *gyrA* gene in *Campylobacter jejuni* isolates (%)		Detection of the *tetO* gene in *Campylobacter jejuni* isolates (%)	
Cipro-R	Cipro-S	Tet-R	Tet-S
78.3	35.5	43.6	21.7

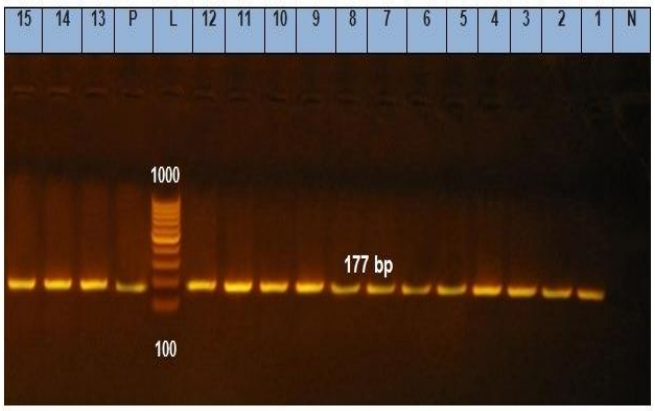
Cipro-R: Isolate with phenotypic resistance to ciprofloxacin. Cipro-S: Isolate with phenotypic susceptibility to ciprofloxacin. Tet-R: Isolate with phenotypic resistance to tetracycline. Tet-S: Isolate with phenotypic susceptibility to tetracycline

A



***tetO* gene** samples were positive at 559bp except sample at lane 1

B



***gyrA* gene all** samples were positive at 177bp

Figure 2. Genotypic characterization of antimicrobial resistance in *Campylobacter jejuni*. **A:** Detection of *tetO* gene, **B:** Detection of *gyrA* gene.

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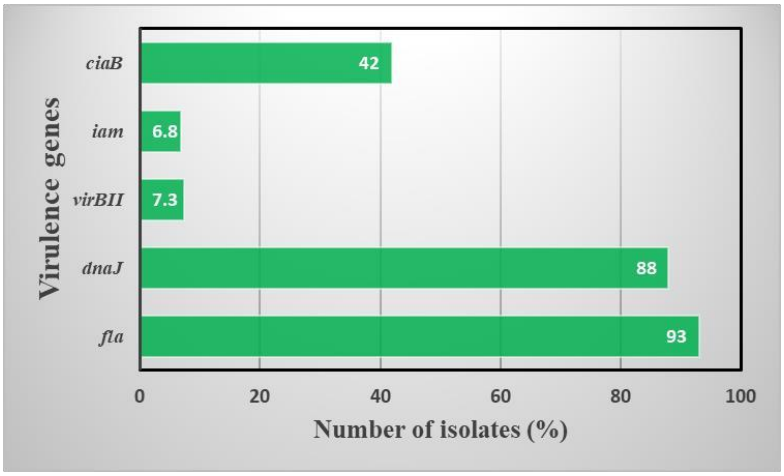
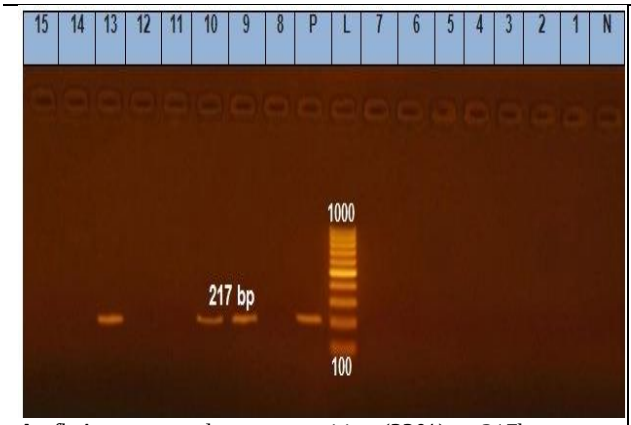
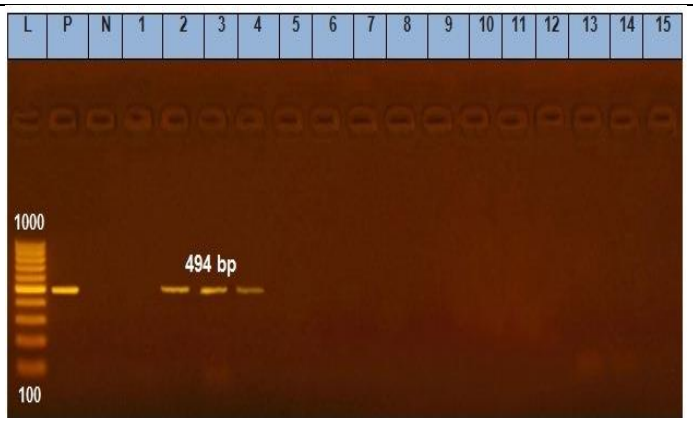


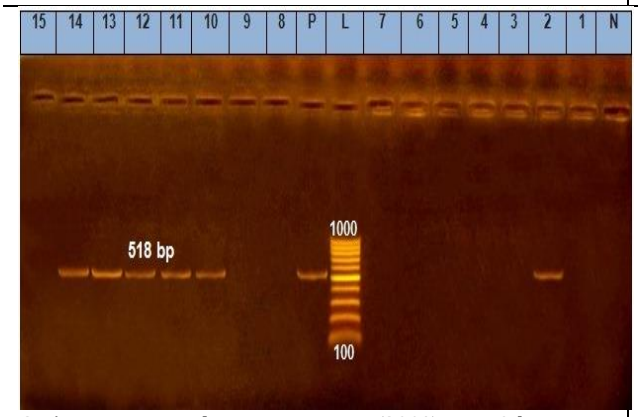
Figure 3. Prevalence of virulence genes in *Campylobacter jejuni* from Barki sheep stool



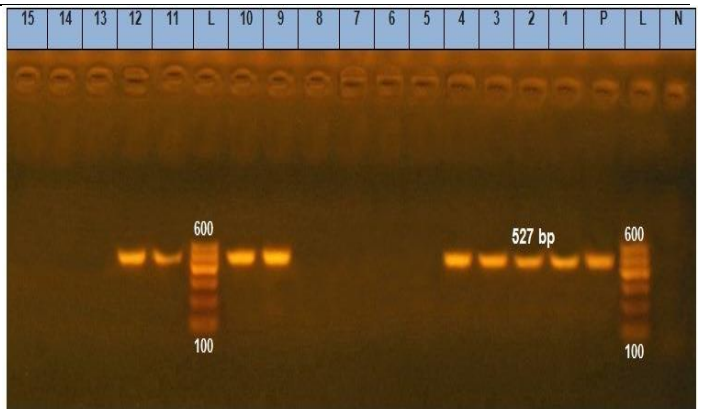
A: *flaA gene* samples were positive (**32%**) at 217bp except sample at lanes 1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 14, and 15



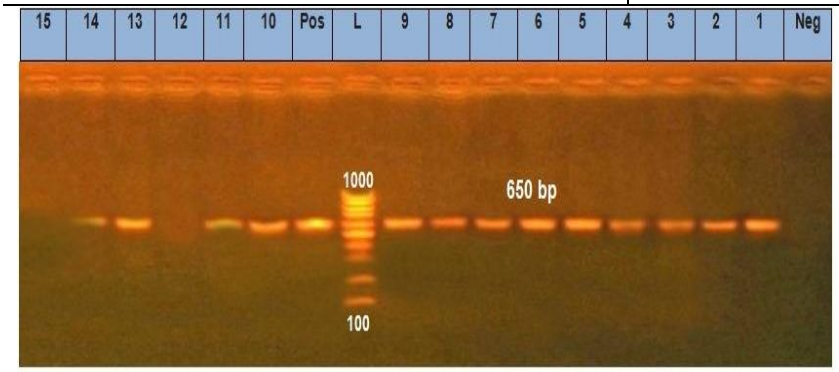
B: *virBII gene* samples were positive (**15%)** at 494 bp except sample at lanes 1, , 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15



C: *iam gene* samples were positive (**29%**) at 518 bp except sample at lanes 1, 3, 4, 5, 6, 7, 8, 9, and 15



D: *ciaB gene* samples were positive (**27%**) at 518 bp except sample at lanes 5, 6, 7, 8, 13, 14, and 15



E: *23S rRNA* samples were positive at 650 bp except sample at lanes 12 and 15

Figure 4. Detection of virulence genes and 23S rRNA of *Campylobacter jejuni* isolates from Barki sheep stool. **A:** Detection of *flaA* gene. **B:** Detection of *virBII*. **C:** Detection of *iam* gene. **D:** Detection of *ciaB* gene. **E:** Detection of 23S rRNA

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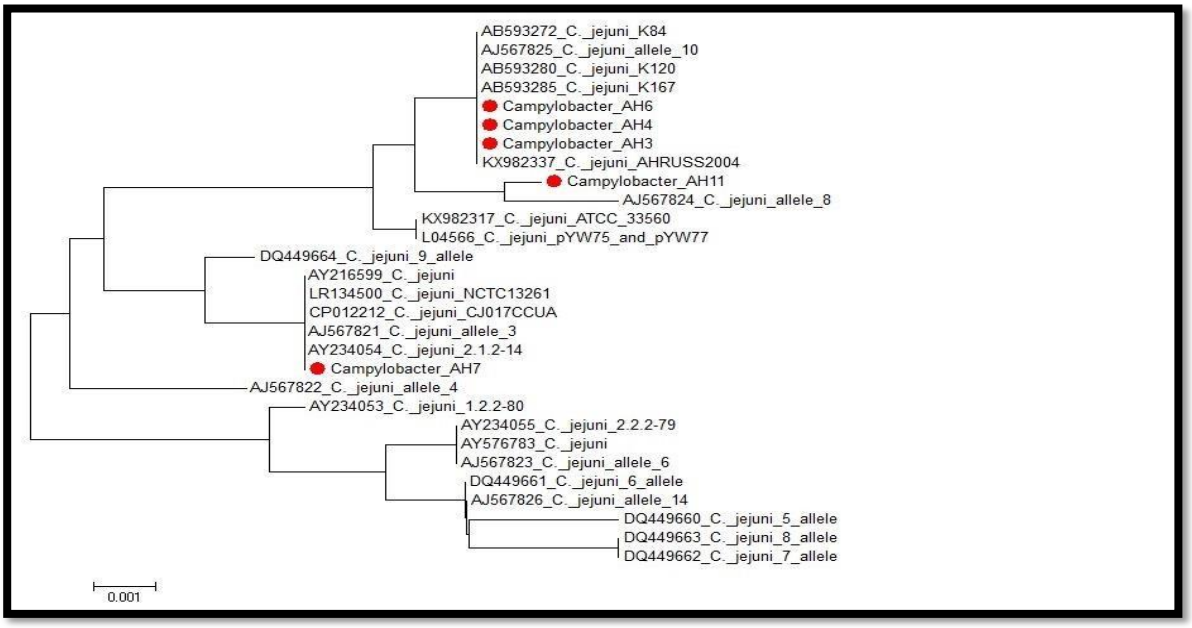

Figure 5. *Campylobacter jejuni* phylogenetic relationship

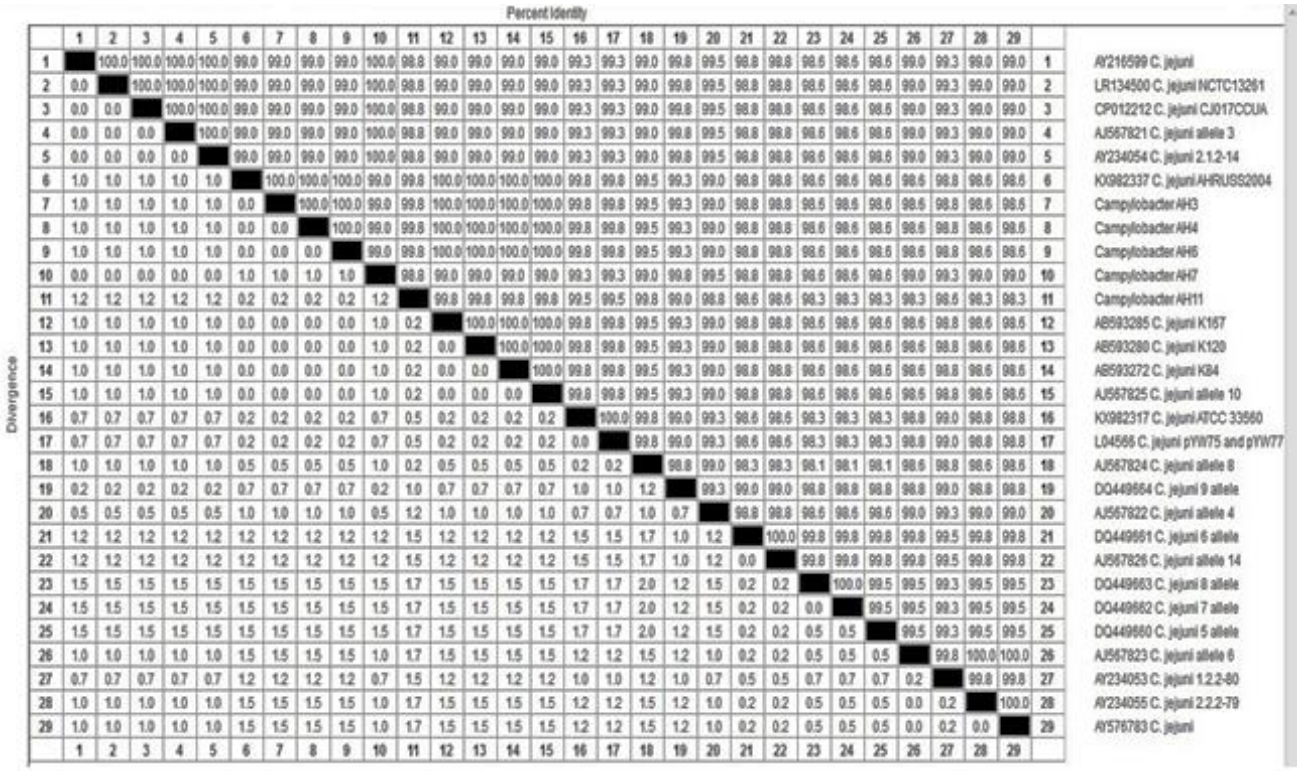

Figure 6. Sequence distance of 5 *Campylobacter jejuni* isolates from Egypt, compared with 24 *Campylobacter jejuni* in the gene bank

DISCUSSION

The intermittent nature of *Campylobacter* infection explains why there are different reports on this type of infection and impeding the discovery of its source (Havelaar et al., 2013). It is established that poultry is the main source of human infection (Ranjbar and Babazadeh, 2017; Nur-Aziera-Aina et al., 2020), *Campylobacter* spp. is also highly prevalent in ruminants all over the world (Babazadeh and Ranjbar, 2022). There is growing data that the ruminants play a pivotal role in the spreading campylobacteriosis to humans as cattle and sheep are considered the second most important reservoir after broiler for the transmission of *C. jejuni* infection to humans (Roux et al., 2013).

Of 302 fecal samples from sheep in Shiraz, Iran, 67.8% were positive for the presence of *Campylobacter* species isolates showed high resistance to cephalothin (83%) and ciprofloxacin (67.7%, Khoshbakht et al., 2016). Regarding the

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obtained results of the current study, there was no antibiotic that could trigger the sensitivity of all *Campylobacter* isolates. Fluoroquinolones are one of the recommended drugs for campylobacteriosis treatment. The *Campylobacter* strains resistant to ciprofloxacin were established in the late 1980s, indicating that the animals play a key role in generating and transmitting the resistant bacteria. At Present, *C. jejuni* resistance to fluoroquinolones is increasing worldwide which poses a threat to public health (Wieczorek, 2011). The current findings indicated that the treatment with fluoroquinolones has become inefficient as some *Campylobacter* strains are resistant to this class of antibiotics (Bolinger and Kathariou, 2017).

Antibiotic resistance was high against the ciprofloxacin (68.3%), and this is consistent with a previous prevalence report in South Africa where rates of *Campylobacter* resistance to fluoroquinolones have been reported between 14.8% and 51.3% (Kepner et al., 2003). These results show that *Campylobacter* resistance to fluoroquinolones can increase over the years. Therefore, constant monitoring is necessary as *Campylobacter* species can mutate (Luo et al., 2003).

High tetracycline resistance has been recognized globally. Modifications in media to test *C. jejuni* isolates from several countries in the European Union indicated a resistance of about 45% (Aleksić at al., 2021). In the present study, there was relatively higher tetracycline resistance (48.4%). The higher resistance of *C. jejuni* to the tetracycline group may be due to the overuse of this group of antibiotics as they are given to treat most infections in the veterinary field in Egypt (Schiaffino et al., 2018). The comparatively high co-resistance of some strains of *C. jejuni* to tetracycline and/or ciprofloxacin is also important due to their clinical significance in the treatment of severe cases of campylobacteriosis. Therefore, the best solution is to use other groups of antibiotics, such as aminoglycosides and macrolides, and to use them only in severe cases where mild forms of *Campylobacter* should be considered a self-limiting infection.

In the current study, *C. jejuni* strains from sheep stool were examined for resistance to ciprofloxacin. An increase in *C. jejuni* resistance to ciprofloxacin was detected (68.3%), which was similar to previous studies in Poland (Wieczorek and Osek, 2013) and other EU countries (EFSA, 2014a; EFSA, 2014b). It must be taken into consideration that the prevalence of resistance can change significantly over time ($p < 0.05$), amino acids substitution is the main cause of fluoroquinolones resistance in *Campylobacter* (Wieczorek and Osek, 2013). The most common silent mutations in the quinolone resistance determination region of gyrA are presented in Table 3. Thr86Ile substitution in the gyrase reveals high-level resistance to this antibiotic group (Payot et al., 2006). In agreement and confirmation of this and other similar studies (Duarte et al., 2014), the Thr86Ile substitution was the most detected amino acid change. On the other hand, further mechanisms of resistance, such as alteration in the outer membrane permeability and efflux systems, have been reported (Charvalos et al., 1996) and these may explain the detection of phenotypic resistance without amino acid changes in *gyrA* in the tested strains.

It is noteworthy that the silent mutation in *Campylobacter* species was reported in both resistant and sensitive strains to ciprofloxacin and a high number of combinations of transitions and mutations may exist. The current work confirmed these results, and some silent mutations that are frequently observed at Ser-119 → His, Glu-131 → Glu, and Ser-157 → Ser correspond to mutations detected in *Campylobacter* strains isolated in Finland and Brazil (Hakanen et al., 2002).

In the present study, gentamycin and erythromycin exhibited a lower resistance at 6.3% and 4.5%, respectively. Therefore, they offered a better alternative drug for the treatment of campylobacteriosis. It is interesting to note that when using macrolides (erythromycin) in treatment, attention should be devoted to testing resistance to erythromycin.

The mechanism through which the *Campylobacter* species cause enteritis is a complex process depending on many factors where specific genes are implicated in all virulence stages of adhesion, colonization, invasion, and toxin production (Bolton, 2015). To evaluate the pathogenicity of the *Campylobacter* isolates in the present study, the existence of five essential genes coding the virulence factors, such as the motility (*flaA*), invasive (*iam* and *ciaB*), ATPase activity (*dnaJ*), and adhesion (*virBII*) genes in the isolates have been investigated.

The first step in pathogenesis is intestinal colonization. This requires the motility of the microbe into the mucus layer that covers the enterocytes. *Campylobacter* motility is granted by the polar flagella in 'cork-screw' shape movement allowing them to effectively penetrate and overcome this mucus barrier (Haag et al., 2012). The flagellin protein encoded by the *flaA* gene is considered the most virulence factor that has been studied and characterized in *Campylobacter* species (Hermans et al., 2011).

The higher prevalence of *flaA* gene (93%) among the *Campylobacter* isolates in the present study is nearly consistent with an Egyptian study by Abd El-Hamid et al. (2019), where the *flaA* gene was detected in all isolates (100%). On the other hand, the *flaA* gene prevalence was inconsistent with other published studies where the prevalence was 87.5%. This discrepancy can be attributed to the identification of a higher number of virulence genes.

The second step in pathogenesis is adhesion. Gene liable for the adhesion of *C. jejuni* is *virBII* that is responsible for producing the IV secretory system protein and is located on the pVir plasmid. It has been reported that strains that show a mutation in the *virB11* sequence have a much lower ability in adhesion and penetration when compared with the original strains, and hence, lower pathogenicity (Bacon et al., 2000). In the present study, the gene was detected in 7.3% of the isolates, which was in accordance with the previous work with a prevalence rate of 9.7% (Abd El-Hamid et al.,

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2019). One of the most crucial genes for adhesion and invasion is the *ciaB* gene *(Campylobacter* invasive antigen B). The *ciaB* gene has been reported to be involved in the invasion of the enterocytes and plays a significant role in colonization (Ó Cróinín and Backert, 2012). and was detected in 42% of the tested strains. The low frequency of the *ciaB* gene in the clinical isolates disagrees with other previously published data reporting 100% detection of this virulence gene in their isolates (Biswas et al., 2011). Therefore, the results obtained from the current study confirm the claim that not all *Campylobacter* strains having the *ciaB* gene can adhere to or invade enterocytes. This indicates the presence of other factors than those coded by the *ciaB* gene on *Campylobacter* surfaces, and this perception is confirmed by previous studies (Bolton, 2015). In accordance with the current study, a lower prevalence of *ciaB* (76.4%) has been measured in Qatar, while the prevalence range of 52.4-71.4% was reported in Asia and 51.5-66.7% in the Arabian Peninsula (Carvalho et al., 2001).

Another invasion-associated marker (*iam*) gene is one of the most essential factors for the invasion of the host cell and was detected in the current study with a prevalence of 6.8%. This prevalence is considered too low, compared to earlier studies where the prevalence reaches 85%. This divergence may be due to the scarcity of *iam* in the isolated *Campylobacter* strains and therefore, its role in the *Campylobacter* pathogenesis should be further assessed (Wieczorek et al., 2018).

The bacterial response to the thermal stress is mainly via the expression of heat shock proteins. These proteins play an important role in thermotolerance. They act as chaperones to improve the folding of cellular protein, and degradation of possibly deleterious misfolded proteins. Several heat shock proteins were identified in the *C. jejuni*, including *DnaJ, DnaK, GroESL,* and *ClpB* genes. However, the most important one is *dnaJ* gene, as any mutation in *C. jejuni* unable the bacteria to colonize the enterocytes (Konkel et al., 1998). In the present work, *dnaJ* gene was detected in 88% of all tested sheep fecal samples. Relatively similar results were reported by many authors (Redondo et al., 2019) who verified the importance of *dnaJ* gene for colonization.

CONCLUSION

Campylobacteriosis control and prevention in sheep requires an understanding of the transmission routes, antibiogram, and virulence abilities of the isolates. The results gained in the current study demonstrated the presence of *Campylobacter* isolates and different degrees of resistance. The prevalence of the resistance may mainly be attributed to the misuse of antibiotics used for the treatment of *Campylobacter* infections, such as ciprofloxacin and tetracycline. Although the isolated strains carried both virulence and antibiotic resistance genes, continuous monitoring of the prevalence of *Campylobacter* strains and identification of associated genes for virulence and antibiotic resistance is urgently required to update effective treatment schedules for *Campylobacter* infection.

Finally, it is worth mentioning that the presence of virulence genes is an important predictor of strain virulence although it may not exactly predict the virulence of the isolated *Campylobacter* strains. Additionally, negative PCR results do not mean that there is no gene but could be attributed to a different primer binding site sequence or the presence of another gene with a similar function. To generalize the findings, extensive profiling that includes more virulence genes is required for other strains of *Campylobacter*.

DECLARATIONS

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Author's contribution

Amani Hafez performs collection, preparation, processing, and analysis of samples, isolation of bacteria, data acquisition, writing, preparation, and revision of the manuscript. The author has read and approved the data and final draft of the manuscript.

Competing interests

The author has declared no conflict of interest.

Ethical consideration

The author checked the manuscript for ethical issues, such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publishing and/or submission, and redundancy.

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REFERENCES

Abd El-Hamid MI, Abd El-Aziz NK, Samir M, El-Naenaeey EY, Abo Remela EM, Mosbah RA, and Bendary MM (2019). Genetic diversity of *Campylobacter jejuni* isolated from avian and human sources in Egypt. Frontiers in Microbiology, 10: 2353. DOI: https://www.doi.org/10.3389/fmicb.2019.02353

Aleksić E, Miljković-Selimović B, Tambur Z, Aleksić N, Biočanin V, and Avramov S (2021). Resistance to antibiotics in thermophilic campylobacters. Frontiers in Medicine. 8: 763434. DOI: https://doi.org/10.3389/fmed.2021.763434

Altschul SF, Gish W, Miller W, Myers EW, and Lipmanl DJ (1990). Basic Local Alignment Search Tool. Journal of Molecular Biology, 215: 403-410. DOI: https://www.doi.org10.14202/vetworld.2019.664-670

Babazadeh D and Ranjbar R (2022). *Campylobacter* species in the Middle East. Journal of Veterinary Physiology and Pathology. 2022; 1(1): 1-9. Available at: https://jvpp.rovedar.com/article_120791.html

Bacon DJ, Alm RA, Burr DH, Hu L, Kopecko DJ, Ewing CP, Trust TJ, and Guerry P (2000). Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. Infection and Immunity Journal, 68(8): 4384-4390. DOI: https://www.doi.org/10.1128/IAI.68.8.4384-4390.2000

Biswas D, Hannon SJ, Townsend HGG, Potter A, and Allan BJ (2011). Genes coding for virulence determinants of *Campylobacter jejuni* in human clinical and cattle isolates from Alberta, Canada, and their potential role in colonization of poultry. International Journal of Microbiology, 14: 25-32. DOI: https://www.doi.org/10.2436/20.1501.01.132

Bolinger H and Kathariou S (2017). The Current State of Macrolide Resistance in *Campylobacter* spp. Trends and Impacts of Resistance Mechanisms. Applied and Environmental Microbiology, 83: e00416-e00417. DOI: https://www.doi.org/10.1128/AEM.00416-17

Bolton DJ (2015). *Campylobacter* virulence and survival factors. Food Microbiology, 48: 99-108. DOI: https://www.doi.org/10.1016/j.fm.2014.11.017

Carvalho ACT, Ruiz-Palacios GM, Ramos-Cervantes P, Cervantes LE, Jiang X, and Pickering LK (2001). Molecular characterization of invasive and non-invasive *Campylobacter jejuni* and *Campylobacter coli* isolates. Journal of Clinical Microbiology, 39(4): 1353-1359. DOI: https://www.doi.org/10.1128/JCM.39.4.1353-1359.2001

Chansiripornchai N and Sasipreeyajan J (2009). PCR detection of four virulence-associated genes of *Campylobacter jejuni* isolates from Thai broilers and their abilities of adhesion to and invasion of INT-407 cells. Journal of Veterinary Medical Science, 71(6): 839-844. DOI: https://www.doi.org/10.1292/jvms.71.839

Charvalos E, Peteinaki E, Spyridaki I, Manetas S, and Tselentis Y (1996). Detection of ciprofloxacin resistance mutations in *Campylobacter jejuni* gyrA by nonradioisotopic single-strand conformation polymorphism and direct DNA sequencing. Journal of Clinical Laboratory Analysis, 10: 129-133. DOI: https://www.doi.org/10.1002/(SICI)1098-2825(1996)10:3<129::AID-JCLA3>3.0.CO;2-6

Dasti JI, Tareen AM, Lugert R, Zautner AE, and Gross U (2010). *Campylobacter jejuni*: a brief overview on pathogenicity associated factors and disease-mediating mechanisms. International Journal of Medical Microbiology, 300: 205-211. DOI: https://www.doi.org/10.1016/j.ijmm.2009.07.002

Datta S, Niwa H, and Itoh K (2003). Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. Journal of Medical Microbiology, 52(4): 345-348. DOI: https://www.doi.org/10.1099/jmm.0.05056-0

Duarte A, Santos A, Manageiro V, Martins A, Fraqueza MJ, Caniça M, Dominguesa FC, and Oleastrob M (2014). Human, food and animal *Campylobacter* spp. isolated in Portugal: high genetic diversity and antibiotic resistance rates. International Journal of Antimicrobial Agents, 44: 306-313. DOI: https://www.doi.org/10.1016/j.ijantimicag.2014.06.012

EFSA (2014a). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. EFSA Journal, 12(2): 3547. DOI: https://www.doi.org/10.2903/j.efsa.2014.3547

EFSA (2014b). The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012. EFSA Journal, 12(3): 3590. DOI: https://www.doi.org/10.2903/j.efsa.2014.3590

Gibreel A, Tracz DM, Nonaka L, Ngo TM, Connell SR, and Taylor DE (2004). Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet(O)-mediated tetracycline resistance. Antimicrobial Agents and Chemotherapy, 48(9): 3442-3450. DOI: https://www.doi.org/10.1128/AAC.48.9.3442-3450.2004

Haag LM, Fischer A, Otto B, Grundmann U, Kühl AA, Göbel UB, Bereswill S, and Heimesaat MM (2012). *Campylobacter jejuni* infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses. European Journal of Microbiology and Immunology, 2(1): 2-11. DOI: https://www.doi.org/10.1556/eujmi.2.2012.1.2

Hagos Y, Gugsa G, Awol N, Ahmed M, Tsegaye Y, Abebe N, and Bsrat A (2021). Isolation, identification, and antimicrobial susceptibility pattern of *Campylobacter jejuni* and *Campylobacter coli* from cattle, goat, and chicken meats in Mekelle, Ethiopia. PloS one, 16(2), e0246755. DOI: https://doi.org/10.1371/journal.pone.0246755

Hakanen A, Jalava J, Kotilainen P, Jousimies-Somer H, Siitonen A and Huovinen P (2002). *gyrA* polymorphism in *Campylobacter jejuni*: detection of *gyrA* mutations in 162 *C. jejuni* isolates by single-strand conformation polymorphism and DNA Sequencing. Antimicrob Agents Chemother, 46: 2644-2647. DOI: https://www.doi.org/10.1128/AAC.46.8.2644-2647.2002

Havelaar AH, Ivarsson S, Löfdahl M, and Nauta MJ (2013). Estimating the true incidence of campylobacteriosis and salmonellosis in the European Union, 2009. Epidemiology and Infection, 141: 293-302. DOI: http://www.doi.org/10.1017/S0950268812000568

Hermans D, Van Deun K, Martel A, Van Immerseel F, Messens W, Heyndrickx M, Haesebrouck F, and Pasmans F (2011). Colonization factors of *Campylobacter* jejuni in the chicken gut. Veterinary research, 42(1): 1-14. DOI: http://www.veterinaryresearch.org/content/42/1/82

To cite this paper: Hafez A (2022). Antimicrobial Resistance and Virulence Genes of *Campylobacter jejuni* Isolates from Diarrheic Sheep. *World Vet. J.*, 12 (2): 186-196. DOI: https://dx.doi.org/10.54203/scil.2022.wvj24

İlhan Z, Ekin İH, Gülaydın Ö (2021). Determination of Campylobacter fetus subsp. fetus and Campylobacter jejuni in Aborted Sheep Fetuses by Multiplex PCR Assay. Israel Journal of Veterinary Medicine, 76 (4): 161-167. Available at: http://www.ijvm.org.il/sites/default/files/ilhan.pdf

Indykiewicz P, Andrzejewska M, Minias P, S´pica D, and Kowalski J (2021). Prevalence and Antibiotic Resistance of *Campylobacter* spp. in Urban and Rural Black-Headed Gulls *Chroicocephalus ridibundus*. EcoHealth, 18: 147-156. DOI: https://www.doi.org/10.1007/s10393-021-01540-0

Iraola G, Hernandez M, Calleros L, Paolicchi F, Silveyra S, Velilla A, Carretto L, Rodríguez E, and Pérez R (2012). Application of a multiplex PCR assay for *Campylobacter fetus* detection and subspecies differentiation in uncultured samples of aborted bovine fetuses. Journal of Veterinary Science, 13(4): 371-376. DOI: https://www.doi.org/10.4142/jvs.2012.13.4.371

Kepner DE, Turnidge J, McCarthy LR, and Master RN (2003). Low levels of fluoroquinolone resistance in *Escherichia coli*. A five-year trend in Australia measured through the use of TSN Database Australia. Communicable diseases intelligence quarterly report, 27: 89-91. PMID: 12807281.

Khoshbakht R, Tabatabaei M, Hoseinzadeh S, Raeisi M, Aski HS, and Berizi E (2016). Prevalence and antibiotic resistance profile of thermophilic *Campylobacter* spp. of slaughtered cattle and sheep in Shiraz, Iran. Veterinary Research Forum, 7(3): 241. PMCID: PMC5094166.

Konkel ME, Kim BJ, Klena JD, Young CR, and Ziprin R (1998) Characterization of the thermalstress response of *Campylobacter jejuni*. Infection and Immunity Journal, 66(8): 3666-3672. DOI: https://www.doi.org/10.1128/IAI.66.8.3666-3672.1998

Lior H (1984). New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter* laridis. Journal of Clinical Microbiology, 20: 636–640. DOI: https://www.doi:10.1128/jcm.20.4.636-640.1984

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 gyrAmutations and the function of the CmeABC e_ux pump. American Society for Microbiology, 47: 390-394. DOI: https://www.doi.org/10.1128/AAC.47.1.390-394.2003

McDermott PF, Bodeis-Jones SM, Fritsche TR, Jones RN, Walker RD (2005). Broth microdilution susceptibility testing of *Campylobacter jejuni* and the determination of quality control ranges for fourteen antimicrobial agents. Journal of clinical microbiology, 43(12): 6136-6138. DOI: https://www.doi.org/10.1128/JCM.43.12.6136-6138.2005

Nur-Aziera-Aina CMN, Nur-Syafiqah MN, and Zaidah AR (2020). Detection of *campylobacter jejuni* among commercial broiler chickens in east-coast Malaysia. Journal of World`s Poultry Research, 10 (2): 367-370. DOI: https://dx.doi.org/10.36380/jwpr.2020.42

Ó Cróinín T and Backert S (2012). Host epithelial cell invasion by *Campylobacter jejuni*: Trigger or zipper mechanism?. Front Cell Infection Microbiology, 2: 25. DOI: https://www.doi.org/10.3389/fcimb.2012.00025

Pao S, Hagens BE, Kim C, Wildeus S, Ettinger MR, Wilson MD, Watts BD, Whitley NC, Porto-Fett ACS, Schwarz JG, et al. (2014). Prevalence and molecular analyses of *Campylobacter jejuni* and *Salmonella* spp. in co-grazing small ruminants and wild-living birds. Livestock Science, 160 (1): 163-171. DOI: https://doi.org/10.1016/j.livsci.2013.11.020

Park SF (2002). The physiology of *Campylobacter* species and its relevance to their roleas foodborne pathogens. International journal of Food Microbiology, 74: 177-188. DOI: https://www.doi.org/10.1016/S0168-1605(01)00678-X

Payot S, Bolla JM, Corcoran D, Fanning S, Mégraud F, and Zhang Q (2006). Mechanisms of fluoroquinolone and macrolide resistance in *Campylobacter* species. Microbes and Infection, 8: 1967-1971. DOI: https://www.doi.org/10.1016/j.micinf.2005.12.032

Pitkänen T (2013). Review of *Campylobacter* spp. in drinking and environmental waters.Journal of Microbiological Methods, 95(1): 39-47. DOI: https://www.doi.org/10.1016/j.mimet.2013.06.008

Quino W, Caro-Castro J, Hurtado V, Flores-León D, Gonzalez-Escalona N, and Gavilan RG (2022). Genomic Analysis and Antimicrobial Resistance of *Campylobacter jejuni* and *Campylobacter coli* in Peru. Frontiers in microbiology, 12. DOI: https://www.doi.org/10.3389/fmicb.2021.802404

Ranjbar R and Babazadeh D (2017). Contact with poultry and animals increases risk of Campylobacter infections in adults of Ardabil province, Iran. Universa Medicina, 36(1): 59–67. https://doi.org/10.18051/UnivMed.2017.v36.59-67

Redondo N, Carroll A, and McNamara E (2019). Molecular characterization of *Campylobacter* causing human clinical infection using whole-genome sequencing: Virulence, antimicrobial resistance and phylogeny in Ireland. PloS one, 14(7): e0219088. DOI: https://www.doi.org/10.1371/journal.pone.0219088

Roux F, Sproston E, Rotariu O, MacRae M, Sheppard SK, Bessell P, Smith-Palmer A, Cowden J, Maiden MCJ, Forbes KJ et al. (2013). Elucidating the etiology of human *Campylobacter coli* infections. PLoS ONE, 8: e64504. DOI: https://www.doi.org/10.1371/journal.pone.0064504

Rukambile E, Sintchenko V, Muscatello G, Kock R, and Alders R (2019). Infection, colonization and shedding of *Campylobacter* and *Salmonella* in animals and their contribution to human disease: a review. Zoonoses Public Health, 66: 562-578. DOI: https://www.doi.org/10.1111/zph.12611

Schiaffino F, Colston JM, Paredes-Olortegui M, François R, Pisanic N, Burga R, Peñataro-Yori P, and Kosek MN (2018). Antibiotic resistance of *Campylobacter* species in a paediatric cohort study. Antimicrob Agents Chemother, 63(2): e01911-01918. DOI: https://www.doi.org/10.1128/AAC.01911-18

Sheppard SK and Maiden MC (2015). The evolution of *Campylobacter jejuni* and *Campylobacter coli*. Cold Spring Harbor Perspectives in Biology, 7: a018119. DOI: https://www.doi.org/10. 1101/cshperspect.a018119

Sproston EL, Ogden ID, MacRae M, Forbes KJ, Dallas JF, Sheppard SK, Cody A, Colles F, Wilson MJ, and Strachan NJC (2010). Multi- locus sequence types of *Campylobacter*s carried by flies and slugs acquired from local ruminant faeces. Journal of applied microbiology, 109(3): 829-838. DOI: https://www.doi.org/10.1111/j.1365-2672.2010.04711.x

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30: 2725-2729. DOI: https://www.doi.org/10.1093/molbev/mst197

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Thompson JD, Higgins DG, and Gibson TJ (1994). Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22(22): 4673-4680. DOI: https://www.doi.org/10.1093/nar/22.22.4673

Wang L, Yaeger MJ, Hoffman LJ, and Zhang Q (2008). Emergence of a tetracycline- resistant *Campylobacter jejuni* clone associated with outbreaks of ovine abortion in the United States. Journal Clinical Microbiology, 46(5): 1663-1671. DOI: https://www.doi.org/10.1128/JCM.00031-08

Wieczorek K (2011). Resistance to quinolones and tetracycline and its molecular background among *Campylobacter* strains isolated in Poland. Biology Bulletin of The Veterinary Institute, 55(4): 613-618. Available at: https://www.semanticscholar.org/paper/Resistance-to-quinolones-and-tetracycline-and-its-Wieczorek/8cf3ac15dad942ce13cf02efe1f3606cf1782269

Wieczorek K and Osek J (2013). Antimicrobial resistance mechanisms among *Campylobacter*. BioMed Research International, 2013: e340605. DOI: https://www.doi.org/10.1155/2013/340605

Wieczorek K, Denis E, Lachtara B, and Osek J (2017). Distribution of *Campylobacter jejuni* multilocus sequence types isolated from chickens in Poland. Poultry Science, 96: 703-709. DOI: https://www.doi.org/10.3382/ps/pew343

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://www.doi.org/10.3389/fmicb.2018.01508

World Health Organisation (2018). Critically Important Antimicrobials for Human Medicine 6th Revision. Available at: https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/

Zheng J, Meng J, Zhao S, Singh R, and Song W (2006). Adherence to and invasion of human intestinal epithelial cells by *Campylobacter jejuni* and *Campylobacter coli* isolates from retail meat products. Journal of Food Protection, 69(4): 768-774. DOI: https://www.doi.org/10.4315/0362-028X-69.4.768

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Effect of Oral Administration of Honey on Hemato-biochemical Parameters of Dogs with Atopic Dermatitis

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ABSTRACT

Honey from *Trigona* species is widely used as herbal medicine in humans due to its antimicrobial, anti-inflammatory, and antioxidant effects as well as the potential to increase body resistance and boost blood formation. The current study aimed to determine the hemato-biochemical profile of dogs with atopic dermatitis treated with *Trigona* honey. The hematology profile included the measurement of erythrocytes, hemoglobin, hematocrit, and erythrocyte index, as well as blood biochemical parameters, including aspartate aminotransferase (AST), alanine transaminase (ALT), and blood sugar. A total of 12 local dogs aged 4 months old were divided into two treatment groups, namely the control group (G1) and treatment with liquid *Trigona* honey at a dosage of 5 ml/dog/day (G2) for 35 days. Then, blood was collected and tested for routine and chemical blood assay. The results showed that the administration of fresh *Trigona* honey (5 ml/day for 5 weeks) exhibited a significant increase in most of hematological variables of dogs with atopic dermatitis, compared to G1. The results of blood biochemical profiles (AST, ALT, and blood glucose) remained unaffected by the treatment of *Trigona* honey. It can be concluded that honey from *Trigona* spp. was safe to be given to the dogs with dermatitis and no adverse physiological effects were observed during the present study.

Keywords: Blood, Dermatitis, Dog, Hemato-biochemical, *Trigona* species honey

INTRODUCTION

Honey from *Trigona* spp. is widely used as herbal medicine in humans. It was reported to have antimicrobial, anti-inflammatory, and antioxidant effects, as well as the potential to increase body resistance and boost blood formation (McLoone et al, 2016; Baby et al., 2018). It has no or very low side effects (Erejuwa et al., 2012; Chuttong et al., 2015). Honey from *Trigona* has strong antioxidant potential because it contains polyphenols in the form of phenolic acids (chlorogenic, ferulic, caffeic, ellagic, vanillic, benzoic, cinnamic, and coumaric acids) and bioflavonoids (pinocembrin, apigenin, hesperetin, chrysin, quercetin, luteolin, myricetin, pinobanksin, galangin, and kaempferol) to scavenge free radicals (Nayik and Nanda, 2016; Baby et al., 2018). High levels of antioxidants also have a hepatoprotective function (Visweswara et al., 2016) that may be evident by hemato-biochemical alterations. Antioxidant activity is related to the breakdown of free radicals (Ahmend and Rao, 2013), and has an ameliorative effect on the clinical signs of canine atopic dermatitis (De Santiago et al., 2021). In addition, *Trigona* honey is rich in organic acids, proteins, amino acids, minerals, and Vitamin C. This type of honey is also reported to function as antibiotics, antitoxins, anti-inflammatory (Criner et al., 2014), immunostimulants, and blood-forming (Baby et al., 2018).

There are a few reports on the use of honey for the treatment of dermatitis in dogs. The use of honey as alternative medicine in dogs is effective to manage otitis externa (Maruhashi et al., 2016). However, the use of honey from *Trigona* spp. for the nutrition and treatment of diseases in dogs has not been widely investigated. Stray dogs are reported to have a very high incidence of dermatitis due to poor maintenance, irregular food, and high chances of infection from the environment (Purnama et al., 2019). Infections on external parts of the body can also impact physiological changes in animals, which can be seen from the hematological and biochemical profiles of the blood. Therefore, the current study aimed to determine the effects of oral administration of honey from *Trigona* spp. on hematological and biochemical profiles of blood as liver function indicators in dogs with atopic dermatitis.

MATERIALS AND METHODS

Ethical approval

The procedures performed in this study were guided by the principles of animal welfare, the Animal Welfare Act of the Faculty of Veterinary Medicine of Udayana University, Bali, Indonesia (No B/81/UN14.2.9/PT.01.04/2021).

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Research design

This study used 12 local dogs (2 males and 10 females) aged 2-6 months old diagnosed with atopic dermatitis with clinical signs of itching, redness, and swelling on the skin (Junaedi et al., 2020). Two dogs were served as a control group (G1) while 10 dogs were treated orally with fresh honey from *Trigona* spp. (G2, 5 ml/dog/day for 5 weeks). The honey was collected from a local farmer in Badung regency, Bali, Indonesia. All dogs were placed in separate cages and adapted to the study environment with a room temperature of 26-30°C and relative humidity of 71-85% for 14 days before the treatment. The dogs were given commercial dog dry food (PEDIGREE® Puppy, United Kingdom) for 14 days. Dogs were bathed regularly every week. The hemato-biochemical investigations were carried out three times on day 1 as well as on weeks 3 and 5.

Collecting blood samples

The blood samples were collected from the cephalic vein in a non-EDTA vial for routine hemato-biochemical examination without using any medication. The collected blood samples were stored in a cooler box and transported to the laboratory for blood examination on the same day within 30 minutes.

Routine blood tests

Routine blood tests were performed using an automated hematology analyzer (BC-2800Vet Mindray, Nanshan, Shenzhen, China). The hematologic investigation includes red blood cell count, hemoglobin, hematocrit, white blood cell count, lymphocytes, monocytes, and neutrophils.

Blood serum biochemical assay

Blood biochemical assays for aspartate aminotransferase (AST), and alanine transaminase (ALT) were performed using an automated chemistry analyzer (chemray 120; Rayto Co., Shenzhen, China). Evaluation of Blood glucose was performed using glukometer GlucoDr^{TM} (All Medicus Co., Ltd., Anyang, South Korea). Albumin levels were determined using a Spectrophotometer (Rayto life and AnalyticalScience Co., Ltd, Shenzhen, China). All tests were done in the Laboratory of Veterinary Internal Medicine, Faculty of Veterinary Medicine, University of Udayana, Bali, Indonesia.

Statistical analysis

All data were analyzed by using SPSS for windows 25 (SPSS Inc., Chicago, IL, USA). In order to investigate the statistical difference between the treatment and control group, Student's T-test was applied. The t-test probability result values greater than 0.05 were considered non-significant.

RESULT AND DISCUSSION

The results showed that the hematology profile values for all parameters were below the normal value range (Table 1). This means that the hematological profile, including red blood cells, hemoglobin, and hematocrit values, of dogs who suffered from atopic dermatitis (control group), was below the normal range for healthy dogs. The Hemoglobin and red blood cell (RBC) values below normal range indicated that dogs were anemic. This result indicated that the control dogs with atopic dermatitis had anemia at the beginning of the study. It could result from receiving no treatment and the presence of atopic dermatitis at the beginning of the study (Figure 1a). Atopic dermatitis usually causes anemia because the dogs feel uncomfortable with the itching leading to stress. Widyanti et al. (2018) reported that most dogs with dermatitis had anemia. Stress conditions also trigger anemia due to problems in the erythropoiesis process. Iron deficiency can be caused by ectoparasite infection, leading to low hemoglobin conditions which were previously reported by Sakina and Mandial (2013) in dogs with scabies and demodicosis. Bacterial infections also play a role in the occurrence of anemia, as bacteria use heme as a source of iron in their growth (Widyanti et al., 2018). This anemia condition was also confirmed by the hematocrit value below the normal range. Hematocrit value can be used to measure the degree of anemia or polycythemia.

The administration of honey from *Trigona* improved the hematological profile of dogs with dermatitis although the increase was not statistically significant ($p > 0.05$). The improvement of the hematological profile of dogs treated with honey indicated that honey from *Trigona* spp. contains 85% glucose and fructose, which can be used as a ready-to-use energy source in the body. This type of honey is rich in organic acids, proteins, amino acids, minerals, and vitamin C, which triggers the formation of erythrocytes and is easily absorbed into the gastrointestinal tract (Criner et al., 2014: Baby et al., 2018).

In addition, the high polyphenol content in honey from *Trigona* spp. did not damage internal organs, such as the liver and pancreas (Nayik et al., 2016; Baby et al., 2018). This is evident from AST, ALT, and blood glucose values which were recorded within the normal range (Table 1).

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Table 1. The hematological parameters of dogs with dermatitis and treated with *Trigona* spp. honey

Parameter	Treatment	Day 1	Week 3	Week 5	Normal Range*
RBC ± SD (x10^6/ µL)	G1	6.21 ± 2.56	4.10 ± 1.27	4.51 ± 1.09	5.5-8.5
	G2	4.60 ± 1.39	4.75 ± 0.39	4.84 ± 0.59	
Hemoglobin ± SD (g/dL)	G1	11.2 ± 2.26	8.9 ± 2.82	8.5 ± 1.55	12-18
	G2	8.98 ± 1.67	10.3 ± 0.92	9.35 ± 1.10	
PCV ± SD (%)	G1	30 ± 4.24	26 ± 8.48	30.35 ± 6.4	37-55
	G2	25.16 ± 6.49	30.8 ± 2.22	30.85 ± 3.4	
WBC± SD (x10^3/ µL)	G1	31.15 ± 251[a]	32.5 ± 0.98	28.39 ± 6.93	6.0-17.0
	G2	19.76 ± 7.45[b]	19.58 ± 6.44	15.93 ± 7.3	
Monocyte (%)	G1	8.00 ± 0.00	7.5 ± 3.53	7.01 ± 1.4	3-10
	G2	9.16 ± 6.61	6.33 ± 4.08	8.50 ± 2.73	
Eosinophil (%)	G1	3.50 ± 3.53	10.5 ± 2.12	9.23 ± 3.1	2-10
	G2	5.33 ± 5.68	12.6 ± 12.19	11.6 ± 5.46	
Lymphocyte (%)	G1	14.00 ± 2.82	19 ± 1.41	33.4 ± 6.27	12-30
	G2	12.66 ± 4.17	14.33 ± 5.85	26.8 ± 11.4	
Neutrophil (%)	G1	74.5 ± 0.70	60.5 ± 3.53	48.1 ± 13.9	60-77
	G2	72.83 ± 8.9	65.83 ± 10.9	53.00 ± 5.00	

G1: Control, G2: Treatment *Trigona* spp. honey, SD: Standard deviation, [a,b]: Different letters in column mean significant ($p < 0.05$), RBC: Red blood cell, PCV: Packed cell volume, WBC: White blood cell. *Reference: Weiss et al. (2010).

Table 2. The blood biochemical parameters of dogs with dermatitis and treated with honey from *Trigona* spp.

Parameter	Treatment	Day 1	Week 3	Week 5	Normal Range*
AST ± SD (u/L)	G1	55.0 ± 1.41	53.5 ± 7.7	44.20 ± 10.18	10-62
	G2	63.3 ± 23.61	51.83 ± 8.54	51.66 ± 13.86	
ALT ± SD (u/l)	G1	33.5 ± 3.53	31 ± 4.24	30.25 ± 8.84	10-94
	G2	35 ± 10.05	26.83 ± 7.19	30.16 ± 5.56	
Albumin (g/dL)	G1	2.75 ± 0.70[a]	1.9 ± 0.56[b]	2.20 ± 0.56[b]	3.2-4.7 (g/dL)
	G2	2.11 ± 0.37[a]	1.88 ± 0.19[b]	2.08 ± 0.14[b]	
Glucose (mg/dL)	G1	105.5 ± 9.19	105.5 ± 9.19	71.36 ± 9.00	53-117
	G2	93.5 ± 31.3	93.5 ± 31.3	72.83 ± 17.12	

G1: Control, G2: Treatment *Trigona* spp. honey, SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, [a,b]: Different letters in a row mean significant ($p < 0.05$). *Reference: Willard and Tvedten (2012)

Figure 1. Balinese local dog with atopic dermatitis before and after treatment with honey from *Trigona* spp.

A: Dog before treatment with the lesion of alopecia and visible erythema at the beginning of the study;
B: Three weeks after treatment, the fur starts to grow and the lesion of alopecia is smaller than before, the erythema is not as visible as day 1;
C: Five weeks after treatment, the soft fur is fully grown and no sign of erythema

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The administration of *Trigona* honey in dogs with dermatitis had a significant effect on the total WBC, monocytes, eosinophils, basophils, lymphocytes, and neutrophils ($p < 0.05$). However, there was an increase in eosinophils and lymphocytes and a decrease in total white blood cells (WBC) and neutrophils, which indicated a healing response in dogs suffering from dermatitis (Ferrer et al., 2014). An increase in WBC number has many potential etiologies. The Increase of WBC (Leukocytosis) is a common sign of bacterial infection, chronic inflammatory conditions, or some medications. In the acute stage of several bacterial infections, primarily mature and immature neutrophils can be found. The obtained results of the current study indicated an increased number of WBC in the first week of the study in both groups. Stressors also can trigger acute leukocytosis. The most common type of leukocytosis is neutrophilia, which can be due to infections, stress, chronic inflammation, and medication use. Leukocytosis occurs due to hypersensitivity reactions, immune dysregulation, and/or bacterial and fungal infections on the skin surface. The total WBC value at the next week of honey administration leads to normal values. In other words, honey as an anti-inflammatory agent also has the potential to increase leukocytes (Majtan, 2014).

An increase in monocytes (monocytosis) followed by neutropenia indicates recovery from inflammation and infection. Honey plays an important role in activating macrophages, which play an active role in wound healing due to inflammation in the skin (Majtan, 2014). Although uncommon, eosinophilia may suggest allergic conditions such as dermatologic conditions and parasitic infections (Alangari et al., 2017). Lymphocytosis can occur in patients with viral infections and hypersensitivity reactions. The administration of honey could increase lymphocytes which act as the immune system of the body (Ahmed et al., 2018). Flavonoids from honey stimulate antibody production and antibacterial activity (Yaghoobi and Kazerouni, 2013). In patients with dermatitis, there is a strong corticoid reaction with systemic stress affecting lymphopenia and eosinophilia as well as decreasing neutrophil values (Dulman et al., 2015). Neutrophilia can occur because a secondary pathogen infects the dog's skin (Breathnach et al., 2011).

The results of the AST and ALT values showed an average decrease after being given honey from *Trigona* spp. for 5 weeks, but the decrease was still within the normal range (Table 2). The administration of fresh *Trigona* honey to dogs with dermatitis had no significant effect ($p > 0.05$) on AST and ALT activity. The decrease in AST and ALT values showed that honey does not cause interference with liver function. Factors that cause increased AST activity are tissue inflammation, toxicity, malnutrition, and hypo-albumin. Therefore, at the beginning of the study, the AST value was higher than the weeks after the administration of honey. This is because the dogs used in this study were stray dogs without owners and roamed in the wild, with a high level of stress. With the administration of honey, skin tissue damage was reduced, leading to a decrease in AST values at week 5. The clinical sign showed that the fur started to grow, erythema decreased, and dogs did not show any itching signs after the treatment (Figures 1b and 1c). This condition shows that honey from *Trigona* contains high levels of antioxidants and has a hepatoprotective effect (Visweswara et al., 2016) by scavenging free radicals (Nayik et al., 2016; Baby et al., 2018) because it contains hydroxyl groups that act as reducing agent and as a hydrogen donor against free radicals (Palupi and Martosupono, 2009). Honey from *Trigona* contains small amounts of sucrose, so it does not increase AST values (Botezelli et al., 2012), and triggers inflammation in the liver (Fu et al., 2010; Sanchez-Lozada et al., 2010).

The ALT activity of dogs with dermatitis after administration of *Trigona* honey for 5 weeks was 30.16 ± 5.564 (u/L). This value was still within the normal range for healthy dogs (5-60 u/l). An increase in ALT and AST activity up to 2-4 times the normal value indicates liver damage. ALT enzymes are known to be found in liver cells, and used as an indicator of hepatocellular destruction. If the ALT value is higher than the AST, it indicates acute liver parenchymal damage, while the chronic process shows the opposite result. The increase in ALT in this study did not show mild or severe damage because it was still within normal limits. Polyphenol compounds as antioxidants effectively maintain enzyme activity in the blood (Kikuzaki and Nakatami, 1993). Likewise, honey from *Trigona* spp. did not trigger an increase in the amount of blood glucose as an indication that there was no disruption of insulin hormone activity in glycogenesis. This is also due to the high fructose content in *Trigona* honey.

Fructose stimulates glucokinase enzymes to increase glucose uptake by the liver, glycogen storage, and stimulates hexokinase enzymes that play a role in the phosphorylation of glucose by adenosine triphosphate into glucose-6-phosphate when blood sugar levels are high (Erejuwa et al., 2012). Fructose has an important role in producing energy in the liver. Fructose does not require insulin to enter the cells. Furthermore, the metabolic process of fructose is also different from that of glucose. The rate of absorption of fructose is slower than that of glucose. If fructose is not absorbed completely, then fructose is fermented by fungi or bacteria and then converted into ethanol and carbon dioxide (Prahastuti, 2011). The content of flavonoids lowers blood sugar levels by donating one electron to an unpaired electron in free radicals formed through the pathway of glucose metabolic activity. The antioxidant content works to fight against free radicals and oxidative stress by providing one Hydrogen atom to convert free radicals into neutral and non-destructive compounds.

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CONCLUSION

Dogs with dermatitis were found with anemia and had a low number of erythrocytes, hemoglobin levels, and PCV values. The administration of fresh *Trigona* honey resulted in a numerical increase in the number of erythrocytes, hemoglobin, and hematocrit. The administration of *Trigona* honey for 35 days exhibited no significant changes in the values of AST, ALT, and blood glucose. Thus, administration of *Trigona* honey in liquid form at a dosage of 5 mL daily for 5 weeks is safe to give to dogs with atopic dermatitis. Further research is needed to find the most optimal dosage of *Trigona* honey to treat atopic dermatitis in dogs.

DECLARATIONS

Authors' contributions

I Nyoman Suartha designed the study, analyzed it, and wrote the manuscript. Luh Made Sudimartini and Putu Devi Jayanti collected the samples and analyzed the data. Ni Putu Ayu Dewi Wijayanti supports the conduct of the study. All authors have read and approved the data and final draft of the manuscript.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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Competing interests

The authors declare that there are no competing interests.

REFERENCES

Ahmend MF, and Rao AS (2013). Comparative hepatoprotective activities of selected Indian medicinal plants. Global Journal of Medicinal Research, 13(2): 14-21. Available at: https://globaljournals.org/item/1632-comparative-hepatoprotective-activities-of-selected-indian-medicinal-plants

Ahmed S, Sulaiman SA, Baig AA, Ibrahim M, Liaqat S, Fatima S, Jabeen S, Shamim N, and Othman NH (2018). Honey as a potential natural antioxidant medicine: An insight into its molecular mechanisms of action. Oxidative Medicine and Cellular Longevity, Article ID: 8367846. DOI: https://www.doi.org/10.1155/2018/8367846

Alangari AA, Morris K, Lwaleed BA, Lau L, Jones K, Cooper R, and Jenkins R (2017). Honey is potentially effective in the treatment of atopic dermatitis: Clinical and mechanistic studies. Immunity, Inflammation and Disease, 5(2): 190-199. DOI: https://www.doi.org/10.1002/iid3.153

Baby N, Anil KV, and Minol V (2018). Biological and pharmacological potentials of *trigona irridipennis* bee products: A review. World Journal of Pharmaceutical Research, 7(17): 651-663. DOI: https://www.doi.org/10.20959/wjpr201817-13452

Botezelli JD, Cambri LT, Ghezzi AC, Dalia RA, Voltarelli FA, and de Mello MAR (2012). Fructose-rich diet leads to reduced aerobic capacity and to liver injury in rats. Lipids Health Diseases, 11(78): 1-9. DOI: https://www.doi.org/10.1186/1476-511X-11-78

Breathnach RM, Quinn, PJ, Baker, KP, Mc Geady T, Strobl E, Abbott Y, and Jones BRJ (2011). Association between skin surface ph, temperature and staphylococcus pseudintermedius in dogs with ImR-LPP. Veterinary Dermatology, 22(4): 312-318. DOI: https://www.doi.org/10.1111/j.1365-3164.2010.00946.x

Criner GJ, Connett JE, Aaron SD, Albert RK, Bailey WC, Casaburi R, Cooper JAD, Curtis JL, Dransfield MT, Han MK et al. (2014). Simvastatin for the prevention of Exacerbations in Moderate-to-Severe COPD. The New England Journal of Medicine, 370: 2201-2210. DOI: https://www.doi.org/10.1056/NEJMoa1403086

Chuttong B, Chanbang Y, Sringarm K, and Burgett M (2015). Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand). Food Chemistry, 192: 149-155. DOI: https://www.doi.org/10.1016/j.foodchem.2015.06.089

De Santiago MS, Arribas JLG, Llamas YM, Becvarova I, and Meyer H (2021). Randomized, double-blind, placebo-controlled clinical trial measuring the effect of a dietetic food on dermatologic scoring and pruritus in dogs with atopic dermatitis. BMC Veterinary Research, 17(1): 1-8. DOI: https://www.doi.org/10.1186/s12917-021-03063-w

Dulman OM, Anton A, and Solcan G (2015). Variations in standard blood count and biochemical parameters in dogs with atopic dermatitis. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 72(1): 86-89. DOI: https://www.doi.org/10.15835/buasvmcn-vm:10808

Erejuwa O, Sulaiman SA, and Wahab AMS (2012). Honey: A novel of antioxidant. Molecules, 17(4): 4400-4423. DOI: https://www.doi.org/10.3390/molecules17044400

To cite this paper: Suartha IN, Sudimartini LM, Jayanti PD, and Wijayanti NPAD (2022). Effect of Oral Administration of Honey on Hemato-biochemical Parameters of Dogs with Atopic Dermatitis. *World Vet. J.,* 12 (2): 197-202. DOI: https://dx.doi.org/10.54203/scil.2022.wvj25

Ferrer L, Raverat I, and Silbermayr K (2014). Immunology and pathogenesis of canine demodicosis. Journal of Veterinary Sciences, 3(71): 1324-1331. DOI: https://www.doi.org/10.1111/vde.12136

Fu J, Sun H, Wang Y, Zheng W, Wang Q, and Shi Z (2010). The effects of fat and sugar-enriched diet and chronic stress on nonalcoholic fatty liver disease in male wistar rats. Digestive Diseases Sciencei, 55(8): 2227-2236. DOI: https://www.doi.org/10.1007/s10620-009-1019-6

Junaedi PA, Suartha IN, and Sudimartini LM (2020). Healing complex dermatitis lesions with herbal cream preparations. Buletin Veteriner Udayana, 12(1): 19-23. DOI: https://www.doi.org/10.24843/bulvet.2020.v12.i01.p04

Kikuzaki H, and Nakatami N (1993). Antioxidant effect of some ginger constituents. Journal of Food Science, 58(6): 1407-1410. DOI: https://www.doi.org/10.1111/j.1365-2621.1993.tb06194.x

Majtan J (2014). Honey: An immunomodulator in wound healing. The International Journal of Tissue Repair and Regeneration, 22(2): 187-192. DOI: https://www.doi.org/10.1111/wrr.12117

Maruhashi E, Braz BS, Nunes T, Pomba C, Belas A, Duarte-Correia JH, and Lourenço AM (2016). Efficacy of medical grade honey in the management of canine otitis externa–a pilot study. Veterinary Dermatology, 27(2): 93-8e27. DOI: https://www.doi.org/10.1111/vde.12291

McLoone P, Oluwadun A, Warnock M, and Fyfe L (2016). Honey: A therapeutic ant for disorders of the skin. Central Asian Journal of Global Health, 5(1): 241-248. DOI: https://www.doi.org/10.5195/cajgh.2016.241

Nayik GA, Dar BN, and Nanda V (2016). Optimization of the process parameters to establish the quality attributes of dpph radical scavenging activity, total phenolic content, and total flavonoid content of apple (malusdomestica) honey using response surface methodology. International Journal of Food Properties, 19(8): 1738-1748. DOI: https://www.doi.org/10.1080/10942912.2015.1107733

Palupi IA, and Martosupono M (2009). Buah merah potensi dan manfaatnya sebagai antioksidan (Red fruit: Potential and benefits as antioxidant). The Journal of Indonesian Medical Plant, 8(2): 42-48. Available at: http://ejournal.litbang.kemkes.go.id/index.php/toi/article/view/1279

Prahastuti S (2011). Consuming excessive amount of fructose may affect our health. Maranatha Journal of Medicine and Health, 10(2): 173-189. Available at: https://www.neliti.com/publications/151132/konsumsi-fruktosa-berlebihan-dapat-berdampak-buruk-bagi-kesehatan-manusia#cite

Purnama KA, Winaya IBO, Suartha IN**,** Mirah Adi AAA, Erawan IGMK, and Kardena IM (2019). Histopathological features of dog's skin with dermatitis. Journal Veteriner, 19(3): 608-615. DOI: https://www.doi.org/10.19087/jveteriner.2019.20.4.486

Sakina A, and Mandial RK (2013). Haematobiochemical changes in canine scabies. Vetscan, 7(2): 27-30. Available at: https://journal.vetscan.co.in/index.php/vs/article/view/134

Sanchez-Lozada LG, Mu W, Roncal C, Sautin YY, Abdelmalek M, Reungjui S, Le M, Nakagawa T, Lan HY, Yu X et al. (2010). Comparison of free fructose and glucose to sucrose in the ability to cause fatty liver. European Journal of Nutrition, 49(1): 1-9. DOI: https://www.doi.org/10.1007/s00394-009-0042-x

Visweswara P, Thevan K, Salleh N, and Hua S (2016). Biological and therapeutic effects of honey produced by honey bees and stingless bees: Comparative review. Revista Brasileira de Farmacognosia, 26(5): 657-664. DOI: https://www.doi.org/10.1016/j.bjp.2016.01.012

Weiss DJ, Wardrop KJ, and Schalm OW (2010). Schalm's veterinary haeamtology. 6th edition. Ames, Iowa, Wiley–Blackwell, pp. 799-810. Available at: https://www.noor-book.com/en/ebook-Schalm%E2%80%99s-Veterinary-Hematology-6th-ed--pdf

Widyanti A, Suartha IN, Erawan IGMK, Anggreni LD, and Sudimartini LM (2018). Dog hemogram with complex dermatitis. Indonesia Medicus Veterinus, 7(5): 576-587. DOI: https://www.doi.org/10.19087/imv.2018.7.5.576

Willard MD and Tvedten H (2012). Small animal clinical diagnosis by laboratory methods. Elsevier Health Sciences Misouri, pp. 399-402. Available at: https://www.sciencedirect.com/book/9781437706574/small-animal-clinical-diagnosis-by-laboratory-methods

Yaghoobi R, and Kazerouni A (2013). Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory anti-oxidant and anti-viral agent: A review. Jundishapur Journal of Natural Pharmaceutical Products, 8(3): 100-104. DOI: https://www.doi.org/10.17795/jjnpp-9487

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The Transmission Pattern of Amoebiasis in Bale Zone, South East Ethiopia

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ABSTRACT

Amoebiasis is a primarily zoonotic disease, mainly transferred through the fecal-oral route and waterborne. Amoebiasis is still a big challenge for human and animal health and is a major cause of diarrhea in developing countries, including Ethiopia. Therefore, the study was conducted to assess the epidemiology of the disease in humans, dogs, and the occurrence of the parasite in water bodies. A prospective cross-sectional study was conducted in selected districts of the Bale zone in southeastern Ethiopia. Pet owners were selected randomly. Socio-demographic data were collected using a questionnaire and fecal samples were used to perform microscopic examination. A total of 383 fecal samples of humans, 383 fecal samples of dogs, and 58 water samples were studied from December 2019 to July 2020. Of 383 humans, 179 were males and 186 were females, while 94 individuals were grouped as children younger than 8 years, 164 were grouped as youth within the age range of 8-18 years, and 125 were grouped as adults who were older than 18 years. Of 383 local breeds, dogs were grouped as 87 puppies younger than one year, 192 young dogs with the age range of 1-2 years, and 104 adult dogs who were older than 2 years. Fecal samples were taken from 173 male and 210 female dogs. The water samples were taken randomly from the water sources (river, lake, pond, or water tank) at different sites where dogs and humans can easily contact water to use for different purposes. Of the total samples, 70 humans (18.3%), 63 dogs (16.5%), and 16 water samples (27.6%) were contaminated with the parasite. The major risk factors for the transmissions of parasites were contaminated drinking water, large family size, open-air defecation, and improper handwashing. The present study revealed that the human reservoir was a major risk factor for the spread and transmission of amoebiasis in dogs. The high prevalence of the disease might be due to open-air defecation, unhygienic health practices, domestic animals inside the houses, and using local water bodies as a drinking source.

Keywords: Amoeba, Dog, Human, Transmission, Water

INTRODUCTION

Amoebiasis has been introduced to the scientific community since 300 BC. *Entamoeba histolytica* (*E. histolytica*) was identified in human fecal samples in 1875. The genus *Entamoeba* comprises several species, such as *E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. polecki*, *E. nutalli*, *E. chattoni*, *E. coli*, and *E. Hartmanni* (Ekanayake et al., 2006), which infects a wide range of mammals, including humans, non-human primates (dogs, cats, swine, rats, rabbits), and reptiles (such as snakes and lizards). Recent studies revealed the presence of *E. polecki* in swine, goats, and humans (Abioye et al., 2019). Among reptiles, snakes and lizards are infected by *E. invadens* which causes amoebiasis in these animals (Chia et al., 2009). Amoebiasis is a major cause of death in developing countries with an estimated rate of 50 million cases per year and 100000 deaths per year (Xim´enez et al., 2010).

The main hosts of *E. histolytica* are humans and primates. Infected dogs and cats can carry this pathogen. Dogs cannot transmit this pathogen, they only pass non-infective fragile trophozoite. However, dogs and other animals can be infected by humans (Ashar et al., 2014). The resistant cysts are passed through the excreta of chronic carriers and asymptomatic individuals. Seropositive Human immunodeficiency virus (HIV) case is a risk factor for invasive extra-intestinal amoebiasis (Hung et al., 2008). The main reservoir and source of infection are humans (Verkerke et al., 2012).

Amoeba produces cysts during unfavorable conditions and spreads to hosts due to unhygienic conditions. Infective cysts reach the hosts through unprotected water and contaminated food (Prakash and Bhimji, 2022). The life cycle of amoebiasis starts with the ingestion of infective cysts. The cysts germinate out and become feeding trophozoites, and then the trophozoites multiply asexually (Tanyuksel and Petri, 2003). The drinking water contaminated with fecal matter, human carriers (such as food handlers and livestock workers), and poor hygienic conditions all become major causes of the infection spread (Ashar et al., 2014). Microscopic examination of fecal samples needs three specimens taken on a

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separate day to increase the sensitivity of the test (Fotedar et al., 2007). The molecular diagnosis is the best method of identification; however, it cannot be used as a routine technique to identify the parasite due to the high cost and lack of standardization (Ngui et al., 2012).

The epidemiological studies of amoebiasis in domestic animals, reptiles, and other animals are rare. In Nigeria, the prevalence rate of amoebiasis in drill monkeys and chimpanzees was reported as 40% and 66%, respectively (Akpan et al., 2010). In a study conducted in the United Kingdom at Twycross zoo, the prevalence of amoebiasis was 81.7% in primates among which Old World Colobinae primates showed the highest prevalence of *Entamoeba* infection (Regan et al., 2014). Studies from Pakistan indicated that the prevalence of amoebiasis was 6% for local dogs, 2% for exotic, and 1% for crossbreeds (Ashar et al., 2014). Appropriate health practices (food hygienic practice, controlling open field defecation, hand washing, maintaining good environmental sanitation, especially in controlling the quality of drinking water, health education to the general public and food trade on the observance of good personal, environmental, and food hygiene) are the best methods to prevent this disease (Dickson et al., 2017). There is a gap in studies addressing the prevalence of amoebiasis in humans and dogs interface to measure the effect of the human amoebiasis on dogs amoebiasis as well as the effect of environmental health on the prevalence of amoebiasis in the study area. Therefore, the current study was designed to measure the prevalence and associated risk factors of amoebiasis in humans and dogs, and the occurrence of *Entamoeba* species in water bodies.

METHODS AND MATERIALS

Study area and duration

The present study was carried out in Delomena and its surrounding districts (Haranabuluk and Medawalabu) of the Oromiya regional state, Southeast Ethiopia about 430 km away from Addis Ababa, Ethiopia, from December 2019 to July 2020. The area was selected due to the proximity of Delomana Hospital, Ethiopia, to sample examination and ease of access to transportation. The altitude of the selected districts is between 850 m and 2800 m above sea level. The area gains a bimodal rainfall rained from September to November and March to June. An average annual temperature of 20-25°C and rainfall of 200 mm were recorded in 2020 (BZANRO, 2020).

Study population and sources

The study population consisted of dogs with their owners. The dog owners were included in the study using a simple random sampling technique. A total of 383 dog owners with their dogs (383 human and 383 dogs) were included in the study. Out of 383 humans (179 males and 186 females), 94 were grouped as children under the age of 8 years old, 164 individuals with the age range of 8-18 years were grouped as young, and 125 were grouped as adults who aged over 18 years old. Regarding the dogs, they were grouped as 87 puppies younger than one year, 192 young dogs with the age range of 1-2 years, and 104 adult dogs who were older than 2 years. All dogs included in the current study were local breeds, among which there were 173 males and 210 females. The owner's sociodemographic data were collected. Dogs under different management systems were sampled. The dog's demographic characteristics, management practices, and history of deworming and vaccination were also recorded. The questionnaires were distributed among the dog owners. The plastic container of 1-liter volume was used for water sampling from different water sources (river, lake, wells, springs, and piped water supply). Using this technique, 58 water samples were collected.

Study design

A cross-sectional study was conducted based on a questionnaire survey (Thrusfield, 2005) and microscopic examination of stool samples by direct fecal smear and floatation method. The presence of *Entamoeba* cysts and trophozoites in fecal and water samples were recorded as positive for *Entamoeba* species and further investigation of the historical background of dog owners with their dogs and the conditions of water bodies were carried out to identify the risk factors for the disease through questioner surveys.

Sampling methods and sample size

A total of 24 kebeles were selected by simple random sampling from the study area. A random sampling method was used to select the pet owners from the selected kebele. The desired sample size was calculated using a standard method based on the previous finding with a 5% expected prevalence of amoebiasis in Delomana town by Begna et al. (2014) results with 5% absolute precision. So, the calculated sample size included 383 dog owners, 383 dogs, and 58 water samples.

Data collection and analysis

Data were collected through direct smears and floatation method of fecal samples (microscopical examination) and a questionnaire survey. Each participant was asked to provide the fecal sample three times on different days to avoid

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false-negative results. All samples were placed in the air. Watertight samples and the vials were labeled with specific codes. Then, all stool samples kept in dry universal bottles were sent immediately to the Delomena Hospital Laboratory, Mena town, Ethiopia, for further studies. Ritchie's fecal concentration technique (Ritchie, 1948) and polyvinyl alcohol fixation of stools were carried out to obtain maximum sensitivity (Jensen et al., 2000). The fresh stool specimen was preserved with polyvinyl alcohol, and kept cool (4°C). Microscopic examination of fresh stool samples was carried out according to Soulsby (2006). A saline method and Lugol's iodine method were used to evaluate trophozoite and cysts. Motile trophozoites were observed and identified. All fecal samples were examined for *E. histolytica* analysis as described by Adam et al. (1979) and Soulsby (2006). A positive sample would contain motile amoebic trophozoites measuring 10-60 µm in size. The number of trophozoites and cysts was counted as described by Soulsby (2006). Stool specimens were stained with Lugol's iodine for the identification of cysts. The ocular micrometer was used to measure the size of the trophozoite or cysts. The *Entamoeba* cysts were identified based on the size and number of nuclei, as well as the presence and shape of the glycogen mass of chromatid bars. Maximum care was taken to select pathogenic *E. histolytica*. However, the result was confirmed as *Entamoeba* species due to the lack of confirmation by molecular diagnosis.

A questionnaire was prepared based on the standard questionnaire of the World Health Organization (WHO) and demographic information (sociodemographic factors, issues related to amoebiasis, hygiene, food handling system, drinking water sources, dog management practices, knowledge of parasitic diseases, and use of anthelmintics, the purpose of keeping dogs and the breeds of dogs) were collected.

Methods for detecting *Entamoeba* cyst in water

A total of 58 samples of high-turbidity water (100 mL) were collected from different sites of water bodies, including sludge, surface water, and groundwater. The water samples were taken randomly from the water sources at five different locations. Four different specimens, namely wet soil, mud, turbid water, and clean water were collected from each sample location. Each sample was then subjected to centrifugation and floatation techniques (Xiao et al., 2004). Slides were prepared, stained, and subjected to microscopy (XSZ-210, China).

Statistical analysis

Data were analyzed by the SPSS statistical software, version 23. The Chi-Square test and multivariable logistic regression were performed. Odds ratios were recorded. The Chi-Square test was used to evaluate the association between hypothesized risk factors and amoebiasis infection. Further analysis of the association was made by the multivariable logistic regression, and the odds ratio (OR), computed as the exponent of the respective regression coefficients, was used to quantify the effect of risk factors on the likelihood of amoebiasis. The confidence level was held at 95% and $p \leq 0.05$ was used to check the significance level in the analysis of generated data by logistic regression.

RESULTS

The overall prevalence of *Entamoeba* species in humans, dogs, and the water bodies in study areas is presented in Table 1. Of the samples, 70 (18.3%) humans, 63 (16.5%) dogs, and 16 (27.6%) water samples were positive for *Entamoeba* species.

Table 1. The overall prevalence of *Entamoeba* species in humans, dogs, and the water bodies in Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Study Districts	Humans		Dogs		Water bodies	
	Tested samples (N)	Positive (prevalence percentage)	Tested samples (N)	Positive (prevalence percentage)	Tested samples (N)	Positive (prevalence percentage)
Medawalebu	105	26 (24.8)	105	25 (23.8)	12	5 (41.7)
Haranabuluk	125	24 (19.2)	125	20 (16)	20	5 (25)
Delomena	153	20 (13.1)	153	18 (11.8)	26	6 (23.1)
Total	383	70 (18.3)	383	63 (16.5)	58	16 (27.6)

N: Number

Risk factors and prevalence of *Entamoeba* species in humans

The prevalence of amoebiasis was determined based on family size, living conditions, district, sources of drinking water, age, and educational status of the study population. Of those risk factors, family size, age, district, standards of

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living condition, sources of drinking water, and educational status were statistically significant ($p \leq 0.05$, Tables 2 and 3). The prevalence of human amoebiasis was higher in participants with the largest family size, defecation in the field, poorest living conditions, age range of 8-18 years, being users of the lake as water sources for household consumption, and illiteracy ($p \leq 0.05$), compared to other groups. However, there was no significant difference between male and female participants as shown in Table 3 ($p > 0.05$).

Statistical analyses of risk factors (demographics of participants) with amoebiasis through logistic regression analysis are illustrated in Tables 2 and 3. The contamination of drinking water (occurrences of *Entamoeba* species in water bodies) was statistically associated with the prevalence of human amoebiasis ($p \leq 0.05$). The prevalence of amoebiasis was higher in individuals who used contaminated drinking water (OR= 67.05, CI= 31.303- 143.618, $p \leq 0.05$), compared to those who used clean water for household consumption (absence of *Entamoeba* species in water bodies).

In terms of defecation, the open-air defecation group showed a higher infection rate of 26.4% (OR= 0.367, CI = 0.195- 0.689, $p \leq 0.05$), compared to the users of a toilet with a 6.4% infection rate of *Entamoeba* species. Moreover, a univariate analysis of the socio-demographic factors indicated that the prevalence rate of amoebiasis was associated with the living condition and the family size of participants.

The users of lake, river, well and spring water for household consumption were at higher risk, compared to the users of the piped water supplied by the municipalities. A significant association was observed between the sources of drinking water (lakes, rivers, and spring uses for drinking water, OR = 0.762, CI = 0.633-0.917, $p \leq 0.05$) and the prevalence of amoebiasis in participants. It was observed that the prevalence rate decreased from 29.8% to 4.2%, with the lower education level of the participants (OR=0.569, CI = 0.409-0.790; $p \leq 0.05$).

A total of 105 (27.41%) participants were from Medawolebu district, Ethiopia. Infection was higher in respondents from the Medawolebu district, Ethiopia (OR= 1.477, CI = 1.072-2.036, $p \leq 0.05$), compared to those from the Delomena and Heranabuluk districts of Ethiopia. The prevalence rate confirmed an age dependency association, with a significantly highest prevalence of amoebiasis in respondents of the age group between 8-18 years old (OR = 1.588, CI = 1.015-2.485, $p \leq 0.05$), compared to other age groups.

There was no significant association between the prevalence of *Entamoeba* species infection and the sex of the participants, however, males (19.8%) had a slightly higher prevalence rate, compared to females (16.7%).

Table 2. The potential risk factors associated with *Entamoeba* species in human Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Variable		Tested samples (N)	Number of positive (%)	X^2 (Chi-square)	Logistic regression	
					OR (95% CI)	P-value
Presence of *Entamoeba* species in waterbody						
	Occurrence	314	54 (78.3)	202.743	67.050 (31.303-143.618)	0.001
	Absence	69	16 (5.1)		57.968 (25.652-130.994)	
Defecation						
	Open-air	193	51 (26.4)	20.463	0.367 (0.195-0.689)	0.001
	Sometimes use toilet	65	11 (16.9)		0.352 (0.174-0.710)	
	Use toilet	125	8 (6.4)		0.337 (0.172-0.659)	
Family size						
	<6	307	32 (10.4)	63.883	6.513 (2.787-15.220)	0.001
	>6	76	38 (50)			
Income						
	High income	117	11 (9.4)	8.884	0.252 (0.111-0.568)	0.001
	Low income	266	59 (22.2)		0.257 (0.108-0.610)	
Handwashing						
	Proper handwashing	168	18 (10.7%)	11.459	0.46 (0.23- 0.9)	0.0016
	Improper handwashing	215	52 (24.2%)			

N: Number

The prevalence and risk factors of amoebiasis in dogs

The result of the microscopic examination of the fecal samples of the dogs is presented in Table 1. The results indicated that out of 383 fecal samples examined, 63 (16.5%) were positive for *Entamoeba* species. The present study revealed a significant association between amoebiasis in dog owners and amebiasis in dogs ($p \leq 0.05$). A higher prevalence of amoebiasis was observed in dogs whose owners had amoebiasis than in dogs whose owners did not have amoebiasis. The age and sex-wise prevalence of *Entamoeba* species were observed as the pups and females had higher prevalence, compared to adults and males (Table 4).

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Table 3. The potential risk factors (sociodemographic) factor associated with *Entamoeba* species in human Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Variable	Tested samples (N)	Positive samples (%)	X^2 (Chi-square)	Logistic regression	
				OR (95% CI)	P-value
Sources of water					
River	199	40 (20.1)	23.496	0.762 (0.633- 0.917)	0.004
Lake	40	15 (37.5)			
Well	28	7 (25)			
Spring	14	3 (21)			
Piped-water supply	102	5 (4.9)			
Literacy					
Illiterate	141	42 (29.8)	23.083	0.569 (0.409-0.790)	0.008
Read and write	112	17 (15.2)		0.563 (0.401-0.789)	
Elementary school	63	8 (12.7)		0.536 (0.382-0.751)	
High school	43	2 (4.7)		0.518 (0.366-0.735)	
Professional	24	1 (4.2)		0.536 (0.376-0.765)	
District					
Delomena	153	20 (13.1%)	5.803	1.47 7(1.072-2.036)	0.017
Heranabuluk	125	24 (19.2%)			
Medawolebu	105	26 (24.8%)			
Age					
Child<8	94	9 (9.6)	8.08	1.59 (1.01- 2.5)	0.043
Young (>8<18 yr)	164	41 (23.6)			
Adult (> 18yr)	125	20 (17.4)			
Sex					
Male	179	39 (19.8)	0.628	1.0	0.428
Female	186	31 (16.7)			

N: Number

Table 4. The potential risk factors associated with *Entamoeba* species in dogs of Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Variable	Tested samples (N)	Positive samples (%)	X^2 (Chi-square)	Logistic regression	
				OR (95% CI)	P-value
Owners' health status					
Infected by *Entamoeba*	70	18.3	299.01	620 (165.71-2319.73)	0.001
Not infected by *Entamoeba*	313	81.7			
Age					
≤1 yr	87	10 (11.5)	15.9	1.993 (1.324-3.00)	0.001
> 1yr ≤2	192	23 (12)		2.158 (1.417-3.285)	
>3	104	30 (28.8)			
Sources of drinking water					
River	199	37 (18.6)	17.443	0.69 (0.57- 0.85)	0.001
Lake	40	12 (30)		0.72 (0.59- 0.87)	
Well	28	5 (17.9)		0.71 (0.57-0.88)	
Spring	14	4 (28.6)		0.69 (0.54-0.86)	
Piped water supply	102	5 (4.9)			
District					
Delomena	153	18 (11.8)		1.673 (1.180-2.371)	0.004
Heranabuluk	125	20 (16)			
Medawolebu	105	25 (23.8)			
Sex					
Male	348	36 (17.1)	0.163	2.0 (0.48-8.14)	0.340
Female	173	27 (15.6)			

N: Number

Occurrence of *Entamoeba* species in water and its risk factor

A total of 58 samples of high-turbidity water (100 mL) were collected at various points of a water body (lake, river, well, spring, and pipe water), where the water was used for household consumption. Out of the 58 water samples,16 (27.6%) were positive for *Entamoeba* species based on direct microscopical examination (Table 5). During the study, it

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was found that 26.63% of domestic households in study areas were supplied with treated water from piped water supply and there were other remote villages with a sparse population not being covered by the main water supply system. The risk factors, sources of drinking water (lakes, rivers, springs, and wells, versus piped water supply), hygienic (poor hygienic surroundings versus good hygienic surroundings), human activities around water bodies (higher human activities around water bodies versus lower human activities around water bodies), and turbidity (lower turbidity of water bodies versus higher turbidity of water bodies) were significantly associated with the occurrence of *Entamoeba* species in water bodies ($p \leq 0.05$). The study revealed that the occurrences of the parasite in water bodies with poor hygienic surroundings (OR = 39, CI=7.635-199.208, $p \leq 0.05$) were highly comparable to that of water bodies with good hygienic surroundings. The occurrences of the *Entamoeba* species were higher in water bodies with a history of high human activities in and surrounding water bodies (OR = 9.667, CI = 2.347-39.817, $p \leq 0.05$) and lower turbidity (OR = 0.064, CI = 0.007-0.617, $p \leq 0.05$), compared to minimal human activities in and surroundings water bodies and higher turbidities, respectively.

Table 5. The potential risk factors associated with *Entamoeba* species in water bodies of Delomena, Haranabuluk, and Medawalabu districts from December 2019 to July 2020

Variable	Tested samples (N)	Positive samples (%)	X^2(Chi square)	Logistic regression	
				OR (95% CI)	P-value
Surrounding hygiene					
Good	43	4 (9.3)	27.82	39.0 (7.635-199.208)	0.001
Poor	15	12 (80)			
Population and animal activity					
High	26	13 (50%)	11.851	9.667 (2.347-39.817)	0.002
Low	32	3 (9.4%)			
Turbidity					
High	27	3 (9.7)	10.692	0.064 (0.007-0.617)	0.017
Low	31	13 (48.1)			
Protection					
Unprotected	40	15 (37.5)	6.341	0.098 (0.012-0.813)	0.031
Protected	18	1 (5.6)			
Sources of water					
River	25	8 (33.3)	8.358	0.686 (0.472-0.998)	0.049
Lake	2	2 (66.7)			
Well	9	4 (44.4)			
Spring	4	1 (25)			
Piped water supply	18	1 (5.6)			

N: Number

DISCUSSION

In the study area, the high activities of domestic animals and humans surrounded by unprotected water sources caused frequent contamination of surface water, ponds, wells, rivers, and lakes with cysts of *Entamoeba* species. The occurrence of *Entamoeba* species in the water body depended on open-air defecation and high human activity in the surrounding water body. The overall prevalence of amoebiasis in humans was found to be 18.3% which agreed with the findings of previous studies conducted in Omo valley in southern Ethiopia with a prevalence of 16% (Teklehaymanot, 2009). Comparatively, the obtained result of the present study indicated a lower prevalence rate, compared to previous studies conducted in different parts of Ethiopia, such as 27.3% in Gondar (Ayelaw et al., 2011), 33.7% in Diredawa (Dawit, 2006), and in other countries, Kenya which was 74.1% (Robert et al., 2008) and 25.9% in Tajikistan (Matthys et al., 2011). However, the prevalence rate of *Entamoeba* species was higher in the current study, compared to other parts of Ethiopia, including 5% in Delomenatown (Begna et al., 2014), 5.6% in Jimma (Amare et al., 2007), 2.2% in South Wollo (Haji, 2016) and other countries, such as 6.2% in Thai district, Myanmar (Simona et al., 2005),4.1% in Parma Italy (Chukiat et al., 2003), and 0.4% in Kuala Lumpur, Malaysia (Jamaiah and Rohela, 2005). This higher prevalence of *Entamoeba* species might be due to low sanitary conditions and contamination of drinking water.

The results of the present study indicated a significantly higher prevalence of *Entamoeba* species among large-size families, compared to the small families. This finding was similar to a study in Derna city, Libya (Sadaga and Kassem, 2007) which reported higher infection of intestinal parasites in big families. Similarly, exposure to *E. histolytica* was positively associated with overcrowding at home or more household members in the family in the Orang Asli Ethnic Group in Malaysia (Shahrul et al., 2012) and Mexico (Alvarado et al., 2015). However, Gelaw et al. (2013) and Nath et

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al. (2015) reported that infection of *E. histolytica* was independent of family size in Ethiopia and India, respectively. The results of the present study indicated that participants both humans and pets belonging to large size families might be favoring the transmission of *Entamoeba* species. This might be due to overcrowding near homes.

Toilet usage and open-air defecations were statistically significant. This further increased the risk of the high prevalence of *Entamoeba* species. Similar findings were reported by Haji (2016). Indiscriminate stool elimination increased the risk of *Entamoeba* prevalence among communities. A study conducted on antiretroviral-treated HIV/Acquired Immunodeficiency syndrome patients in Ethiopia also reported that unavailability of latrines and lack of handwashing with soap was associated with *E. histolytica*/*dispar* infections (Mahmud et al., 2014). Open field defecation is found to be an important determinant in Nigeria, for *E. histolytica/ E. dispar* infestation which was similar to the findings of the present study (Idowu and Rowland, 2006).

It has been noted that those who did not wash their hands properly were at higher risk of being infected with *Entamoeba* species. The main role of dirty hands in the fecal-oral spread of the disease has been well reported in low-income countries and washing hands before eating or after defecation have been regarded as a secondary barrier. The result agrees with previous studies (Gasem et al., 2001). Poor personal hygiene, poor living conditions, unwashed hands after playing with soil or gardening, and the presence of already infected family members were important predictors for intestinal protozoan infections including *Entamoeba* species (Zhang et al., 2013; Nath et al., 2015; Joyobrato et al., 2018). In Vietnam, the transmission route via contaminated hands is of significant importance through which the infection risk increases by three times if hands are not washed properly (Pham et al., 2011). Previous studies in Italy and Yemen also show the significance of handwashing prior to food (Rinne et al., 2005; Naelahet al., 2011). Hand washing and diarrhea were significantly associated (Knight et al., 1992) as was reported in Myanmar (Han and Hlaing, 1989) and Indonesia (Gasem et al., 2001).

The present finding indicated that the factors responsible for infection by *Entamoeba* species were related to poor living conditions, unhygienic toilet facilities, without habits of washing hands before taking food. The result agrees with other studies from India, Italy, and Yemen (Rinne et al., 2005, Naelah et al., 2011; Joyobrato et al., 2018). The place of defecation and the presence of pets in the houses were found as significant contributing factors. The type of water used for washing utensils was a determinant of disease transmission. The pond water (27%) is a major determinant of parasitic infestation, compared to river water (25.8%) and tube wells 14.9%, (Sintu et al., 2011). The literacy of the parents was a crucial contributing factor in *Entamoeba* transmission. This was confirmed by the recent studies conducted in Ethiopia and other countries (Wordemann et al., 2006; Ayalew et al., 2011; Begna et al., 2014).

A significant difference in the prevalence of amoebiasis was observed among the three age groups. The highest prevalence rate of amoebiasis was seen among the young group (8-18 years), and the lowest infection rate was seen among children. Similar age distribution of infection had been observed in Dire Dawa, Ethiopia (36.1%), where children within the age range of 6-14 years had the highest infection rate, compared to other age groups (Dawit, 2006). In Kenya, individuals within the age range of 10-14 years recorded a higher prevalence and intensity of *E. histolytica* infection, compared to the older age group, 15 years and over. Garmie (2016) also reported that children of 8-18 years were the most affected group with *E. histolytica* infection. Generally, young children have been reported to be more exposed to *Entamoeba* species infection than adults. The reason might be that children have very active playing habits at home/school and more chances to get in contact with food and drinks that were contaminated with the infective cyst of the parasite.

The prevalence of *Entamoeba* species among different sex was not statistically significant. The present study indicated that 19.8% of males and 16% of females were positive for *Entamoeba* species. This finding was in agreement with the previous studies (Ohnishi and Murata, 1997; Sharma et al., 2004; Ozyurt et al., 2007). Other studies conducted in Turkey (Ogzumusand Efe, 2007) have shown a high prevalence of amoebiasis among females. The higher prevalence of *Entamoeba* species in males could be explained on the basis that males are more susceptible than females to infections caused by parasites because males generally exhibit reduced immune responses and increased intensity of infection compared to females (Klein, 2000). These differences were usually attributed to ecological, sociological, and physiological factors.

The prevalence of *Entamoeba* species between humans using pipe water and a hand pump (protected) and lake, river, well, and spring (unprotected water sources) showed a significant difference. In the study area, different activities of humans and livestock surrounding the unprotected water sources led to continuous contamination of surface water, wells, rivers, and the lake with cysts of *Entamoeba* species. A similar observation was recorded by Dawit (2006) in Diredawa. According to Dawit, there is a significant difference in infection of *Entamoeba* species among children using protected (23.3% for amoebiasis) and unprotected (33.3% for amoebiasis) water sources. Besides, a study conducted in the United Kingdom showed that a large number of intestinal parasites, including *E. histolytica/dispar* were detected in drinking water samples from water sources where agricultural and human activities were high. On the other hand, the prevalence of *Entamoeba* species and other intestinal parasites was low in the protected water bodies with no agricultural activities and minimal human activities. The prevalence of amoebiasis was low in areas with access to clean water and

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improved environmental hygiene. In the present study, water sample analysis indicated that the occurrence of *Entamoeba* species cyst in both protected and unprotected water sources was 27.6%. This occurrence of *Entamoeba* species in the water body caused infection in humans and dogs in the study area. The prevalence of *Entamoeba* species in water bodies of the study area depended on the level of protection, which stresses the importance of the water quality and the transmission of *Entamoeba* species. The occurrence of *Entamoeba* species in the water body depended on open-air defecation, and high human activity in and surrounding the water bodies (fetching, swimming, washing, and bathing). These results agree with the report of Ben and Sabbahi (2017) indicating that the occurrence of amoebiasis in waterbodies was significantly higher in uncovered wells (43.3%) than in covered wells (35%), in unclean surroundings (48.9%) than in clean surroundings (20%), in turbid well water (51.9%) than relatively clear well water (30.2%) and wells frequently used by grazing animals (53.1%).

The present study revealed an overall prevalence of *Entamoeba* species in pets was 16.5% in Delomena, Haranabuluk, and Medaworeda districts, Ethiopia. The study reported a lower level of infection with a prevalence of enteric protozoan infections in dogs at 50.9% in Hawassa, Ethiopia (Mekibib and Sheferaw, 2018). These findings were relatively similar to the reports from various areas as 12.11% in Spain by Segovia et al. (2010), 13.4% in Bangladesh by Mahmud et al. (2014), and relatively higher than those reported by Alam et al. (2015) in dogs (7%) and Gillespie et al. (2017) in Australia (1%), and Justin et al. (2020) in Cameroon (1.5%). The differences in the prevalence of enteric protozoa could be due to variation in geographic location, owner awareness, and the number of stray dogs in an area. The prevalence of enteric protozoan parasites in dogs was significantly higher in younger dogs. A higher prevalence of *Entamoeba* species in puppies was reported by other studies (Adejinmi et al., 2001, Sager et al., 2006, Lorenzini et al., 2007). This might be due to either immune incompetence or a low level of passive immunity in puppies received from their dam. The prevalence of amoebiasis was higher in the dogs whose owners had a history of *Entamoeba* species infection, compared to the dogs whose owners were free from the amoebiasis. This appeared to be due to the cross-infection of *Entamoeba* species from the owner to the pet. Most of these dogs became chronic carriers of *Entamoeba* species. The dogs that were kept by low-income groups of people receive less medical care. The prevalence of *Entamoeba* species in the dog was related to the income of the owner. The prevalence of the dog amoebiasis was low, in which the pet owners had a high income (2%), compared to those with low income.

During a cross-sectional study of dog owners in the study area, the analyses of fecal samples of dogs and their owners revealed that both harbor the *Entamoeba* species. Both the samples from the drinking water source and the stool samples of dog owners showed the presence of cysts or trophozoites. This result indicated that the source of infection was contaminated drinking water, poor management practice, and level of awareness of dog owners about dog parasites and associated risk factors, in addition to the lack of veterinary attention. These exacerbated the risk of transmission of canine parasitic zoonoses to the human community or vice versa.

In the present study, it was surprising to see that amoebiasis was transmitted between humans and dogs. Additionally, the prevalence of amoebiasis in humans and dogs had a great contribution to the contamination of water bodies by cysts of *Entamoeba* species. *Entamoeba* was identified or diagnosed in both pets and pet owners from the same households with similar sources of drinking water. During a cross-sectional survey of pet owners in the study area, 63 individuals and their dogs were positive for *Entamoeba* species. This result agrees with the findings of Endrias et al. (2010). It is now accepted that *E. dispar* infection is much more common than *E. histolytica* worldwide (Ramos et al., 2005). Human infections with *E. moshkovskii* have also been reported in Tanzania, Bangladesh, India, Iran, Australia, and Turkey, and in general, they are not associated with the disease (Solaymani et al., 2006; Tanyuksel et al., 2007; Fotedar et al., 2008).

CONCLUSION

In the present study, a higher prevalence and occurrence of *Entamoeba* species have been found among humans, dogs, and water bodies with the average prevalence rates of 18.3%, 16.5%, and 27.6%, respectively. Amoebiasis was identified or diagnosed in both dogs and dog owners from the same households with similar sources of drinking water. This indicated that the transmissions and spread of the amoebiasis occurred among humans, dogs, and water bodies. The increasing prevalence of *Entamoeba* species in the present study was associated with the socioeconomic burden of the community. Improved personal and environmental sanitation is the most effective sustainable option for controlling and preventing amoebiasis in humans and dogs. People often depend on open-air defecation systems might lead to infection in humans, dogs, and contamination of water bodies by *Entamoeba* species. The microscopic examination for intestinal parasites and comparison with symptom profiles of the patient remains the crucial routine diagnostic tool. However, further studies using molecular approaches are needed to distinguish the morphologically identical pathogenic and non-pathogenic species in the studied area.

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Authors' contribution

Sufian Abdo Jilo generated the idea, proposal, and paper write-up and completed the paper. Mukarim Abdurahman Kadir, Johar Aliye Hussein, and Sureshkumar P. Nair have taken part in the paper write-up, data analysis, and edition of the manuscript. All authors read and approved the final version of the manuscript and conceived the study.

Competing interests

The authors have not declared any conflict of interest.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

REFERENCES

Abioye JOK, Mbagwu TT, and Babatunde S (2019). Prevalence of *Entamoeba histolytica* in Bingham University and Environs. ECo. Microbiology, 15(4): 242-250. Available at: https://www.ecronicon.com/ecmi/volume15-issue4.php

Adam KM, Paul J, and Zaman V(1979). Medical and Veterinary Protozology: An illustrated guide. Churchill Livingstone Edi., London, pp. 32-49. Available at: https://www.sid.ir.com

Adejinmi JO, Okewole EA, and Obideyi SA (2001). Prevalence of Giardia species in dogs in Ibadan, South-Western Nigeria. Nigeria Veterinarian Journal, 22: 54-59. Available at: https://www.slideshare.net

Akpan P, Abraham AP, and Ekwetiong O (2010). Survey of Gastro-Intestinal Parasites of Chimpanzees and Drill Monkeys in Drill Ranch, Calabar, Cross River State-Nigeria. African Research Revision, 4(3): 334-340. Available at: https://www.docplayer.net.com

Alam MA, Maqbool A, Nazir MM, Lateef M, Khan MS, and Lindsay DS (2015). *Entamoeba* infections in different populations of dogs in an endemic area of Lahore, Pakistan. Veterinary Parasitology, 207: 216-219. DOI: https://www.doi.org/10.1016/j.vetpar.2014.12.001

Alvarado EC, Hernandez TJ, and Sanchez AF (2015). Seroepidemiology of *Entamoeba histolytica* infection in general population in rural Durango, Mexico. Journal of Clinical and Medical Research, 7: 435-439. DOI: https://www.doi.org/10.14740/jocmr2131w

Amare M, Solomon G, and Tesfaye K (2007). Prevalence of intestinal parasitic infections among urban dwellers in southwest Ethiopia. Ethiopian Journal of Health and Development, 21(1): 12-17. DOI: https://www.doi.org/10.4314/ejhd.v21i1.100266

Anuar TS, Al-Mekhlafi HM, Ghani MKA, Osman E, Yasin AM, and Nordin A (2012). Prevalence and Risk Factors Associated with Entamoeba *histolytica*/*dispar*/*moshkovskii* infection among Three Orang Asli Ethnic Groups in Malaysia. PLoS One, 7(10): e48165. DOI: https://www.doi.org/10.1371/journal.pone.0048165

Ashar M. Muhammad Al, Tanveer A, Sultan M, Muhammad Nisar K, Asif, I, Faisal S, Ghulam A, and Muhammad IS (2014). Amoebiasis: Prevalence, Treatment with Metronidazole and alidixic Acid in Dogs. Advanced Animal and Veterinary Research, 1(1): 34-37. DOI: https://www.doi.org/10.5897

Ayalew A, Debebe T, and Worku A (2011). Prevalence and risk factors of intestinal parasites among Delgi school children, North Gondar, Ethiopia. Journal of Parasitological and Vector Biology, 3(5): 75-81. DOI: https://doi.org/10.1186/1471-2164-14-53

Bale Zone Agriculture and Natural Resource Office (BZANRO)(2020). Annual plan of the year 2012 E.C document, pp. 34-55

Begna T and Taye S, and A Eden (2014). Prevalence and its associated risk factors of intestinal parasitic infections among Yadot primary school children of South Eastern Ethiopia: a cross-sectional study. BMC Research Notes, 7: 848. DOI: https://doi.org/10.1186/1756-0500-7-848

Ben AL and Sabbahi S (2019). *Entamoeba histolytica*. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Global Water Pathogen Project. DOI: https://www.doi.org/10.14321/waterpathogens.34

Chia MY, Jeng CR, Hsiao SH, Lee AH, Chen CY, and Pang VF (2009). *Entamoeba invadens* Myositis in a Common Water Monitor Lizard (Varanus salvator). Veterinary Pathology, 46:673-676. DOI: https://www.doi.org/10.1354/vp.08-VP-0224-P-CR

Chukiat S, Chanathep P, Pataraporn W, Chutatip S, and Pornthep C (2003). Prevalence of Intestinal Parasitic Infection among Thai People with Mental Handicaps Southeast Asian. Journal of Tropic Medicine and Public Health, 34: 259-263. Available at: https://www.pubmed.ncbi.nlm.nih.gov/12971546

Dawit A (2006). Association Of Cryptosporidium Parvum, Giardia Lamblia And *Entamoeba histolytica*/*dispar* Infection With Drinking Water Sources Among Children in Rural Part Of Dire-Dawa, Eastern Ethiopia. Thesis, pp. 31-45. Available at: Https://www.daneprairie.com

Dickson, D. Despommier R, Gwadz W, Daniel OG, Peter JH, and Charles AK (2017). Parasitic Diseases. Protozoal Disease. 7th Edition, Springer Verlag, New York, pp. 155-163. Available at: http://www.web.natur.cuni.cz

Ekanayake DK, Arulkanthan A, Horadagoda NU, Sanjeevani GKM, Kieft R, Gunatilake SW, and Dittus PJ (2006). Prevalence of cryptosporidium and other enteric parasites among wild non-human parasites in Polonnaruwa, Sri Lanka. American. Journal. of Tropical Medicine and Hygiene 74: 322-329. DOI: https://www.doi.org/10.4269/AJTMH.2006.74.332

Endrias Z, Yohannes S, and Berhanu M (2010). Prevalence of helminth parasites of dogs and owners' awareness about zoonotic parasites in Ambo town, Central Ethiopia. Ethiopian Veterinary Journal, 14(2): 17-30. Available at: https://www.ajol.com

Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, and Harkness J (2007). Laboratory diagnostic techniques for *Entamoeba* species. Clinical Microbiology Revision, 20(3): 511-532. DOI: https://www.doi.org/10.1128/CMR.00004-07

Fotedar R, Stark D, Marriott D, Ellis, and Harkness JJ (2008). *Entamoeba moshkovskii* infections in Sydney, Australia. European Journal of Clinical Microbiology and Infectious Disease, 27:133-137. DOI: https://www.doi.org/10.1007/s10096-007-0399-9

Garmie V (2016). Prevalence and Intensity of *Entamoeba histolytica* in Patients Attending Health Centres in Mathare Slums, Nairobi County, Kenya. Available at: http://Ferepository.uonbi.ac.ke

Gasem M, Dolmans W, Keuter M, and Djokomoeljanto R (2001). Poor food hygiene and housing as risk factors for typhoid fever in Semarang, Indonesia. Tropical Medicine and International Health, 6: 484-490. DOI: https://www.doi.org/10.1046/j.1365-3156.2001.00734.x

Gelaw A, Anagaw B, Nigussie B, Silesh B, Yirga A, and Alem M (2013). Prevalence of intestinal parasitic infections and risk factors among schoolchildren at the University of Gondar Community School, Northwest Ethiopia: A cross‐sectional study. BMC Public Health, 13: 304. DOI: https://www.doi.org/10.1186/1471-2458-13-304

Gillespie S and Bradbury SR (2017). A Survey of Intestinal Parasites of Domestic Dogs in Central Queensland. Tropic Medical Infectious Disease, 2: 60. DOI: https://www.doi.org/10.3390/tropicalmed2040060

Haji M (2016). Status of Sanitary Practices and Amebiasis and Giardiasis Among Patients Visiting Haik Health Center, South Wollo, Northeast Ethiopia. Available at: https://www.semanticscholar.org/

Han A and Hlaing T (1989). Prevention of diarrhoea and dysentery by hand washing. Transformative. Research Society Tropic. Medicine and Hygiene, 83: 128-131. DOI: https://www.doi.org/10.1016/0035-9203(89)90737-2

Idowu OA and Rowland SA (2006). Oral fecal parasites and personal hygiene of food handlers in Abeokuta, Nigeria. African. Health. Science, 6(3): 160-164. Available at: https://www.pubmed.ncbi.nlm.nih.gov/17140338

Jamaiah I and Rohela M (2005). Prevalence of Intestinal Parasites among Members of the Ppublic in Kuala Lumpur, Malaysia. Southeast Asian. Journal Tropical Medicine and Public Health, 36: 68-71. Available at: https://www.pubmed.ncbi.nlm.nih.gov/15906644

Jensen B, Kepley W, Guarner J, Anderson K, Anderson D, Clairmont J, De L'aune W, Austin EH, Austin GE (2000). Comparison of polyvinyl alcohol fixative with three less hazardous fixatives for detection and identification of intestinal parasites. Journal of Clinical Microbiology, 8(4): 1592-1598. DOI: http://www.doi.org/10.1128/JCM.38.4.1592-1598.2000.

Joyobrato N, Baby S, Jaishree P, and Sankar KG (2018). Diagnosis and epidemiology of amoebiasis in India. Current Science, 114: 2045-2052. DOI: https://www.doi.org/10.18520/cs/v114/i10/2045-205

Justin K, Alain GDK, and Carène CZD (2020). The retrospective study of prevalence and associated risk factors of diseases and others clinical conditions diagnosed in dogs and cats in Douala City, Cameroon. DOI: https://www.doi.org/10.21203/rs.3.rs-43889/v1

Klein SL (2000). Hormones and mating system affect sex and species differences in immune function among vertebrates. Behavioral Process, 51(1): 149-160. DOI:https://www.doi.org/10.1016/s0149-7634(00)00027-0

Knight S, Toodayan W, Kyi CW, and Barnes WA (1992). Risk factors for the transmission of diarrhoea in children: a case-control study in rural Malaysia. International Journal of Epidemiology, 21: 812-818. DOI: https://www.doi.org/10.1093/ije/21.4.812

Lorenzini G, Tasca T, and De Carli GA (2007). Prevalence of intestinal parasites in dogs and cats under Veterinary care in Porto Ategre, Rio Grande do Sul, Brazil. Brazilian Journal of Veterinary Research and Animal. Science, 44: 137-145. DOI: https://www.doi.org/10.11606/issn.1678-4456.bjvras.2007.26652

Mahmud MA, Belal SH, and Uddin FM (2014) .Prevalence of protozoan diseases in pet dogs at district veterinary hospital, Sirajganj, Bangladesh. Bangladesh Journal of Veterinary Medicine, 12(2): 191-196. DOI: https://doi.org/10.3329/bjvm.v12i2.21290

Mahmud MA, Bezabih AM, and Gebru RB (2014). Risk factors for intestinal parasitosis among antiretroviral treated HIV/AIDS patients in Ethiopia. International Journal of STD AIDS, 25: 778-784. DOI:https://www.doi.org/10.1177/0956462414520803

Matthys B, Bobieva M, Karimova G, Mengliboeva Z, Jean-Richard V, Hoimnazarova M, Kurbonova M, Lohourignon LK, Utzinger J, and Wyss K (2011). Prevalence and risk factors of helminths and intestinal protozoa infections among children from primary schools in western Tajikistan. Parasites Vectors, 4: 195-208. DOI: https://www.doi.org/10.1186/1756-3305-4-195

Mekibib B and Sheferaw D (2018). Enteric protozoa of dogs: prevalence, associated risk factors and owners' awareness in and around Hawassa town, Ethiopia. Ethiopian Veterinary Journal, 22 (1): 59-73. DOI: https://www.dx.doi.org/10.4314/evj.v22i1.5

Naelah AA, Mohammed AKM, Rohela M, and Yvonne LAL (2011). Factors associated with high prevalence of intestinal protozoa infections among patients in Sana'a City, Yemen. PLoS One, 6(7): e22044. DOI: https://www.doi.org/10.1371 /journal.pone.0022044

Nath J, Banyal N, Gautam D, Ghosh S S, Singha KB, and Paul J(2015). Systematic detection and association of *Entamoeba* species in stool samples from selected sites in India. Epidemiology Infectious, 143: 108-119. DOI: https://www.doi.org/10.1017/S0950268814000715

Ngui R L, Angal SA, Fakhrurrazi YL, Lian LA, Ling Y, Ibrahim J, and Mahmud R (2012). Differentiating *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* using nested polymerase chain reaction (PCR) in rural communities in Malaysia. Parasites and Vectors, 5: 187. DOI:https://www.doi.org/10.1186/1756-3305-5-187

Ogzumus OB and Efe U (2007). Distribution of intestinal parasites detected in Camlihemsin healthcare center during the period from July 2005 to January 2007. Turkish Society for Parasitology, 31: 142-144. Available at: https://cms.galenos.com.tr/Uploads/Article_22828/TPD-31-142.pdf

Ohnishi K and Murata M (1997). Present characteristics of symptomatic amebiasis due to *Entamoeba histolytica* in the east-southeast area of Tokyo. Epidemiology Infectious, 119: 363–367. DOI: https://www.doi.org/10.1017/s0950268897008236

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Ozyurt M, Kurt O, Yaman O, Ardic N and Haznedaroglu T (2007). Evaluation of intestinal parasites in a period of four years in the coprology laboratory of a training hospital. Türkiye Parazitoloji Dergisi, 31: 306-308. Available at: https://www.pubmed.ncbi.nlm.nih.gov/18224623

Pham Duc P, Nguyen-Viet H, Hattendorf J, Zinsstag J and Dan Cam P (2011). Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. Parasites Vectors, 4: 102. DOI: https://www.doi.org/10.1186/1756-3305-4-102

Prakash V and Bhimji SS (2022). Amebic Liver. Stat Pearls Publishing, 2017. Available at: https://www.ncbi.nlm.nih.gov/books/NBK4. Accessed on 22/3/2022

Regan CS, Maqsud LY, and Hany ME (2014). Prevalence of *Entamoeba* species in captive primates in zoological gardens in the UK. Peer Journal, 2: 2-12. DOI: https://www.doi.org/10.7717/peerj.492

Ritchie LS (1948). An ether sedimentation technique for routine stool examination. Bulletin of the United States Army Medical Department, 8(4): 326. Available at: https://www.cabdirect.org/cabdirect/abstract/19480801031

Rinne S, Rodas EJ, Galer-Unti R, Glickman N, and Glickman LT (2005). Prevalence and risk factors for protozoan and nematode infections among children in an Ecuadorian highland community. Trans. Research Society Tropical Medicine and Hygiene, 99: 585-592. DOI: https://www.doi.org/10.1016/j.trstmh.2005.01.003

Robert MN, Peninah AA, EphantusWK, Benson O, and Nyanchongi (2008). The risk of Pathogenic Intestinal Helminthiasis in Kisii Municipality Kenya. BMC Public Health, 14(8): 237. DOI: https://www.doi.org/10.1186/1471-2458-8-237

Rugaia MA, ESalem SA, A Gahgah, Aisha SH, Ali A, and Samah RAl (2020). Prevalence and risk factors associated with *Entamoeba histolytica* infection among children in Sebha, Libya. Available at: https://www.dmrjournal.org

Sadaga GA and HH Kassem (2007). Prevalence of intestinal parasites among primary schoolchildren in Derna District, Libya. Journal of Egyptian Society Parasitology, 37: 205-214. Available at: https://www.pubmed.ncbi.nlm.nih.gov/17580578

Sager H, Steiner MC, Multer N, Staubli D, Esposito M, Schares G, Hassig M, Stark K, and Gottsterin B (2006). Incidence of Neospora caninum and other intestinal protozoan parasites in populations of Swiss dogs. Veterinary Parasitology, 139: 84-92. DOI: https://www.doi.org/10.1016/j.vetpar

Segovia G, Cristina N, Chavez MB, Medina FY, Cazares RF, Elena HR, Vonica I, Martinez PA, and Talamas RP (2010). *Entamoeba Invadens*, encystation process and enolase. Experimental Parasitology, 125(2): 63-69. DOI: https://www.doi.org/10.1016/j.exppara

Shahrul AT, Al‑Mekhlafi MH, Abdul Ghani MK, Osman E, Mohd Yasin A, Nordin A Azreen SN, Md Salleh F, Ghazali N, Bernadus M, et al. (2012). Prevalence and risk factors associated with *E. histolytica*/*dispar*/*moshkovskii* infection among three Orang Asli ethnic groups in Malaysia. PLoS One, 7: e48165. DOI: https://www.doi.org/10.1371/journal.pone.0048165

Sharma BK, Rai SK, Rai DR, and DR Choudhury (2004). Prevalence of intestinal parasitic infestation in schoolchildren in the northeastern part of Kathmandu Valley, Nepal. Southeast Asian Journal of Tropical Medicine and Public Health, 35: 501-505. Available at: https://www.pubmed.ncbi.nlm.nih.gov/15689056

Simona P, Ch Gorrini, G Piccolo, A Calderaro, and G Dettori (2006). Prevalence of intestinal parasites in the area of Parma during the year 2005. Acta Biomedicine, 77: 147-151. Available at: https://www.pubmed.ncbi.nlm.nih.gov/17312984

Sintu S, Soumyendu M, TK Maiti1, Pramit G, and KG Sudip (2011). A Community Based Epidemiological Study on Intestinal Amoebiasis in Rural West Bengal, Ind. Asian Journal of Medicine Science, 2: 164-168. DOI: https://www.doi.org/10.3126/ajms.v2i3.4375

Solaymani M S, Rezaian M, Babaei Z, Rajabpour A, and Meamar AR Pourbabai AA, and Petri Jr WA (2006). Comparison of a stool antigen detection kit and PCR for diagnosis of *Entamoeba histolytica* and *Entamoeba dispar* infections in asymptomatic cyst passers in Iran. Journal of Clinical Microbiology, 44: 2258-2261. DOI: https://www.doi.org/10.1128/JCM.00530-06

Soulsby EJL(2006). Helminths. Arthropods and protozoa of domesticated animals.Bailliere Tindall., London, pp. 276-284. Available at: https://www.cabi.org/isc/abstract/19840510866

Tanyuksel M. and Petri WA Jr (2003). Laboratory diagnosis of Amoebiasis. Clinical Microbiology Revision, 16(4): 713-729. DOI: https://www.doi.org/10.1128/CMR.16.4.713-729.2003

Tanyuksel M, Ulukanligil M, Guclu Z, Araz E, Koru O, and Petri Jr WA (2007). Two cases of rarely recognized infection with *Entamoeba moshkovskii*. American Journal of Tropical Medicine and Hygiene, 76: 723-724. Available at: https://www.pubmed.ncbi.nlm.nih.gov/17426178

Teklehaymanot T (2009). Intestinal parasitosis among Kara and Kwego semi pastoralist Tribes in lower Omo valley, Southwestern Ethiopia. Ethiopian Journal of Health Development, 23: 57-62. DOI: https://www.doi.org/10.4314/EJHD.V23l1.44838

Thrusfield M (2005). Veterinary Epidemiology. Sampling method. Blackwell Science Ltd., Iowa, pp. 628. Available at: https://www.scirp.org/(S(czeh2tfqyw2orz553k1w0r45)

Verkerke HP, Petri WA Jr, and CS Marie (2012). The dynamic interdependence of amebiasis, innate immunity, and under nutrition. Seminar Immunopathology, 34(6): 771-785. Available at: https://www.ncbi.nlm.nih.gov/3510265

Wo¨rdemann M, Polman K, Heredia L, Diaz R, Madurga A, Ferna´ndez F, Prado R, Espinosa A, Duran L, Gorbea M, et al. (2006). Prevalence of intestinal parasite infections and their risk factors in Cuba children. Tropical Medicine of International Health, 11: 1813-1820. DOI: https://www.doi.org/10.1111/j.1365.2006.01745.x.

Xiao L, Alderisio KA, and Jiang J (2004). Detection and differentiation of cysts in water by PCR-RFLP. Methods. Molecular Biology, 268: 163-176. Applied and Environmental Microbiology. DOI: https://www.doi.org/10.1128/AEM.00927-06

Ximénez C, Cerritos R, and Rojas L (2010). Human amebiasis: breaking the paradigm? International Journal of Environment and Research and Public Health, 7(3): 1105-1120. DOI: https://www.doi.org/10.1016/j. meegid.2009.06.008.

Zhang H, Ehrenkaufer GM, Hall N, and Singh U (2013). Small RNA pyrosequencing in the protozoan parasite *Entamoeba histolytica* reveals strain-specific small RNAs that target virulence genes. BMC Genomics, 14: 53. DOI: https://www.doi.org/10.1186/1471-2164-14-53

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Diagnosis and Control of Peste des Petits Ruminants Disease in Small Ruminants: A Review

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ABSTRACT

Peste des Petits Ruminants (PPR) is an acute highly contagious febrile disease of sheep and goats characterized by erosive and necrotizing stomatitis and associated with severe pneumo-enteritis and bronchopneumonia ended by recovery or death. The aim of the present study was to throw light on the diagnosis and control of PPR. Diagnosis of PPR depends on clinical signs, pathological lesions, and specific detection of the viral antigen, viral genome, or specific antibodies by serological tests and nucleic acid-based assays. The most commonly used diagnostic techniques are cell culture isolation, agar gel immunodiffusion, hemagglutination tests, immunocapture ELISA, and competitive ELISA. In addition to the abovementioned techniques, virus neutralization tests and reverse transcriptase PCR are used. Peste des Petits Ruminants is characterized by high fever associated with watery nasal and ocular discharges, mucopurulent stomatitis, and broncho-pneumonia. Moreover, severe bloody diarrhea and the disease associated with high levels of mortality reached up to 90%. The diagnosis of viral diseases is important in determining the control strategies. Therefore, it can be concluded that recent diagnostic tools are urgently needed not only for the diagnosis but also for following-up combating programs and control of viral diseases. Early and rapid complete identification of infectious viral agents in small ruminants as well as in the surrounding environment is recommended for effective control of PPR. The control program depends mainly on vaccination, hygiene and sanitation measures, and effective quarantine measures.

Keywords: Control, Dairy Ruminants, Diagnosis, Goat, Peste des Petits, Sheep

INTRODUCTION

Sheep and goats are susceptible to infection by many viruses. Viral diseases affect the productivity of both species and sometimes induce severe losses which appear in the form of high mortalities in the newborns, low growth rates, loss in body gain in adult animals in addition to early embryonic deaths, newborn mortalities, abortions, abnormal weak lambs and persistently infected animals (Onono et al., 2013). The clinical signs of viral diseases differ according to the type of the disease. Peste des Petits Ruminants (PPR) is an acute highly contagious febrile disease of sheep and goats characterized by erosive and necrotizing stomatitis and associated with severe pneumo-enteritis and bronchopneumonia ended by recovery or death (Ismail et al., 1995). The PPR is reported in most African countries, south of the Sahara and north of the equator, and in nearly all Middle Eastern countries and South-West Asia (OIE and FAO, 2015). The PPR virus (PPRV) is related to the family *Paramyxoviridae* that consists of two subfamilies, *Paramyxovirinae* and *Pneumovirinae*. Large, negative-strand RNA viruses characterize this family. The two subfamilies include several genera with important human and veterinary pathogens that cause diseases, such as canine distemper and rinderpest (RP). Although PPRV is famous as one serotype, the partial sequencing of the fusion protein (F) gene reveals the existence of 4 lines (I, II, III, and IV) (Banyard et al., 2010; Kardjadj et al., 2015). Commonly, the first two lineages II and I are existing in West Africa, while lineage III is present in East Africa and the Middle East, and lineage IV is present in Asia (Wasim et al., 2015). The first case of PPR in Egypt was recorded in 1988 by Ikram et al. (1988) in goats located in Giza Governorate. The disease is responsible for high rates of morbidity and mortality, leading to severe economic losses (El-Allawy et al., 1993). The disease is considered endemic in Egypt and has a seasonal occurrence in winter in most of the country (Fayed et al., 1994). Mouaz et al. (1995) reported an outbreak of PPR affecting sheep in 1994 in Giza province. The isolated virus was designated as Giza-94 after confirmation by ELISA and VNT (Abd El-Rahim et al., 2010). A recent study applied competitive ELISA on sheep and goat samples in two Egyptian governorates and recorded the prevalence of PPR antibodies. Giza governorate showed positivity of 63.8% in sheep and 45.7% in goats. Moreover, 71.7% of sheep and 45% of goats in the Beni-Suef governorate were positive (Mahmoud et al., 2017). Diagnosis of PPR depends on clinical signs, epidemiology, pathological lesions, and specific detection of the viral antigen, viral genome, or the specific antibodies in the tested samples by different serological tests and nucleic acid-based assays (Balamurugan et al., 2014; Kinimi et al., 2020). The most commonly used diagnostic techniques are culture

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isolation, agar gel immunodiffusion (AGID), hemagglutination assay (HA), immunocapture enzyme-linked immunosorbent assay (IC-ELISA), and competitive ELISA. In addition to the previously mentioned techniques, virus neutralization test (VNT) and reverse transcriptase polymerase chain reaction (RT-PCR) are used (Forsyth and Barrett 1995; Anderson et al., 2006). Infectious viral diseases in small ruminants such as PPR are difficult to control because of the insufficient data of the epidemiology, diagnosis and different control strategies in the herds where the problems are occurring. Infectious viral diseases of small ruminants hinder the expected benefits from these animals (Mahmoud et al., 2017). The diagnosis of viral diseases is important in determining of the control strategies. Therefore, the aim of this study was to throw light on the diagnosis and control of PPR in sheep and goats.

DIAGNOSIS OF PESTE DES PETITS RUMINANTS DISEASE

Sheep and goat susceptibility

The disease attacks primarily goats and sheep. Goats are more liable than sheep and show heavy losses. Goat breeds have different susceptibilities to PPR. Guinean breeds show more susceptibility than Sahelian breeds. The European breeds are readily susceptible. The age of the animal has also a significant effect, where lambs are highly susceptible than adults or unweaned young animals (Kardjadj et al., 2015). Sheep or goats aged 3–4 months are more susceptible to PPRV infection due to the diminishing the maternal antibodies (Burezq et al., 2020).

Clinical signs

The disease is characterized by a high fever reach to 40ºC, associated with watery nasal and ocular discharges which later change to mucopurulent, stomatitis, and bronchopneumonia (Balamurugan et al., 2014). Moreover, severe diarrhea with an offensive odor has also been reported which may be bloody and often lead to death (Farougou and Gagara, 2013). This disease is characterized by high levels of morbidity and mortality up to 90% (Kwiatek et al., 2011). Recent researchers recorded deviations in the distribution and the varieties of clinical signs of the disease, represented in the emergence of PPRV lineage IV in northeastern and northern Africa **(**Albina et al., 2013; Kardjadj, 2018).

Post-mortem findings

The post-mortem findings include intensive red areas and congestion in the lungs with pneumonic changes. Large intestines and colon show hemorrhages (Zebra markings). Besides, enlargement of the spleen and lymph nodes in association with abomasal erosion has been reported by Abubakar et al. (2011) and Abubakar et al. (2015)**.**

Differential diagnosis

Differential diagnosis must be applied to differentiate the other diseases that can be misdiagnosed with PPR and these include; signs of lameness differentiate Foot and Mouth Disease (FMD), there is no diarrhea in bluetongue disease, pock lesions differentiate capripox viruses, and necrotic stomatitis is not found in contagious ecthyma. Pneumonic pasteurellosis and caprine pleuropneumonia are characterized by respiratory illness alone. Although some PPR-infected cases do not show all the clinical signs, so differential diagnoses in these cases cannot be applied. As a result, laboratory confirmation of PPR is recommended for the definitive diagnosis (Radostits et al., 2006).

Virus identification

Virus isolation

Virus isolation represents the gold standard method for the diagnosis of PPR. Different types of cell cultures like Vero cells (African green monkey kidney cells) were used for PPRV isolation (Durojaiye, 1987). The characteristic specific cytopathic effect is in the form of cell rounding followed by grape-like formation, vacuolation, cytoplasmic granulation, and syncytia formation that appears after 3–5 days of infection. Virus isolation is not applicable in routine diagnostic assays as it is slow and needs special facilities and lacks the same sensitivity as RT-PCR (Santhamani et al., 2016).

Agar gel immunodiffusion

The comparative efficiency of competitive ELISA and the standard AGID for the diagnosis of PPR was inspected and the results showed that competitive ELISA has high sensitivity and specificity than AGID and could be used as a standard test, especially for the testing of the improperly kept samples. Some common serological tests like AGID and indirect ELISA were effective in the past for the primary diagnosis of PPR but there is a cross-reaction between PPR and RP bodies (Santhamani et al., 2016).

Sandwich ELISA

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Sandwich ELISA ***(s-ELISA)*** is highly sensitive (99.8%) and specific (90.5%), and it is used for the detection of PPRV in the nasal and ocular secretions of infected sheep with PPR PCR (Mahajan et al., 2013; Santhamani et al., 2016; Mahmoud and Galbat, 2017).

Nucleic acid recognition methods

Most of the serological tests are slow, labor exhaustive, and of low sensitivity. Therefore, they are of low value in the primary diagnosis, but of high value in confirmatory steps and in epidemiological studies. Real-time RT-PCR and loop-mediated isothermal amplification (LAMP) assays are used for their quickness and high sensitivity in the detection of PPRV RNA from clinical samples to overcome the problems associated with the serological tests (Santhamani et al., 2016).

Multiplex PCR

The conventional RT-PCR is laborious and unaffordable for routine clinical diagnosis in poorly financed laboratories, especially in large sample numbers. Moreover, this technique is sensitive to cross-contamination during sample collection and processing which may lead to false positives (Santhamani et al., 2016). False negatives may occur due to unexpected alteration in the primer binding sites, destruction of the RNA, in addition to the existence of PCR inhibitors in the samples. To overcome the false negatives results, multiplex PCR must be applied in the presence of multiple primer pairs to amplify not only different but also specific regions of genes. There are two types of multiplex RT-PCRs. Two-step and single-step procedures are directed to the N and M genes to differentiate PPRV from the rinderpest virus (Balamurugan et al., 2006). The multiplex RT-PCR has higher sensitivity for PPR diagnosis than s-ELISA. Cross-contamination is reduced in single-step assays as the same micro-tube was used for the two reverse transcriptions. Lately, all the respiratory diseases of sheep and goats could be diagnosed by a single-step multiplex RT-qPCR including PPRV, *Capripoxvirus*, *Pasteurella multocida*, and *Mycoplasma capricolum* subsp. *capripneumoniae* (Settypalli et al., 2016).

Loop-mediated isothermal amplification

The LAMP applied for PPR diagnosis has a higher sensitivity than RT-PCR. The one-step LAMP technique is applicable for the diagnosis of PPR and reduces the possibility of sample contamination and will be a hopeful tool in low-financed laboratories (Venkatesan et al., 2015).

Serological diagnosis

Virus neutralization test

VNT is the prescribed test for international trade. It was applied as a confirmatory test for the differentiation between RP and PPR, and for PPRV antibodies detection in serum samples (Santhamani et al., 2016). Although PPRV is antigenically related to the RP virus, PPRV could be differentiated serologically from it (Munir, 2014).

Competitive ELISA

Competitive ELISA showed a high specificity (99.85%) and sensitivity (91.5 %) in the detection of PPRV antibodies in tested sera when compared with VNT (Abubakar et al., 2015; Mahmoud and Galbat, 2017). The market contains patent kits that have a high indicative sensitivity and specificity (92.2 % and 98.4 %, respectively) (Santhamani et al., 2016).

Hemagglutination assay

HA has a higher sensitivity than AGID in the diagnosis of PPR (Abubakar et al., 2011). This result agrees with the obtained results by Nussieba et al. (2008). Furthermore, the HA test is a rapid, simple, inexpensive, and reliable confirmatory test for the diagnosis of PPRV (OIE and FAO, 2015**)**. Table 1 illustrates tests commonly used in the diagnosis of PPR.

Table 1. The different diagnostic tests commonly used in the diagnosis of Peste des Petits Ruminants (OIE and FAO, 2015)

Diagnostic tests/tools	Detection target	Application	Advantages	Disadvantages	Relative sensitivity/ detection limit
Virus isolation	Virus	Gold standard test	Useful for virus repository, primary step for virus characterization	Live virus, cell culture facilities required, less sensitive than RT-PCR	More sensitive than sandwich ELISA
Immunocapture ELISA/ sandwich ELISA	N protein	Diagnosis, clinical surveillance	User-friendly test, Preliminary vaccine quality control	Less sensitive than RT-PCR and RT-PCR-ELISA	$10^{1.9}$ $TCID_{50}$/mL 500 $TCID_{50}$/mL
Dot-ELISA	M and N protein	Diagnosis	Easy and quick	Less sensitive than sandwich ELISA	82 % compared to sandwich ELISA

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One-step multiplex RT-PCR	M and N genes	Diagnosis	Sensitive test, potential for Routine diagnosis	May not be economical for routine diagnosis	100 µg of RNA

CONTROL OF PESTE DES PETITS RUMINANTS DISEASE

Prevention and control

The PPR is an important animal disease and its control reflects the economic status of Africa and Southern Asia. Vaccination is the main way to avoid PPR infection. African countries, including West Africa, have succeeded in the control of PPR by the use of the vaccine. As the PPR and RP are antigenically similar, so a live attenuated vaccine prepared from RP was used in the vaccination of goats against PPR and this led to one year of protection (Singh and Bandyopadhyay, 2015). Therefore, different parts of the world controlled PPR by using the tissue culture RP (TCRP) vaccine. This TCRP vaccine is used to protect small ruminants against PPR. Later on, the use of the TCRP vaccine was stopped worldwide to eradicate the RP (OIE and FAO, 2015). The current attenuated vaccines are heat-sensitive, so continuous uninterrupted maintenance of the cold chain until an application to the animal is recommended. Other currently commercially available vaccines are present in freeze-dried form. They are stable for at least two years at 2-8°C and for several years at –20 °C. After reconstitution of the vaccine, it must be utilized within 30 minutes (OIE and FAO, 2015).

The proper control of PPR depends on the solid support of the diagnostic methods and the proper vaccination program. The accessibility of live attenuated tissue culture vaccines and many diagnostic techniques/kits for the diagnosis of PPR facilitate the establishment of a good control program. For controlling the disease in a free area, a stamping out policy is applied, but in a country, like Egypt, this policy is not economic, so PPR control depends primarily on the fast and exact diagnosis or surveillance/monitoring and application of rapid vaccination program (Soltan and Abd-Eldaim, 2014). All the needed elements for a good control program are available in Egypt like an effective vaccine, availability of the diagnostic kits, sero-surveillance, and good infrastructures. Management decisions and control strategies need the development of rapid and accurate diagnostic tools. Immunoenzymatic assays are advantageous because they are cheap, reliable, and quick to perform. Thus, ELISA may be a useful tool in large-scale screening and eradication programs giving insight into the local viral infection status (Ghazy et al., 2007).

Vaccines for peste des petits ruminants

Tissue culture live attenuated vaccine

Tissue culture vaccine was the first produced vaccine for the protection against PPR. It developed from live attenuated Nigerian strain PPRV Nig. 75/1 that has been cultured for 63 passages onto Vero cells. It provokes long-standing immunity for up to 3 years (Diallo et al., 1989; Diallo et al., 2007). Under field conditions, the vaccine was safe for sheep and goats and gave immunity to 98% of the immunized animals even pregnant ones (Diallo et al., 1989). However, the major disadvantage of this type of vaccine is the thermo-liability and inability to distinguish between the vaccinated and naturally infected animals (Venkataramanan et al., 2005). Newborns of vaccinated dams should vaccinate at the age of 4-6 months. Meanwhile, newborns of non-vaccinated dams should vaccinate at the age of two months. The booster dose is recommended after one year.

Recombinant vaccines

The bases of this type of vaccine depend on the insertion of one or both of the F and H glycoproteins producing genes extracted from PPRV RNA into *Capripoxvirus*. The resulting combined vaccine induces protection against PPR and capripox (Berhe et al., 2003). The use of such a vaccine is valuable in regions indicating mixed infection with PPR and sheep or goat pox. Again, the major disadvantage of this type of vaccine is the inability to distinguish between the vaccinated and naturally infected animals (Venkataramanan et al., 2005).

Marker vaccine

In this type of vaccine, a modified live attenuated virus vaccine was developed (Singh et al., 2015). RP vaccine virus genome can be used as the main template for the vaccine besides the replacement of the M, F, and H genes of PPRV. The subsequently produced vaccine is a harmless and active vaccine to protect animals against virulent challenges with PPRV (Mahapatra et al., 2006). In addition, EHV-1 vaccine marker development would help the differentiation between the wild-type virus infection and the mutant virus immunization (Ata et al., 2018).

Vaccines for peste des petits ruminants in Egypt

The vaccination program in Egypt is annual and applied before the season of the disease occurrence, which is usually in September. The Veterinary Serum and Vaccine Research Institute, Cairo, Egypt produces the used vaccine. It

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is a tissue culture attenuated PPR vaccine. It is a free obligatory vaccine for small ruminants. It is applied around the foci of infection (ring vaccination) and as an obligator in case of epidemics. The Center of Food Security and Public Health (CFSPH, 2015) discusses the important vaccines used for PPR as shown in Table 2.

Table 2. The commercially available vaccines for Peste des petits ruminants in Egypt

Producer	Product name	Type	Strain	Adjuvant	Licensed countries
Veterinary Serum and Vaccine Research Institute	Peste Des Petits Ruminants Vaccine (PPR-N75/1)	Live	Nigeria 75/1	Not Available	Egypt
M.C.I. Santé Animale	LYOPOX PPR (Sheep & Goat Pox, Peste des Petits Ruminants)	Live	Romanian (Pox) Nigeria 75 (PPR)	None	Egypt and other
	OVIVAX PPR	Live	Nigeria 75/1	None	Egypt and other
Jordan Bio-Industries Center (JOVAC)	Pestevac	Live	Nigeria 75/1	Non	Egypt and other

Source: The Center of Food Security and Public Health (2015)

Treatment

Peste des petits ruminants like all other viral diseases have no specific treatment. However, affected animals should receive antibiotics (long-acting oxytetracycline, chlortetracycline) to combat secondary bacterial infections, and antidiarrheal drugs with supportive therapy for 5-7 days. The symptomatic treatment reduces the severity of the disease and diminishes the economic losses (OIE and FAO, 2015). Moreover, dietary supplementation with natural antioxidants such as *Nigella sativa*, black cumin seeds, or multi-nutrient antioxidants provides potent immune cell function and has an antibacterial effect against secondary bacterial infections (Abou-Zeina et al., 2013).

CONCLUSION

Sheep and goats represent the essential source of meat and wool production in some countries. Sheep and goat husbandry economically depend on growing these animals in large numbers in a small area (intensive breeding). Several sheep and goat diseases that hinder the intensification of animal husbandry create complex animal health and production problems. Many conventional and modern techniques are used for the diagnosis of PPR. Numerous conventional techniques include isolation on cell culture, serological tests, and immunohistochemical assays usually used in the identification of viral pathogens. However, modern molecular diagnostic techniques are more valuable, more sensitive, fast, and high confident results. Novel methodology such as PCR, DNA probe, and nucleic acid sequencing deliver a detailed understanding of the exact diagnosis and discrimination of the present and emerging diseases. Modern diagnostic tools are urgently needed not only for diagnosis but also for monitoring and controlling viral disease programs. The control program relies mainly on hygiene, vaccination, sanitation, and effective quarantine measures.

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Authors' contribution

All authors, namely Mahmoud MA, Ghazy AA, and Shaapan contributed to developing the concept and designed the review article. Ghazy AA and Shaapan RM. Raafat M. wrote the paper and prepared it for publication.

Competing interests

The authors declare that they have no conflict of interest.

Ethical considerations

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The above-mentioned was ethically cleared by authors including plagiarism checking, the article has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and there is no redundancy

REFERENCES

Abd El-Rahim IH, Sharawi SS, Barakat MR, and El-Nahas EM (2010). An outbreak of peste des petits ruminants in migratory flocks of sheep and goats in Egypt in 2006. Scientific and Technical Review of the Office International des Epizooties, 29(3): 655-662. DOI: http://www.doi.org/10.20506/rst.29.3.2004

Abou-Zeina HAA, Ghazy AA, El-Bayoumy MK, Khairy HA, and Twfik HI (2013). Effects of dietary antioxidants supplementation on cellular immunity response and evaluation of their antimicrobial activity against some enteric pathogens in goats. Global Veterinaria, 11(2): 145-154. DOI: https://www.doi.org/10.5829/idosi.gv.2013.11.2.74153

Abubakar M, Irfan M, and Manzoor S (2015). Peste des petits ruminants in Pakistan ; past , present and future perspectives. Journal of Animal Science and Technology, 57(1): 1-8. DOI: https://www.doi.org/10.1186/s40781-015-0066-0

Abubakar M, Khanb AH, Arsheda MJ, Hussain M, and Ali Q (2011). Peste des petits ruminants (PPR): Disease appraisal with global and Pakistan perspective. Small Ruminant Research, 96(1): 1-10. DOI: https://www.doi.org/10.1016/j.smallrumres.2010.10.006

Albina E, Kwiatek O, Minet C, Lancelot R, de Almeida RS, and Libeau G (2013). Peste des petits ruminants, the next eradicated animal disease. Veterinary Microbiology, 165(1-2): 38-44. DOI: https://www.doi.org/10.1016/j.vetmic.2012.12.013

Anderson J, Corteyn M, and Libeau G (2006). Diagnosis of rinderpest and peste des petits ruminantsvirus. In: Rinderpest and peste des petits ruminant: Virus plagues of large and small ruminants. Academic Press., pp. 163-184. Available at: https://agritrop.cirad.fr/529738/

Ata EB, Zaghawa A, Ghazy AA, Elsify A, and Shaapan RM (2018). Equine herpes virus type-1 infection: Etiology, epidemiology, pathogenesis, identification. Asian Journal of Epidemiology, 11(1): 34-45. DOI: https://www.doi.org/10.3923/aje.2018.34.45

Balamurugan V, Hemadri D, Gajendragad MR, Singh RH, and Rahman H (2014). Diagnosis and control of peste des petits ruminants: A comprehensive review. Virus Disease, 25: 39-56. DOI: https://www.doi.org/10.1007/s13337-013-0188-2

Balamurugan V, Sen A, Saravanan P, Singh RP, Singh RK, Rasool TJ, and Bandyopadhyay SK (2006). One-step multiplex RT-PCR assay for the detection of peste des petits ruminants virus in clinical samples. Veterinary Research Communications, 30: 655-666. DOI: https://www.doi.org/10.1007/s11259-006-3331-3

Banyard AC, Parida S, Batten C, Oura C, Kwiatek O, and Libeau G (2010). Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. Journal of General Virology, 91: 2885-2897. DOI: https://www.doi.org/10.1099/vir.0.025841-0

Berhe G, Minet C, Le Goff C, Barrett T, Ngangnou A, Grillet C, Libeau G, Fleming M, Black DN, and Diallo A (2003). Development of a dual recombinant vaccine to protect small ruminants against peste-des-petits-ruminants virus and capripoxvirus infections. Journal of Virology, 77: 1571-1577. DOI: https://www.doi.org/10.1128/JVI.77.2.1571-1577.2003

Burezq HA, Khalil F, Dashti J, Essawi A, Al-Thefeeri F, and Farouq M (2020). Controlling lambs mortality through ewes vaccination in the farms of Kuwait. Journal of Animal and Plant Sciences, 30(3): 595-602. DOI: https://www.doi.org/10.36899/JAPS.2020.3.0070

Center for Food Security and Public Health (CFSPH) (2015). Peste des petits ruminants. College of Veterinary Medicine, Iowa State University, 12: 1-7. Available at: https://www.cfsph.iastate.edu/Factsheets/pdfs/peste_des_petits_ruminants.pdf

Diallo A, Minet C, Le Goff C, Berhe G, Albina E, Libeau G, and Barrett T (2007). The threat of peste des petits ruminants: Progress in vaccine development for disease control. Vaccine, 25(30): 5591-5597. DOI: https://www.doi.org/10.1016/j.vaccine.2007.02.013

Diallo A, Taylor WP, Lefevre PC, and Provost A (1989). Atténuation d'une souche de virus de la peste des petits ruminants: Candidat pour un vaccin homologue. Revue d'élevage et de Médecine Vétérinaire des Pays Tropicaux, 42(3): 311-319. Available at: http://revues.cirad.fr/index.php/REMVT/article/download/8771/8766

Durojaiye OA (1987). Application of a precipitinogen inhibition test in the detection of antibody to peste des petits ruminants virus. Revue d'élevage et de Médecine Vétérinaire des Pays Tropicaux, 40(1): 17-20. Available at: http://revues.cirad.fr/index.php/REMVT/article/download/8684/8678

El-Allawy TA, Laila SA, and El-Rahim IHA (1993). Serological studies on peste des petits ruminants (PPR) in Upper Egypt. In Proceedings 2nd Scientific Congress, Egyptian Society for Cattle Diseases, Assiut, Egypt, 20: 278-289.

Farougou S, Gagara M, and Mensah GA (2013). Prevalence of peste des petits ruminants in the arid zone in the Republic of Niger. Journal of Veterinary Research, 80(1): 544-550. DOI: https://www.doi.org/10.4102/ojvr.v80i1.544

Fayed AA, Awad F, Abdel O, Hassan HB, and Abd-Alla M (1994). Effect of seasons on the prevalence of antibodies of peste des petits ruminants (PPR) virus in sheep and goats. Veterinary Medical Journal Giza, 42(1): 15-18. Available at: https://vlibrary.emro.who.int/imemr/effect-of-seasons-on-the-prevalence-of-antibodies-of-peste-des-petits-ruminates-pprvirus-in-sheep-and-goats/

Forsyth MA and Barrett T (1995). Evaluation of polymerase chain reaction for the detection and characterisation of rinderpest and peste des petits ruminants viruses for epidemiological studies. Virus Research, 39(2-3): 151-163. DOI: https://www.doi.org/10.1016/0168-1702(95)00076-3

Ghazy AA, Ahmed WM, Mahmoud MA, and Ahmed LA (2007). Prevalence of infectious bovine rhinotracheitis and bovine viral diarrhoea viruses in female buffaloes with reproductive disorders and parasitic infections. International Journal of Dairy Science, 2(4): 339-347. DOI: https://www.doi.org/10.3923/ijds.2007.339.347

Ikram A, Karim IA, El-Danaf NA, and House J (1988). Isolation of a viral agent from Egypt goats suspected to be PPR virus. Journal of Egyptian Veterinaty Medical Association, 48: 429-435.

Ismail TM, Yamanaka MK, Saliki JT, EL-Kholy A, Mebus C, and Yilma T (1995). Cloning and expression of the nucleoprotein of peste des petits ruminants virus in baculovirus for use in serological diagnosis. Virology, 208(2): 776-778. DOI: https://www.doi.org/10.1006/viro.1995.1210

Kardjadj M (2018). Epidemiological situation of transboundary animal diseases in North African countries — proposition of a regional control strategy. Tropical Animal Health and Production, 50: 459-467. DOI: https://www.doi.org/10.1007/s11250-017-1453-y

Kardjadj M, Metref D, Kouidri B, Pam DL, and Ben-Mahdi MH (2015). Seroprevalence, distribution and risk factor for peste des petits ruminants (PPR) in Algeria. Preventive Veterinary Medicine, 122(1-2): 205-210. DOI: https://www.doi.org/10.1016/j.prevetmed.2015.09.002

Kinimi E, Odongo S, Muyldermans S, Kock R, and Misinzo G (2020). Paradigm shift in the diagnosis of peste des petits ruminants: Scoping review. Acta Veterinaria Scandinavica, 62(1): 1-4. DOI: https://www.doi.org/10.1186/s13028-020-0505-x

Kwiatek O, Ali YH, Saeed IK, Khalafalla AI, Mohamed OI, Obeida AA, Abdelrahman MB, Osman HM, Taha KM, Abbas Z et al. (2011). Asian lineage of peste des petits ruminants virus Africa. Emerging Infectious Diseases, 17(7): 1223-1231. DOI: https://www.doi.org/10.3201%2Feid1707.101216

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Mahajan S, Mohapatra JK, Pandey LK, and Sharma GK (2013). Truncated recombinant non-structural protein 2C-based indirect ELISA for FMD sero-surveillance. Journal of Virological Methods, 193(2): 405-414. DOI: https://www.doi.org/10.1016/j.jviromet.2013.07.003

Mahapatra M, Parida S, Baron MD, and Barrett T (2006). Matrix protein and glycoproteins F and H of Peste-des-petits-ruminants virus function better as a homologous complex. Journal of General Virology, 87(7): 2021-2029. DOI: https://www.doi.org/10.1099/vir.0.81721-0

Mahmoud MA and Galbat SA (2017). Outbreak of foot and mouth disease and peste des petits ruminants in sheep flock imported for immediate slaughter in Riyadh. Veterinary World, 10(2): 238-243. DOI: https://www.doi.org/10.14202/vetworld.2017.238-243

Mahmoud MAE, Elbayoumy MK, Sedky D, and Ahmed S (2017). Serological investigation of some important RNA viruses affecting sheep and goats in Giza and Beni-Suef governorates in Egypt. Veterinary World, 10(10): 1161-1166. DOI: https://www.doi.org/10.14202/vetworld.2017.1161-1166

Mouaz MA, Faid AA, Rawwhia ED, and Kodeir MH (1995). Studies on PPR in Egyptian sheep. Veterinary Medical Journal Giza, 43(4): 367-374.

Munir M (2014). Role of wild small ruminants in the epidemiology of peste des petits ruminants. Transboundary and Emerging Diseases, 61(5): 411-424. DOI: https://www.doi.org/10.1111/tbed.12052

Onono JO, Wieland B, and Rushton J (2013). Constraints to cattle production in a semi-arid pastoral system in Kenya. Tropical Animal Health and Production, 45: 1415-1422. DOI: https://www.doi.org/10.1007/s11250-013-0379-2

Osman NA, Rahman MEA, Ali AS, and Fadol MA (2008). Rapid detection of peste des petits ruminants (PPR) virus antigen in Sudan by agar gel precipitation (AGPT) and haemagglutination (HA) tests. Tropical Animal Health and Production, 40(5): 363-368. DOI: https://www.doi.org/10.1007/s11250-007-9106-1

Radostits OM, Gay C, Hinchcliff KW, and Constable PD (2006). Veterinary Medicine: A text book of the diseases of cattle, horses, sheep, pigs and goats. 10th Edition. Saunders Elsevier, London, pp. 1537-1538. Availabe at: https://b2n.ir/e79021

Santhamani R, Singh RP, and Njeumi F (2016). Peste des petits ruminants diagnosis and diagnostic tools at a glance: perspectives on global control and eradication. Archives of Virology, 161: 2953-2967. DOI: https://www.doi.org/10.1007/s00705-016-3009-2

Settypalli TBK, Lamien CE, Spergser J, Lelenta M, Wade A, Gelaye E, Loitsch A, Minoungou G, Thiaucourt F, Diallo A (2016). One-Step Multiplex RT-qPCR assay for the detection of Peste des petits ruminants virus, Capripoxvirus, Pasteurella multocida and *Mycoplasma capricolum* subspecies (ssp.) *capripneumoniae*. PLoS One, 11(4): e0153688.

Singh RK, Rajak KK, Muthuchelvan D, Banyard AC, and Parida S (2015). Vaccines against peste des petits ruminants virus, Peste des Petits Ruminants Virus, 11: 183-194. DOI: https://www.doi.org/10.1007/978-3-662-45165-6_11

Singh RP and Bandyopadhyay SK (2015). Peste des petits ruminants vaccine and vaccination in India: Sharing experience with disease endemic countries. Virus Disease, 26: 215-224. Available at: https://link.springer.com/article/10.1007/s13337-015-0281-9

Soltan MA and Abd-Eldaim MM (2014). Emergence of peste des petits ruminants virus lineage IV in Ismailia. Infection, Genetics and Evolution, 28: 44-47. DOI: https://www.doi.org/10.1016/j.meegid.2014.08.016

Venkataramanan R, Bandyopadhyay SK, and Oberoi MS (2005). Present status and strategies for the control of transboundary and other economically important animal diseases in India: A review. The Indian Journal of Animal Sciences, 75(4): 456-464. Available at: https://epubs.icar.org.in/index.php/IJAnS/article/view/9486

Venkatesan G, Bhanuprakash V, and Balamurugan V (2015). Development and comparative evaluation of loop mediated isothermal amplification (LAMP) assay for simple visual detection of orf virus in sheep and goats. Molecular and Cellular Probes, 29(3): 193-195. DOI: https://www.doi.org/10.1016/j.mcp.2015.03.006

Wasim R, Sen A, Mondal B, Kumar S, Chand K, Kishore K, Gowane GR, Bhusan S, Bihari A, Andavar M et al. (2015). Development of a single-plate combined indirect ELISA (CI-ELISA) for the detection of antibodies against peste-des-petits-ruminants and bluetongue viruses in goats. Small Ruminant Research, 124: 137-139. DOI: https://www.doi.org/10.1016/j.smallrumres.2015.01.007

World Organization of Animal Health (OIE) and Food and Agriculture Organization (FAO) (2015). Global strategy for the control and erradication of PPR. Available at: http://www.fao.org/3/a-i4460e.pdf

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Bats and Antibiotic Resistance: A Culprit or a Victim?

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REVIEW ARTICLE
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ABSTRACT

In the last decades, the increase of antimicrobial resistance bacteria has become a concern for public health. Bats' ability to fly, form colonies for a long lifespan, and inhabit a variety of diverse ecological niches make them successful species in terms of adaptation and distribution on earth. Moreover, these characteristics let them act as the potential natural reservoir of numerous zoonotic pathogens (bacteria, viruses, fungi). Bat bacteriome knowledge is still very scarce, but a few studies have indicated that bats are hosts of antimicrobial resistance and play an important role in the dispersion of resistance in the environment. Moreover, bats are vulnerable to acquiring these pathogens since they sometimes live in close contact with humans and domestic animals. Therefore, the present study aimed to compile the latest studies that describe the presence of antibiotic-resistant in bats. Based on the papers analyzed for this review, it is possible to conclude that bats are hosts of pathogenic bacteria that carry numerous antibiotic resistance. Extended-spectrum b-lactamases (ESBLs) or Methicillin-resistant *Staphylococcus* which nowadays days are a great public health concern, have already been reported in these animals, with some isolated strains being of Human origin. Although not completely understood regarding the dynamics and transmission routes, bats seem to have an important role in the dissemination and acquisition of antibiotic resistance in the environment. They can be contaminated by bacteria with antibiotic resistance and disperse through the environment. However, they also can be the host of bacteria that carry antibiotic resistance.

Keywords: Antibiotic-Resistant, Bacteria, Bat, Chiropters, Zoonoses

INTRODUCTION

Bats are mammals that belong to the order Chiroptera, which is classified into two suborders, including Megachiroptera and Microchiroptera (Fenton and Simmons, 2015; Wilson and Mittermeier, 2019). They are an incredibly diverse group of mammals with the ability to fly and cover long distances during seasonal migrations. Most of the species are nocturnal and prefer to roost in large colonial populations on caves, trees, or human constructions. Some species hibernate during the winter (Neuweiler, 2000; Kunz and Fenton, 2006) and are characterized by using echolocation to capture their prey (Kunz and Fenton, 2006).

This order corresponds to nearly 20% of all classified mammal species worldwide, with over 1400 species reported. Bats are present on almost every continent, except for extremely cold regions, such as Antarctica or the Arctic continent. Their diet is very diverse, depending on the specie can include insects, fruit, nectar and blood or other animals (Neuweiler, 2000; Kunz and Fenton, 2006; Wilson and Mittermeier, 2019). Chiropterans provide humans and ecosystems with various benefits, being essential to the ecosystem's health and economics. Plant pollination, control of insect populations, utilization as fertilizer, rural tourism, and the food source as bushmeat, (Neuweiler, 2000; Kunz and Fenton, 2006; Wilson and Mittermeier, 2019) are some of the services provided by bats.

Antimicrobial resistance (AMR) is a global concern that affects humans, domestic animals, and wildlife. The report of AMR in wildlife has been increasing in the last years, showing their potential role as reservoirs and in the transmission of these pathogens to humans, domestic animals and the environment (Nguema et al., 2020). This subject has been studied under a multidisciplinary and collaborative "One Health" approach, addressing interactions between humans-domestic and non-domestic animals-environment to understand and prevent the dispersion of AMR (Calisher et al., 2006; Mühldorfer, 2013). There is not enough understanding regarding the dynamics and transmission routes among bats and humans (McDougall et al., 2021a).

The ability to fly, form colonies, long lifespan and capability to inhabit a variety of diverse ecological niches make bats one of the most successful species on earth (Neuweiler, 2000) but also made them a potential natural reservoir of numerous zoonotic pathogens (bacteria, viruses, fungi, Mühldorfer, 2013). They have been considered a reservoir of many viruses that occasionally spread into human populations and cause disease outbreaks, such as SARS-CoV-2 (Garcês et al., 2020). The AMR has already been reported in several species of bats. Unfortunately, still little is known regarding bat microbiota and the dynamics of transmission and acquisition of AMR (Calisher et al., 2006; Adesiyun et al., 2009; Nguema et al., 2020). The present study review compiles the latest studies that describe the presence of AMR in bats.

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WEB-BASED LITERATURE SEARCH

To produce this review, we conducted a literature search through the main web search for peer reviews of scientific articles (e.g. original research, case reports, letters, short communications, review articles) and non-peer-reviewed grey bibliographies (e.g. thesis, reports, white papers). To find these references the author placed a specific set of terms in the main search engines available on the internet. The main engines of research were Google Web, ResearchGate, PubMed, and Google Scholer. The search terms included combinations of bats, Chiropterans, antibiotic resistance, zoonoses, bacteria, enteric bacteria, one health, wildlife health, and environment. The languages used in the research were English, Spanish and Portuguese. As inclusion criteria, only works that describe information regarding antibiotic resistance in different bat species were included. Some studies were not used in this review because the information was mixed with other species of mammals or birds and was not possible to retrieve information regarding specie, type of samples or which resistances were observed.

BATS BACTERIOME AND ENTERIC PATHOGENS

Bat bacteriome knowledge is still very scarce. Studies performed have indicated a relatively high diversity and function of bacterial species (Gerbáčová et al., 2020; Nguema et al., 2020). In several studies, it was observed that Gammaproteobacterial (with Enterobacteriaceae family in predominance) appears to be the prevalent bacterial group in the Chiroptera order, while Fusobacteria is less represented in comparison to other mammal species (Gerbáčová et al., 2020). The bacteriome will vary according to several extrinsic and intrinsic factors, such as season, sex, diet type, reproductive stage, and environmental factors (Gerbáčová et al., 2020).

Beyond the bacterial commensal strains, it has been identified in bat's gastrointestinal flora strains similar to the strains of human and animal pathogens in individuals and colonies of different bat species. Bats are a host of pathogenic bacteria such as *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter* (Mühldorfer, 2013). These pathogens usually do not cause disease in the bats but have the potential of causing diseases such as diarrhoea, septicemia, and meningitis, in humans and animals (Nowak et al., 2017; Silveira, 2021). Bats and their guano could serve as reservoirs of numerous pathogenic agents, some of which are zoonotic (Vandžurová et al., 2013). Figure 1 presents some bat-related zoonoses. Bats are hosts of numerous viruses, bacteria, parasites and fungi, that can be transmitted to other animals or Humans and induce diseases.

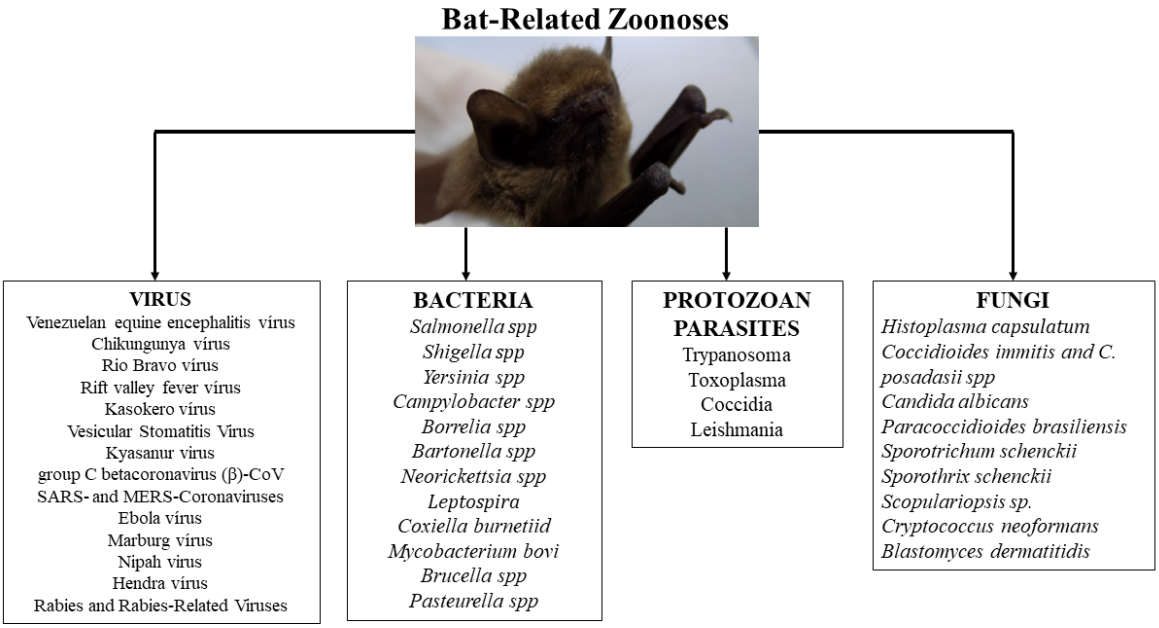

Figure 1. The reports of zoonotic diseases in bat species

BAT DISPERSION AND CONTAMINATION SOURCE OF PATHOGENIC BACTERIA

Due to their specific style of life bats are very prone to spread AMR in the environment and acquire new pathogenic strains. Their flight ability allows them to be in constant movement for food and shelter and to migrate from one region to another (large territorial range of some species). This activity increases the chance of being exposed to antimicrobials, raising the risk of developing, changing, or acquiring a new microbiota (Sens-Junior et al., 2018). Their longevity (some individuals can live up to 30 years) and the realization of periods of hibernation and torpor by some species, allow microorganisms to persist for long periods in the spots where animals are inhabiting and facilitate their transmission between individuals (Sens-Junior et al., 2018).

Figure 2 has represented a scheme considering the possible routes of spread and acquisition of AMR and pathogenic agents by bats. Chiropterans do not build shelters, they often use the natural landscape (e.g., caves or trees) as

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places of shelter, to reproduce and hibernate (Voigt et al., 2015). Many species use artificial hideouts in human environments, such as attics and cellars, as permanent, or temporary shelters (McDougall et al., 2021a). Large colonies can be formed inside of these artificial structures, because there are many benefits to the colony, not only obtaining shelter but also having easier access to food sources (Voigt et al., 2015). The use of shelters located very near to the human population, that is specific synanthropization, raises the change of direct and indirect contact of human and domestic animals with the microbiota of bats. Pets (particularly cats) can hunt the bats. Direct contact with bats when these are captured to be reallocated, or the contact of an aerosol of bat's faeces (guano), are some of the routes by which humans and domestic or non-domestic animals can acquire these pathogenic bacteria from bats (Nowakiewicz et al., 2020; McDougall et al., 2021a).

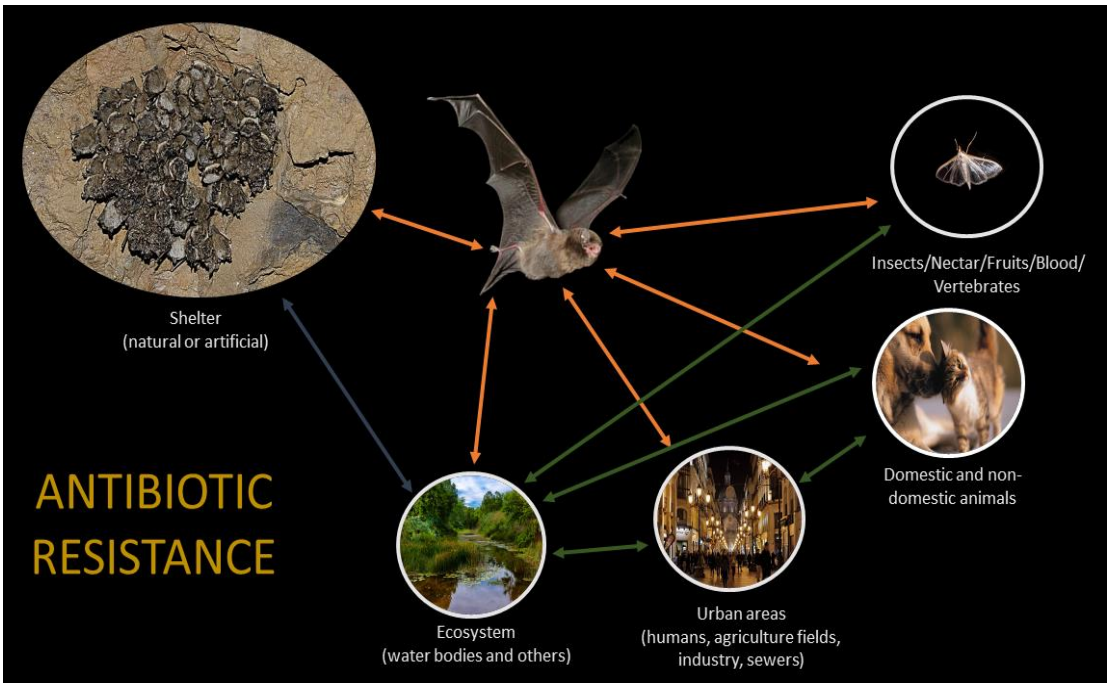

Figure 2. The possible routes of spread and acquisition of antibiotic resistance and pathogenic agents by bats

Another source of contamination due to direct contact with humans is tourists that visit large colonies that are an attraction in some regions. Tourists are responsible for introducing resistant strains of bacteria in and out of the cave, spreading AMR into the ecosystems (Mulec et al., 2020). To prevent the dissemination of these strains should be performed a regular cleaning of the tourist footpaths and a disinfectant barrier at the tourist cave entrance and exit (Mulec et al., 2020). Environmental compartments (e.g., water bodies) can also serve as reservoirs for multiresistant bacteria and resistance genes (Gharout-Sait et al., 2019). The origin of these agents is diverse. Rivers and seas are exposed to discharges with microbial contaminants from industrial, agricultural, and domestic sources, such as faecal sludge from dairy farms, discharges from aquaculture, hospitals, sewers, wastewater treatment plants and surface waters, and others (Sherley et al., 2000; Gharout-Sait et al., 2019). In some countries, antimicrobials agents are still used in domestic animals for their protection and improver, such as prophylaxis of diseases. These animals can act as a source of environmental contamination (Sens-Junior et al., 2018). In many caverns, there is a network of underground water channels that have contact with the exterior environment. These underground water bodies can be contaminated with AMR due to the infiltration of bat faecal matter during the rainy seasons or introduce contaminated water into the caves (Mulec et al., 2020).

The surface of bodies of water is typically rich in insects and other invertebrates, which are an important food source to many species of bats. Bats can acquire and transmit strains of resistant bacteria when in contact with these contaminated water bodies but also from consuming their food source. Insects are also hosts of multidrug-resistant bacteria and have the potential to spread through the environment. Studies have shown that fly guts are carried antimicrobial-resistant bacteria that share the same genotypes as strains isolated on humans and other animals and can occur horizontal transfer of plasmids bearing antibiotic resistance genes inside their gut. (Gharout-Sait et al., 2019). Other sources of food such as fruits, flowers and blood from other animals also can be a source of acquisition of AMR by bats. Plants can be contaminated with water and faecal matter that are contaminated with AMR bacteria. Hematogenous species can also feed on animals and humans that are also carriers of multidrug-resistant bacteria (Nowakiewicz et al., 2020; McDougall et al., 2021b).

ANTIBIOTIC RESISTANCE IN CHIROPTERANS

Overall, the present study presents a total of 32 works, between the years 1985 to 2021. Of the 32 papers (presented in table 1) the majority, 31.3 % (10/32) were performed in individuals from the Pteropodidae family, 25 % (8/32) in the Vespertilionidae Family and the remaining studies in individuals of several families from the Chiroptera Order. In 34.4% (n = 11/32) papers the study was performed in colonies that harboured mixes of individuals from different families. In one study, the specie was not determined.

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Table 1. Antibiotic resistance in Chiropterans regarding specie, family, country, year, type sample, bacteria isolated, antibiotic resistance and resistance genes

Specie	Family	Location of colonies	Country	Year	Type of sample	Isolated Bacteria	Antibiotic resistance	Resistance genes	Reference
Cynopterus tittecheilus, C brachyotis javanicus, C sphinx angulatus, Myotis muricola	Pteropodidae Vespertilionidae	Cave near and in coastal village	Indonesia	1985	Faecal	*Escherichia coli, Klebsiela* spp., *Enterobacter* spp.	*E. coli* to almost every antibiotic tested, with 10 strains resistant to trimethoprim and cephalothin. *Klebsiella* spp. was sensitive to almost every antibiotic except 9 strains resistant to ampicillin, cephalothin, trimethoprim and tetracycline. *Enterobacter* spp. all isolates presented resistance, between 1-6 different agents.		(Graves et al., 1988)
Myotis, M blythii	Vespertilionidae	Church tower	Slovakia	2010	Faecal (guano)	*Staphylococcus nepalensis*	Nalidixic acid, aztreonam, tetracycline, chloramphenicol and erythromycin.		(Vandžurová et al., 2013)
Carollia perspicillata, Sturnira lillium, Artibeus lituratus, Artibeus planirostris	Phyllostomidae	Fragment forest near city	Brasil	2018	Oral and perianal region	*Escherichia coli; Enterobacter aerogenes, Enterobacter agglomerans, Serratia liquefaciens*	All individuals had resistant strains, with a few of them presenting multi-resistance to antimicrobials.		(Sens-Junior et al., 2018)
Rhinolophus pumilus	Rhinolophidae	Cave	Japan	2014	Faecal	*Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii*	One *E. coli* isolate was resistant to chlortetracycline and streptomycin and 4 *K. pneumoniae* and 5 *C. freundii* were resistant to sulfadimethoxine.	Five of the ten resistant isolates exhibited the transmissible R plasmid.	(Obi et al., 2014)
Myotis daubentonii, Eptesicus serotinus, Myotis nattereri, Plecotus auritus, Nathusius' Pipistrellus pipistrellus,	Vespertilionidae	Urban area	Poland	2021	Faecal	*Enterococcus faecalis*	69% of the isolates were multi-drug resistant. Resistance to tetracycline, rifampicin and erythromycin, High-level aminoglycoside resistance (HLAR) was detected in 41.6% of strains.	In all strains the presence of at least one gene encoding resistance: tetM, ermBoraph (30)-IIIaI, gelE, aac (60)-Ie-aph (200)-Ia and ant (6)-Ia).	(Nowakiewicz et al., 2021)
		Urban area	Poland	2020	Faecal	*Escherichia coli*	71% of the isolated strains were multi-drug resistant. The highest resistance was observed in the case of ampicillin, kanamycin, sulfamethoxazole/trimethoprim, and streptomycin.	Two isolates produced extended-spectrum beta-lactamases (ESBL) (bla CTX-M-3, bla CTX-M-15, bla TEM-1).	(Nowakiewicz et al., 2020)
Eidolon helvum, Nycteris hispida	Pteropodidae Nycteridae	Trees near a market and caves	Nigeria	2021	Samples liver, spleen, and intestines	*Escherichia coli*	22.9 % of the isolates were multidrug-resistant-	Two isolates were cefotaxime-resistant, ESBL-producers and harboured the blaCTX-M-15. tet(A), int1 and blaTEM genes were also found. Three isolates contained the eae gene.	(Obodoechi et al., 2021)
Tadarida brasiliensis, Histiotus velatus, Molossus molossus, Eptesicus brasiliensis	Molossidae Vespertilionidae	Urban area	Brasil	2018	Faecal	*Staphylococcus aureus, Yersinia enterocolitica*	70.59% of the *S. aureus* were resistant to methicillin		(Silveira, 2021)
Eidolon helvum, Epomops franqueti, Hypsignathus monstrosus, Myonycteris torquata, Rousettus aegyptiacus	Pteropodidae	National parks and reserves	Republic Congo	2009-2010	Intestine, liver, lung, kidney	*Escherichia coli*	Extended-Spectrum ESBL phenotype was detected in 60% of the samples.	Were detected sequence types (e.g., ST69, ST127, and ST131) and pathotypes (e.g., ExPEC, EPEC and atypical EPEC).	(Nowak et al., 2017)
Barbastella barbastellus, Eptesicus serotinus, Miniopterus schreibersii, Myotis capaccinii,	Vespertilionidae Miniopteridae Rhinolophidae	Caves	Slovenia	2020	Swab samples from tourist	*Escherichia coli*	Several isolates from rivers and swabs exhibited a multidrug-resistant phenotype		(Mulec et al., 2020)

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M. daubentonii, M. oxygnathus, M. myotis, M. nattereri, Nyctalus noctula, Pipistrellus pipistrellus, P. pygmaeus, Rhinolophus ferrumequinum, R. hipposideros					footpaths				
Desmodus rotundus	Phyllostomidae	Farms	Peru	2015	Faecal	*Escherichia coli*	ESBL in 4. 94 % of the isolates	Expression of blaCTXM-15 genes carried by plasmids	(Benavides et al., 2018; Mendoza, 2017)
Pteropus poliocephalus	Pteropodidae	Urban and rural areas	Australia	2017-2018	Faecal	*Klebsiella pneumoniae, Klebsiella africana and Klebsiella variicola subsp. variicola*		Trimethoprim and fluoroquinolone resistance genes in 3 *K. pneumonia* isolates.	(McDougall et al., 2021b)
		Urban and rural areas	Australia	2020	Faecal	*Escherichia coli*	3.8% *E. coli* were amoxicillin-resistant with 38.5% isolates exhibiting multi-resistance. One isolate presented resistance to carbapenem and fluoroquinolone resistance. Two isolates presented resistant to third-generation cephalosporins. 69.2% of isolates presented resistance to tetracycline and trimethoprim-sulfamethoxazole.	38.5% of isolates had class 1 integrons. 69.2% of isolates harboured extraintestinal virulence factors.,	(McDougall et al., 2019; McDougall et al., 2021a)
Epomops franqueti, Megaloglossus woermanni	Pteropodidae	Forest area and trees near village	Gabon	2020	Faecal	*Enterobacteriaceae*	ESBLs in 20.69% E. coli isolates, 13.79% *K. pneumoniae* isolates, and 3.45% *E. cloacae* isolate.	41.67% ESBL had CTX-M-15- gene and, 16.67% CTX-M-15+SHV-11. has presented *E. coli*, In the K. pneumonia isolates 8.33% ha CTX-M-15- and 25% CTX-M-15+SHV-11 CTX-M-15 was detected in 8.33% *E. cloacae.*	(Nguema et al., 2020)
Molossus rufus	Molossidae	Forest area	Brasil	2019	Samples of stomach and intestine	*Lactobacillus* spp. *Enterobacteriaceae*	21% isolates where multiresistant		(Mateus-Vargas et al., 2017)
Chironax melanocephalus	Pteropodidae	National park	Indonesia	2020	Oral and rectal swab	*Proteus mirabilis, Serratia marcescens subsp. marcescens*	*P. mirabilis* and *S. marcescens* are resistant to oxacillin and susceptible to amikacin Antibiotic-resistant profiles also show *P. mirabilis* susceptible against cefoxitin (and amoxicillin-clavulanic acid, while *S. marcescens* resistant against these two antibiotics.	-	(Masrukhin et al., 2021)
Tadarida brasiliensis	Molossidae	Housing ceilings	Brasil	2015-2016	Faeces	*Enterococcus faecalis, E. casseliflavus, E. gallinarum, and E. mundtii*	Resistance to rifampicin, erythromycin, norfloxacin, ciprofloxacin and tetracycline.	Resistance genes detected: tetM, rmC, gyrA, vanA, vanB, vanC1 and vanC2-3 r. Virulence genes observed: gelE, ace, agg, cylA and esp.	(Costa et al., 2019; Frazzon and Alegre, 2018)
Eidolon helvum	Pteropodidae	Urban areas	Nigeria	2017	Faeces	*Citrobacter* spp., *Enterobacter* spp., *Salmonella* spp., *Klebsiella* spp., *and Escherichia coli*	Resistance to multiple antibiotics	35.9% of isolates were ESBL Resistance genes MultiDHA and TEM and virulence Biofilm, PAPC, ISS and eaeAgenes were detected.	(Aladejana, 2019)
		Housing ceilings	Nigeria	2008-2010	Faeces	*Staphylococcus aureus*	The majority are susceptible to the antibiotic tested.	92.9% coagulase type VI, and accessory gene typing classified 69 isolates into the following: type I (12; 17.1%), type II (3; 4.3%), and type III (1; 1.4%) and type IV (53; 75.7%).	(Akobi et al., 2012)

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Artebius sp., Carollia perspicillata, Desmodus rotundus, Diaemus youngi, Glossophaga sp, Molossus major, Molossus ater, Mormoops sp., Noctilio leporinus, Phyllostomus hastatus, Phyllostomus discolor, Pteronotus parnelli	Phyllostomidae Molossidae Noctilionidae Mormoopidae	Urban and rural areas	Trinidade	2006-2006	Gastrointestinal tract	*Salmonella* spp., *Escherichia coli, E. coli O15, Campylobacter* spp.	82% of the *E. coli* isolates exhibited resistance to antibiotic: erythromycin (61%), (27%), gentamycin - (0%) and sulphamethoxazole/trimethoprim (2%).		(Adesiyun et al., 2009)
Myotis myotis, Rhinolophus hipposideros	Vespertilionidae Rhinolophidae	Housing ceilings	Slovakia	2020	Faecal	*Enterococcus faecalis, E. mundtii, Lactobacillus* spp., *Pseudomonas* spp., *Bavillus* spp., *Carnobacterium maltaromaticum, Staphylococcus nepalensis, Arthrobacter sp., Kocuria sp.*	Isolates from the Micrococcaceae family were resistant to ampicillin (7%), chloramphenicol (50%) and vancomycin (50%). *Kocuria sp*. resistance to chloramphenicol (18%) and vancomycin (18%). Vancomycin resistance was observed in *Staphylococcus nepalensis* (94%).	-	(Gerbáčová et al., 2020)
Asellia tridens, Rhinolophus blasii, Rhinolophus clivosus, Rhinolophus euryale	Hipposideridae Rhinolophidae	Cave	Algeria	2016	Guano	*Staphylococcus aureus*	36.3% were resistant to penicillin G, cefoxitin and fusidic acid. Four isolates were resistant to methicillin (MRSA).	MRSA isolates harboured a SCCmecIV Q6GD50 (fusC). Virulence genes including sea, egc enterotoxin locus, sec, sel, tst.	(Mairi et al., 2021)
Tardarita teniotis	Molossidae	Bridges	Portugal		Faecal	*Escherichia coli*	9.6% of samples were ESBL	Detected genes were blaCTX-M-1, blaCTX-M-3, blaSHV, blaTEM, blaOXA, blaCTX-M-9, tet(A), tet(B) and fimA.	(Garcês et al., 2017)
		Bridges	Portugal		Faecal	*Escherichia coli*	Resistance to ampicillin (57.14%) and streptomycin (52.38%)	Virulence gene fimA and antimicrobial resistance *blaOXA, blaSHV, blaTEM, tet(A), tet(B), adA* and *strA-strB*	(Garcês et al., 2019)
Various species	Undetermined	Urban and rural areas	Bangladesh	2016-2017	Faecal	*Salmonella* spp., *Staphylococcus* spp., *Escherichia coli*	*Salmonella* spp. presented resistant to Tetracycline (93%), Sulphamethoxazoal-Trimethoprime (80%), Amoxicillin-Clavulanic acid (42%), Azithromycin (76%) and Chloramphenicol (62%). *Staphylococcus sp.* resistance to Ampicillin (55%), Methicillin, Oxacillin, Streptomycin and Tigecycline by 12%. *E. coli* resistance against Cefepime (16%) and Ampicillin (13%).		(Uddin et al., 2020)
		Trees near urban areas	Nigeria	2012	Faecal	*Escherichia coli*	Resistance to augumentin (92%), to ciprofloxacin, ofloxacin and pefloxacin (1%).		(Oluduro, 2012)
		Urban and rural areas	Nigeria	2012-2014	Fecal	*Escherichia coli*	50% of the isolates were resistant or immediately resistant to cefuroxime, ceftazidime and cefotaxime.		(Ngozi et al., 2018)
		Cave	Algeria	2016	Fecal	*Klebsiella pneumoniae*	2/100 carbapenem-resistant isolates	Carbapenemase-producing isolates harboured the blaOXA-48 gene (CS34) and blaKPC-3 gene (CS63). Other genes observed were blaTEM-1 and aac (6¢)-Ib genes.	(Gharout-Sait et al., 2019)
Undetermined	Undetermined	Veterinary hospital	Germany	2008	Swab wound	*Staphylococcus aureus*	Resistant to Methicillin (MRSA).	mecA gene	(Walther et al., 2008)

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The type of sample predominately was fecal material (anal swabs, droplets, and gastrointestinal samples) in almost every paper, except for two works that had samples from other organs (Walther et al., 2008; Obodoechi et al., 2021). The countries with the greatest number of studies were in decedent order as follows: Nigeria, Brazil (n=5), Slovakia, Australia (n = 3), Indonesia, Peru, Portugal, Poland, Algeria (n = 2), Japan, Republic of Congo, Slovakia, Trinidad, Germany, Bangladesh, Gabon (n = 1). Since most of the studies were performed in fecal samples, the bacteria isolated were mostly the microbiota of faecal flora, and *Escherichia coli(E. coli)*was the most common microorganism in the studies (n=20). The production of extended-spectrum b-lactamases (ESBLs) and carbapenem resistance by Enterobacteriaceae, Methicillin-resistant *Staphylococcus pseudointermedius* (MRSP), and Methicillin-resistant *Staphylococcus aureus* (MRSA) vancomycin-resistant *Enterococci* (VRE), have been some of the main public health concerns in the last years, particularly in human health in the topic of AMR (Garcês et al., 2019; Wangai et al., 2019). Some of these agents have been reported in bats. ESBL was reported in 8 papers and carbapenem resistance in Enterobacteriaceae on 3 as mentioned in Table 1 (Gharout-Sait et al., 2019; McDougall et al., 2019; McDougall et al., 2021a).

A study performed by Obodoechi et al. (2021), detected the blaCTX-M15 gene in ESBL isolated from bats from Nigeria. This gene already had been detected in other bats from Gabon (Nguema et al., 2020), Poland (Nowakiewicz et al., 2020), and Peru (Benavides et al., 2018; Mendoza, 2017). This gene is known for being one of the most predominant ESBL gene types reported in humans, domestic animals and wildlife in many regions of the world. Extended-spectrum b-lactamases type cefotaximase-Munich (CTX-M) is the most widely distributed ESBL in human strains around the world. In the same study, was detected the strain *E. coli* ST2178 was been previously reported in among pets, humans and rodents (Obodoechi et al., 2021). In another study performed in Gabon by Nguema et al., 2020, were detected the presence of blaCTX-M-15 and blaSHV-11 genes in ESBL from insectivorous species. The phylogenetic analyses of the sequences obtained from bats that carry these clustered genes correlate them with one human bacterial strain (Nguema et al., 2020). The presence of these genes in bats populations suggests that the prevalence of antibiotic resistance in bats depends on the antibiotics consumed and the density of human populations in contact with bats.

CONCLUSION

Based on the papers analyzed in the present study, it is possible to conclude that bats do a host of AMR and pathogenic bacteria. Due to their lifestyle bats have an important part in the spread of AMR in the ecosystems. But also, make them more susceptible to acquiring these resistant bacteria from numerous sources such as food, insects, contaminated fruit, animal sources, and environmental factors such as water bodies. Bats are also extremely adaptable animals that sometimes live in close contact with humans and domestic animals. Some colonies of bats are located in urban areas, for example, in old churches or libraries. This proximity to Humans can increase the possibility of the exchange of these bacteria. This cycle of exchange of resistant bacteria between wild animals, including bats, humans, domestic animals and the environment represents a major public health problem. This is because it is extremely difficult to control the flow of these bacteria within wild populations since the mechanism of acquisition and transmission of these bacteria is not yet fully understood.

Bats have undoubtedly an important biological indicator of environmental health, particularly in the case of zoonosis. Nevertheless, there are still gaps in knowledge about the dynamics and mechanisms routes of zoonotic agents and AMR. Often, wild animals such as bats are not included in epidemiological surveillance disease control. In many situations bats have been unfairly accused of being the cause of disease outbreaks, but there is a great possibility that these animals acquired the pathogens elsewhere, for example, in water bodies contaminated with medical wastes, and only act as carriers of those pathogens. As it was possible to observe in this review, numerous EBLS strains of human origin have been reported in different species of bats, from different areas of the globe. Bat are not the cern of the problems, but contribute to its aggravation.

In the future, further studies are needed under the One Health system to determine the role of bats in the dissemination and acquisition of AMR in the ecosystem.

DECLARATIONS

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Competing interests

The author declared no conflict of interest.

Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the author.

REFERENCES

Adesiyun AA, Stewart-Johnson A, and Thompson NN (2009). Isolation of enteric pathogens from bats in Trinidad. Journal of Wildlife Diseases, 45(4): 952-961. DOI: https://www.doi.org/10.7589/0090-3558-45.4.952

Akobi B, Aboderin O, Sasaki T, and Shittu A (2012). Characterization of Staphylococcus aureus isolates from faecal samples of the Straw-Coloured Fruit Bat (Eidolon helvum) in Obafemi Awolowo University (OAU), Nigeria. BMC Microbiology, 12: 279. DOI: https://www.doi.org/10.1186/1471-2180-12-279

Aladejana OM (2019). Isolation and characterization of Enterobacteriaceae from faecal samples of straw-coloured fruit bat (*Eidolon helvum* kerr) in Osun State, Nigeria. PhD dissertation, Obafemi Awolowo University, Nigeria, p. 165. Available at: https://ir.oauife.edu.ng/handle/123456789/6279

Benavides JA, Shiva C, Virhuez M, Tello C, Applegren A, Vendrel J, Solassol J, Godreuil S, and Streicker DG (2018). Extended-spectrum beta-lactamase-producing *Escherichia coli* in common vampire bats Desmodus rotundus and livestock in Peru. Zoonoses Public Health, 65: 454-458. DOI: DOI: https://www.doi.org/10.1111/zph.12456

Calisher CH, Childs JE, Field HE, Holmes KV, and Schountz T (2006). Bats: Important reservoir hosts of emerging viruses. Clinical Microbiology Reviews, 19(3): 531-545. DOI: https://www.doi.org/10.1128/CMR.00017-06

Costa LFX, Grassoti T, Canoni CR, Lira AD, Moura TM, Campos AAS, Frazzon J, and Frazzon APG (2019). Diversidade, perfis de resistência e virulência de *Enterococcus* spp. em fezes de morcegos urbanos *Tadarida brasiliensis* (Brazilian free-tailed bats). Revista Brasileira de Biociências, 17(2): 43-52. Available at: http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/4119

Fenton MB and Simmons NB (2015). Bats : A world of science and mystery. United States: The University of Chicago Press, p. 240. Available at: https://press.uchicago.edu/ucp/books/book/chicago/B/bo17089187.html

Frazzon APG and Alegre P (2018). Caracterização de *Enterococcus* sp. Provenientes de amostras de fezes de morcegos *Tadarida brasiliensis*. Phd dissertation, Federal University of Rio Grande do Sul, Brazil., p. 90. Available at: http://hdl.handle.net/10183/174925

Garcês A, Correia S, Amorim F, Pereira JE, Igrejas G, and Poeta P (2017). First report on extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from European free-tailed bats (*Tadarida teniotis*) in Portugal: A one-health approach of a hidden contamination problem. Journal of Hazardous Materials, 370: 219-224. DOI: https://www.doi.org/10.1016/j.jhazmat.2017.12.053

Garcês A, Correia S, Silva V, Amorim F, Pereira JE, Igrejas G, and Poeta P (2019). Detection of antimicrobial resistance in faecal *Escherichia coli* from European free-tailed bats (*Tadarida teniotis*) in Portugal. Acta Chiropterologica, 21(2): 403-409. DOI: https://www.doi.org/10.3161/15081109ACC2019.21.2.015

Garcês A, Prada J, and Pires I (2020). SARS-CoV-2 and animals: What we known! International Journal of Veterinary Sciences and Animal Husbandry, 5(6): 11-13. DOI: https://www.doi.org/10.22271/veterinary.2020.v5.i6a.304

Gerbáčová K, Maliničová L, Kisková J, Maslišová V, Uhrin M, and Pristaš P (2020). The faecal microbiome of building-dwelling insectivorous bats (*Myotis myotis* and *Rhinolophus hipposideros*) also contains antibiotic-resistant bacterial representatives. Current Microbiology, 77: 2333-2344. DOI: https://www.doi.org/10.1007/s00284-020-02095-z

Gharout-Sait A, Touati A, Ahmim M, Brasme L, Guillard T, Agsous A, and de Champs C (2019). Occurrence of carbapenemase-producing *Klebsiella pneumoniae* in bat guano. Microbial Drug Resistance, 25(7): 1057-1062. DOI: https://www.doi.org/10.1089/mdr.2018.0471

Graves S, Kennelitt-Merrit S, Tideman C, Rawlinson C, Harvey K, and Thornton B (1988). Antibiotic-resistance patterns of enteric bacteria of wild mammals on the Krakatau Islands and West Java, Indonesia. Philosophical Transactions of the Royal Society of London B, 322: 339-353. DOI: https://www.doi.org/10.1098/rstb.1988.0129

Kunz TH and Fenton B (2006). Bat Ecology. United States: The University of Chicago Press. Available at: https://www.amazon.com/Bat-Ecology-Thomas-H-Kunz/dp/0226462072

Mairi A, Touati A, Pantel A, Yahiaoui Martinez A, Ahmim M, Sotto A, Dunyach-Remy C, and Lavigne JP (2021). First report of CC5-MRSA-IV-SCCfus "Maltese Clone" in bat guano. Microorganisms, 9(11): 2264. DOI: https://www.doi.org/10.3390/microorganisms9112264

Masrukhin, Saputra S, Rizal S, Sushadi PS, and Supriatna N (2021). Identification and antibiotic resistance profile of bacteria from fruit bat (*Chironax melanocephalus*). Proceedings of the 3rd KOBI Congress, International and National Conferences, (KOBICINC 2020) Bengkulu, Indonesia, pp. 478-484. DOI: https://www.doi.org/10.2991/absr.k.210621.081

Mateus-Vargas RH, Atanassova V, Reich F, and Klein G (2017). Antimicrobial susceptibility and genetic characterization of *Escherichia coli* recovered from frozen game meat. Food Microbiology, 63: 164-169. DOI: https://www.doi.org/10.1016/j.fm.2016.11.013

Nguema PPM, Onanga R, Ndong Atome GR, Obague Mbeang JC, Mabika AM, Yaro M, Lounnas M, Dumont Y, Zohra ZF, Godreuil S et al. (2020). Characterization of ESBL-producing enterobacteria from fruit bats in an unprotected area of Makokou, Gabon. Microorganisms, 8(1): 138. DOI: https://www.doi.org/10.3390/microorganisms8010138

McDougall F, Boardman W, Gillings M, and Power M (2019). Bats as reservoirs of antibiotic resistance determinants: A survey of class 1 integrons in Grey-headed Flying Foxes (*Pteropus poliocephalus*). Infection, Genetics and Evolution, 70: 107-113. DOI: https://www.doi.org/10.1016/j.meegid.2019.02.022

McDougall F, Wyres KL, Judd LM, Boardman W, Holt KE, and Power ML (2021). Novel strains of *Klebsiella africana* and *Klebsiella pneumonia* in Australian fruit bats (*Pteropus poliocephalus*). Research in Microbiology, 172(7-8): 103879. DOI: https://www.doi.org/10.1016/j.resmic.2021.103879

McDougall FK, Boardman WSJ, and Power ML (2021b). Characterization of beta-lactam-resistant *Escherichia coli* from Australian fruit bats indicates anthropogenic origins. Microbial Genomics, 7(5): 000571. DOI: https://www.doi.org/10.1099/mgen.0.000571

Mendoza MV (2017). Identificación de enterobacterias resistentes a antibióticos en el vampiro común (*Desmodus rotundus*) y en animales de traspatio en el departamento de Lima, Perú. Phd dissertation, Universidad Peruana Cayetano Heredia, p. 34. Avaiable at: https://hdl.handle.net/20.500.12866/758

Mühldorfer K (2013). Bats and bacterial pathogens: A review. Zoonoses and Public Health, 60(1): 93-103. DOI: https://www.doi.org/10.1111/j.1863-2378.2012.01536.x

Mulec J, Kogovšek B, Skok S, Kogovšek B, Tomazin R, Šturm S, Avguštin JA, and Mulec J (2020). Antimicrobial-resistant *Escherichia coli* from karst waters, surfaces and bat guano in Slovenian caves. Acta Carsologica, 49(2-3): 265-279. DOI: https://www.doi.org/10.3986/ac.v49i2-3.9103

Neuweiler G (2020). Biology of bats. New York, United States: Oxford University Press Inc. p. 406. DOI: Https://doi.org/10.1016/B978-0-12-758001-2.X5001-1

Ngozi A, Agabus N, Eucharia O, Onyinyechi UI, Abraham E, Chika E, and Ifeanyichukwu IA (2018). A three-year study on the prevalence and antibiotic susceptibility pattern of *escherichia coli* isolated from cloacal swabs of wild and domestic birds in Ebonyi State, Nigeria. EC Microbiology, 14(5): 266-273. Available at: https://www.ecronicon.com/ecmi/pdf/ECMI-14-00479.pdf

Nowak K, Fahr J, Weber N, Lübke-Becker A, Semmler T, Weiss S, Mombouli JV, Wieler LH, Guenther S, Leendertz FH et al. (2017). Highly diverse and antimicrobial susceptible *Escherichia coli* display a naïve bacterial population in fruit bats from the Republic of Congo. PLoS ONE, 2(7): e0178146. DOI: https://www.doi.org/10.1371/journal.pone.0178146

Nowakiewicz A, Zięba P, Gnat S, Osińska M, Łagowski D, Kosior-Korzecka U, Puzio I, and Króle J (2021). Analysis of the occurrence and molecular characteristics of drug-resistant strains of Enterococcus faecalis isolated from the gastrointestinal tract of insectivorous bat species in Poland: A possible essential impact on the spread of drug resistance?. Environmental Pollution, 269: 116099. DOI: https://www.doi.org/10.1016/j.envpol.2020.116099

Nowakiewicz A, Zięba P, Gnat S, Trościańczyk A, Osińska M, Łagowski D, Kosior-Korzeck U, and Puziod I (2020). Bats as a reservoir of resistant Escherichia coli: A methodical view. Can we fully estimate the scale of resistance in the reservoirs of free-living animals? Research in Veterinary Science, 128: 49-58. DOI: https://www.doi.org/10.1016/j.rvsc.2019.10.017

Obi T, Chibana M, Taira C, Nakayama A, Miyazaki K, Takase K, Nakamura I, Miyamoto A, and Kawamoto Y (2014). Antimicrobial susceptibility in Enterobacteriaceae recovered from Okinawa least horseshoe bat *Rhinolophus pumilus*. Wildlife Biology, 20: 64-66. DOI: https://www.doi.org/10.2981/wlb.13067

Obodoechi LO, Carvalho I, Chenouf NS, Martínez-Álvarez S, Sadi M, Nwanta JA, Chahg KF, and Torres C (2021). Antimicrobial resistance in *Escherichia coli* isolates from frugivorous (*Eidolon helvum*) and insectivorous (*Nycteris hispida*) bats in Southeast Nigeria, with detection of CTX-M-15 producing isolates. Comparative Immunology, Microbiology and Infectious Diseases, 75: 101613. DOI: https://www.doi.org/10.1016/j.cimid.2021.101613

Oluduro AO (2012). Antibiotic-resistant commensal *Escherichia coli* in faecal droplets from bats and poultry in Nigeria. Veterinaria Italiana, 48(3): 297-308. Available at: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.izs.it/vet_italiana/2012/48_3/297.pdf

Sens-Junior H, Trindade WA, Oliveira AF, Zaniolo MM, Serenini GF, Araujo-Ceranto JB, Gonçalves DD, and Germano RM (2018). Bacterial resistance in bats from the Phyllostomidae family and its relationship with unique health. Pesquisa Veterinaria Brasileira, 38(6): 1207-1216. DOI: https://www.doi.org/10.1590/1678-5150-pvb-5185

Sherley M, Gordon DM, and Collignon PJ (2000). Variations in antibiotic resistance profile in Enterobacteriaceae isolated from wild Australian mammals. Environmental Microbiology, 2(6): 620-631. DOI: https://www.doi.org/10.1046/j.1462-2920.2000.00145.x

Débora Rodrigues S (2021). Bactérias relevantes em saúde única isoladas de animais silvestres e domésticos: Similaridade molecular e resistência a antimicrobianos. Phd dissertation, Federal University of Pelotas, p. 116. Available at: http://guaiaca.ufpel.edu.br:8080/handle/prefix/8135

Uddin M, Samad MA, Rahman MK, Islam S, Hossain MS, Sagor MS, Rostal M,

Giasuddi M, Flora MS, Epstein JH et al. (2020). Ecology of bat drinking behaviour and AMR patterns of *Salmonella* spp., *Staphylococcus* spp. *and E. coli* recovered from faecal droppings of bats and water in BD. International Journal of Infectious Diseases, 101: 8. DOI: https://www.doi.org/10.1016/j.ijid.2020.09.060

Vandžurová A, Bačkor P, Javorský P, and Pristaš P (2013). *Staphylococcus nepalensis* in the guano of bats (Mammalia). Veterinary Microbiology, 164(1-2): 116-121. DOI: https://www.doi.org/10.1016/j.vetmic.2013.01.043

Voigt CC, Lehnert LS, Petersons G, Adorf F, and Bach L (2015). Wildlife and renewable energy: German politics cross migratory bats. European Journal of Wildlife Research, 61: 213-219. DOI: https://www.doi.org/10.1007/s10344-015-0903-y

Walther B, Wieler LH, Friedrich AW, Hanssen AM, Kohn B, Brunnberg L, and Lübke-Becker A (2008). Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from small and exotic animals at a university hospital during routine microbiological examinations. Veterinary Microbiology, 127(1-2): 171-178. DOI: https://www.doi.org/10.1016/j.vetmic.2007.07.018

Wangai FK, Masika MM, Maritim MC, and Seaton RA (2019). Methicillin-resistant *Staphylococcus aureus* (MRSA) in East Africa: red alert or red herring?. BMC Infectious Disease, 19: 596. DOI: https://www.doi.org/10.1186/s12879-019-4245-3

Wilson D and Mittermeier R (2019). Handbook of the mammals of the world. Lynx Edicions, 9: 1008. Available at: https://www.lynxeds.com/product/handbook-of-the-mammals-of-the-world-volume-9/

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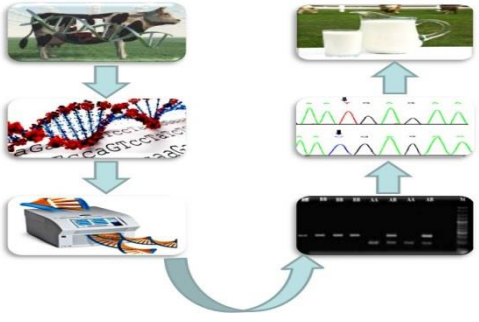

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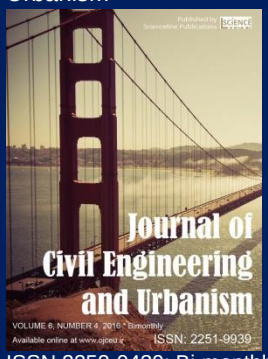

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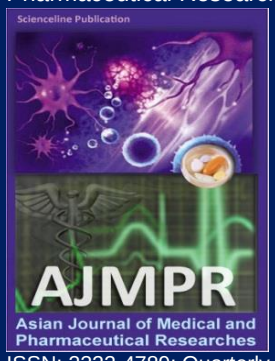

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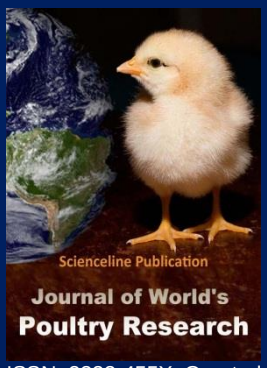

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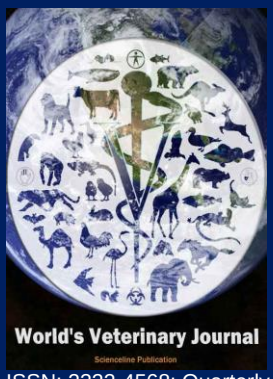

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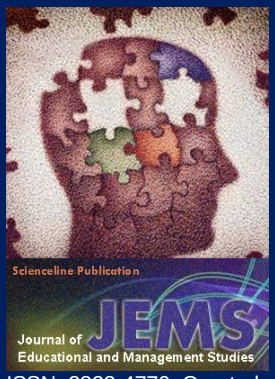

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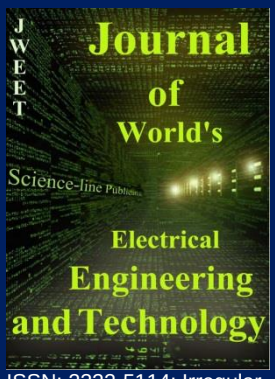

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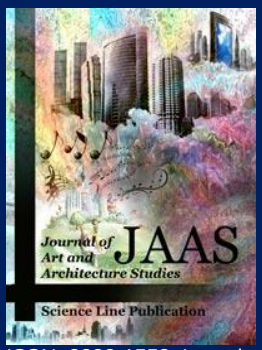

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