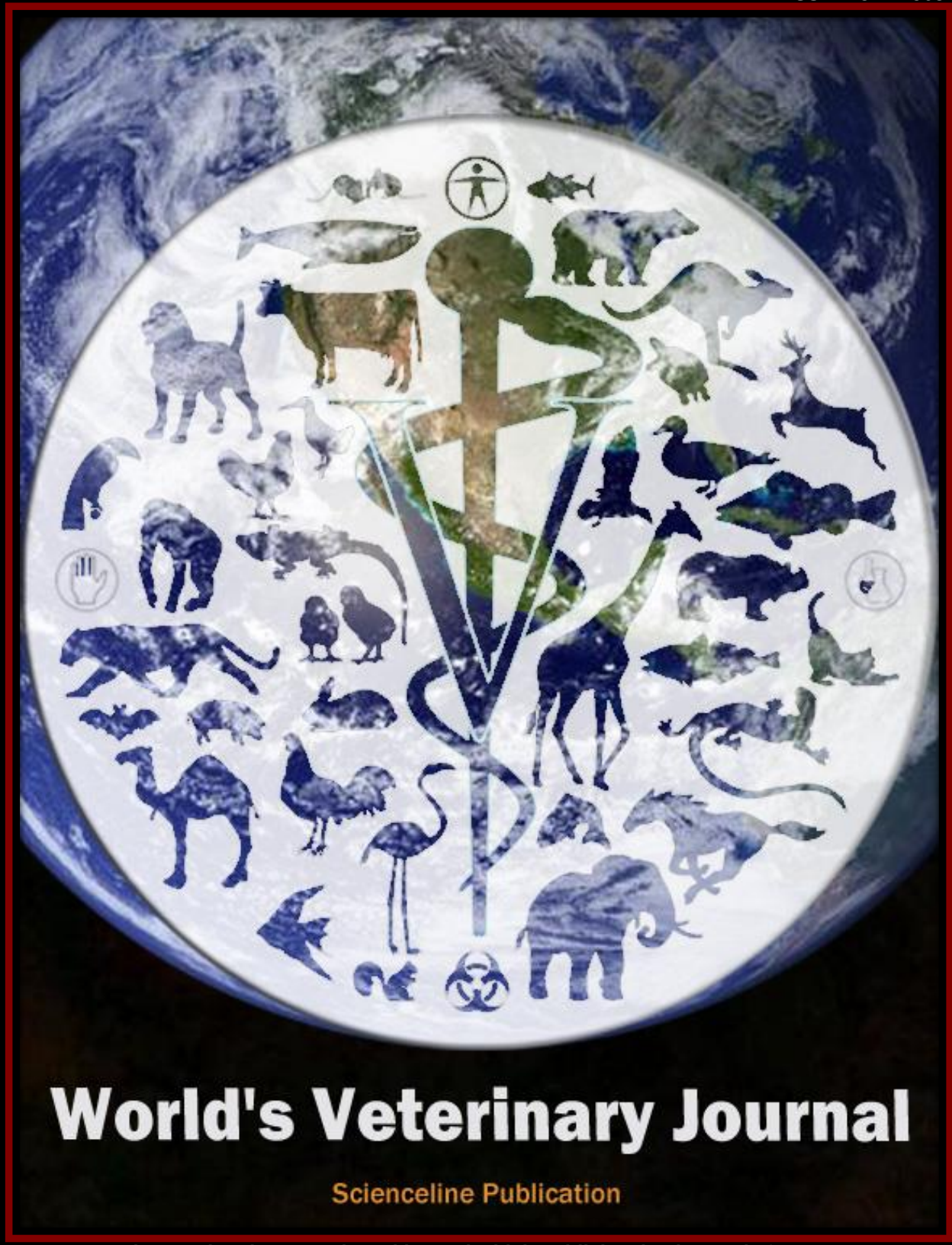
In the Name of God

ISSN: 2322-4568



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Volume 11 (2); June 25, 2021

Systematic Review

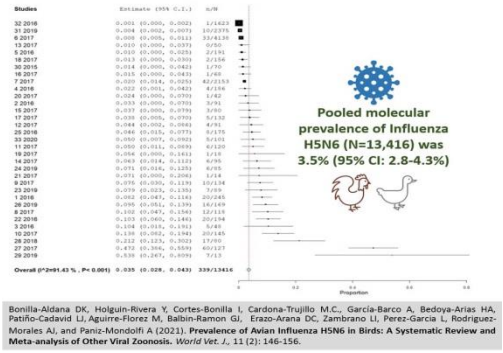
Prevalence of Avian Influenza H5N6 in Birds: A Systematic Review and Meta-analysis of Other Viral Zoonosis

Bonilla-Aldana DK, Holguin-Rivera Y, Cortes-Bonilla I, Cardona-Trujillo M.C., García-Barco A, Bedoya-Arias HA, Patiño-Cadavid LJ, Aguirre-Florez M, Balbin-Ramon GJ, Erazo-Arana DC, Zambrano LI, Perez-Garcia L, Rodriguez-Morales AJ, and Paniz-Mondolfi A.

World Vet. J. 11(2): 146-156, 2021; pii:S232245682100020-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj20

ABSTRACT: Avian influenza viruses (AIV) are zoonotic pathogens that can potentially affect humans and potentially be epidemic in a region. Birds (such as poultry and wild birds) serve as potential reservoirs for these viruses, highlighting the importance of determining AIV prevalence in the avian population. No systematic reviews have been published on this issue in the world so far. The present systematic literature review following the PRISMA standard, with meta-analysis, used three databases to globally assess the Influenza H5N6 infection in birds (including poultry and wild birds). A model of random-effects meta-analysis was performed to calculate the pooled prevalence and 95% Confidence Interval (95% CI) for the prevalence of Influenza H5N6 infection in birds. A total number of 14,605 articles published from 2015 to 2020 were retrieved. After screening the abstract/title, 37 articles were selected for full-text assessment, and 15 were included for qualitative and quantitative analyses. Of the total number of birds (n = 13,416 birds), the pool prevalence by RT-PCR was 3.5% (95% CI: 2.8-4.3%). From the total, 39.67% of the birds assessed were ducks (family Anatidae), in which pool prevalence was 7.7% (95% CI: 4.4-11.0). In chickens (*Gallus gallus domesticus*), the pool prevalence was 3.3% (95% CI 1.9-4.8). Vietnam was the country with the highest pool prevalence; 7.9% (95% CI 4.0-11.7%). Bangladesh was the country with the lowest pool prevalence of 0.4% (95% CI 0.2-0.7%). A considerable proportion of infected birds tested positive highlighted the relevance of individual animals as reservoirs of H5N6. Ducks and chickens were found to be positive by RT-PCR in over 3% of the cases. These data suggest their relevance in maintaining zoonotic transmission and their potential implications for epidemics and even pandemics in the near future.

Keywords: H5N6, Influenza, Meta-Analysis, Molecular diagnosis, RT-PCR, Systematic Review



Bonilla-Aldana DK, Holguin-Rivera Y, Cortes-Bonilla I, Cardona-Trujillo M.C., García-Barco A, Bedoya-Arias HA, Patiño-Cadavid LJ, Aguirre-Florez M, Balbin-Ramon GJ, Erazo-Arana DC, Zambrano LI, Perez-Garcia L, Rodriguez-Morales AJ, and Paniz-Mondolfi A (2021). **Prevalence of Avian Influenza H5N6 in Birds: A Systematic Review and Meta-analysis of Other Viral Zoonosis.** *World Vet. J.*, 11 (2): 146-156.

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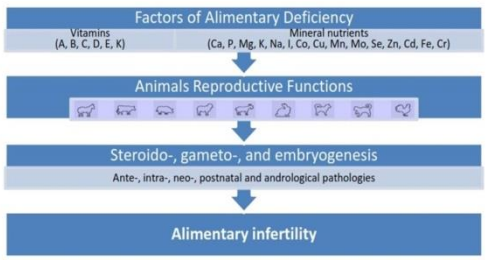
Review

Reviewing Effective Factors of Alimentary Deficiency in Animals Reproductive Functions

Skliarov P, Fedorenko S, Naumenko S, Onyshchenko O, Pasternak A, Roman L, Lieshchova M, Bilyi D, and Bobrytska O.

World Vet. J. 11(2): 157-169, 2021; pii:S232245682100021-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj21

ABSTRACT: Animal reproduction is one of the main factors limiting the efficiency of livestock production. Its optimal level is possibly achieved when certain conditions are created for animals. As reproduction is a complex reflex process depending on neuroendocrine regulatory mechanisms, the character and strength of stimuli, which, in turn, is due to a number of factors. Under normal conditions, the body of animals is affected by many different factors, which are appropriately transformed and specified by positive or negative reactions. Inhibitory factors include air pool, saturated with harmful substances and gases, ionizing radiation, poor water quality along with altered redox properties, hypokinesia combined with poor unbalanced feeding, systematic chronic stress, presence of toxic substances in feed, and the deficiency of vitamins and other bioantioxidants in feed or their excessive spending. Of the wide range of genetic and paratypic factors of negative impacts on reproductive capacity, the most common one is alimentary, which causes impaired reproductive function due to deficiencies in the rules, regulations, and feeding regime of animals. In particular, the alimentary can be associated with both general malnutrition (starvation) and overfeeding (obesity). However, the alimentary form of infertility mostly occurs due to low-quality diets when the diet lacks vital components (mainly



Skliarov P, Fedorenko S, Naumenko S, Onyshchenko O, Pasternak A, Roman L, Lieshchova M, Bilyi D, and Bobrytska O. (2021). **Reviewing Effective Factors of Alimentary Deficiency in Animals Reproductive Functions.** *World Vet. J.*, 11 (2): 157-169.

vitamins, macro-, and micronutrients) or the quantitative ratios of the ingredients are violated. This is possible even if the total nutritional value of the diet meets the established requirements for the physiological needs of the body. Vitamins, micro-, and macronutrients are ecologically deficient factors of disturbance of animal reproductive function, the influence of which is observed on all processes of reproduction, from fertilization to the postpartum period and the preservation of young animals. The pathogenesis of their insufficiency is associated with the violation of steroido-, gameto-, and embryogenesis and the emergence of ante-, intra-, neo- and postnatal pathologies, respectively. Therefore, treatments and prevention measures should be aimed at providing animals with biologically complete balanced feeding and replenishment of the body with vitamins and minerals. However, all these issues remain incompletely studied and need further research.
Keywords: Alimentary deficiency, Animals, Reproductive function.

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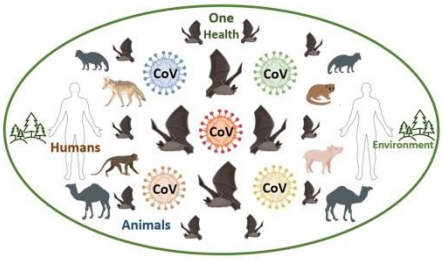
Review

The Broad Range of Coronaviruses Co-existing in Chiropteran: Implications for One Health

Bonilla-Aldana DK, Toro-Ortiz C, Jimenez-Salazar P, Guevara-Manso V, Jimenez-Diaz SD, Bonilla-Aldana JL, Gutierrez-Grajales EJ, Pecho-Silva S, Paniz-Mondolfi A, Suárez JA, Pachar MR, Martinez-Pulgarin DF, Zambrano LI, Soler-Tovar D, Rodriguez-Morales AJ, and Mattar S.

World Vet. J. 11(2): 170-180, 2021; pii:S232245682100022-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj22

ABSTRACT: Bats are a group of mammals that harbor the most significant number of coronaviruses. The aim of present review article was to analyze the broad spectrum of the coronavirus coexisting in Chiropterans hosts. Bats have certain types of cell receptors that allow them to be the potential hosts of a large number of viruses without the presence of any clinical manifestations, and to be a source of contagion infections for other animals and human species. Emphasis can be placed on five coronaviruses, such as Porcine Epidemic Diarrhea Disease, Severe Acute Diarrhea Syndrome, Middle East Respiratory Syndrome, Severe Acute Respiratory Syndrome, and Severe Acute Respiratory Syndrome 2, which have had significant impacts causing epidemic outbreaks in different parts of the world, and generating implications for both human and animal health. In conclusion, recent research indicated the importance of bats as potential hosts of multiple coroaviruses leading to some zoonotic diseases.
Keywords: Bats, Coronaviruses, Cross-species, Evolution, Spillover, Transmission



Bonilla-Aldana DK, Toro-Ortiz C, Jimenez-Salazar P, Guevara-Manso V, Jimenez-Diaz SD, Bonilla-Aldana JL, Gutierrez-Grajales EJ, Pecho-Silva S, Paniz-Mondolfi A, Suárez JA, Pachar MR, Martinez-Pulgarin DF, Zambrano LI, Soler-Tovar D, Rodriguez-Morales AJ, and Mattar S (2021). **The Broad Range of Coronaviruses Co-existing in Chiropteran: Implications for One Health.** *World Vet. J.,* 11 (2): 170-180.

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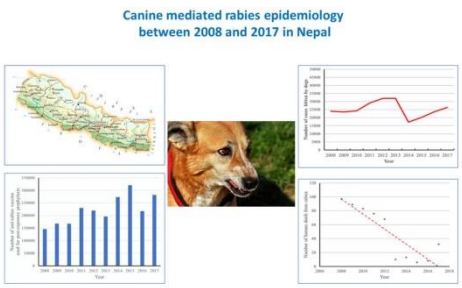
Research Paper

A Retrospective Study on Dog Bite Associated Rabies in Human and the Use of Post-exposure Prophylaxis in Nepal during 2008 to 2017

Pal P, Shimoda H, Bashyal R, Yawongsa A, and Rukkwamsuk Th.

World Vet. J. 11(2): 181-186, 2021; pii:S232245682100023-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj23

ABSTRACT: A 10-year (2008-2017) retrospective canine-mediated human rabies epidemiology was studied to assess the burden of rabies in Nepal. To this end, the number of dog bites, the use of post-exposure prophylaxis (PEP), and human death records from 2008 to 2017 were retrieved from Sukraraj Tropical Hospital, Kathmandu, Nepal. The findings revealed that the number of human rabies occurrences was consistent with minor fluctuations throughout the study period. There were 252,297 dog bite cases in humans recorded between 2008 and 2017. Every month, 2,102 people were bitten by mostly stray dogs. There was a gradual increase in PEP use throughout 10 years. On average, 36,995 PEP dosages were used per year for stray dog bites. The PEP consumption and the number of human deaths were negatively correlated. A total of 482 human rabies deaths were recorded in Nepal during the study period. On average, 49 people died of canine-mediated rabies each year. Although there was an increase in the use of PEP, the number of human deaths and street dog bites recorded were still high. The high mortality due to rabies could then be attributed to the flawed surveillance system and stray dog population management, and not merely the lack of PEP services. Hence, it is recommended that the government agencies and other concerned stakeholders should organize mass vaccination and population management program for stray dogs in order to reduce the country's rabies burden.
Keywords: Dog bite, Epidemiology, Prophylaxis, Rabies



Pal P, Shimoda H, Bashyal R, Yawongsa A, and Rukkwamsuk Th (2021). **A Retrospective Study on Dog Bite Associated Rabies in Human and the Use of Post-exposure Prophylaxis in Nepal during 2008 to 2017.** *World Vet. J.,* 11 (2): 181-186.

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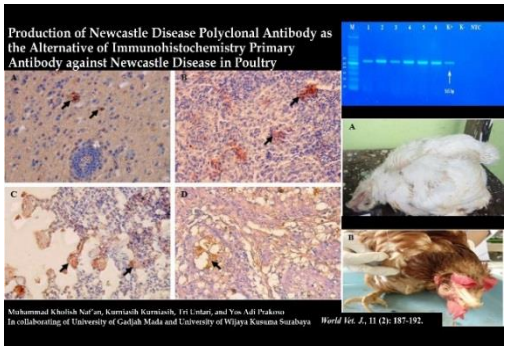
Research Paper

Production of Newcastle Disease Polyclonal Antibody as the Alternative of Immunohistochemistry Primary Antibody against Newcastle Disease in Poultry.

Naf'an MKh, Kurniasih K, Untari T, and Prakoso YA.

World Vet. J. 11(2): 187-192, 2021; pii:S232245682100024-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj24



ABSTRACT: Newcastle disease (ND) is the most pathogenic viral infection in poultry. Furthermore, the availability of laboratories that support the molecular diagnosis of ND is still limited in Indonesia. The present study aimed to produce ND polyclonal antibody as the alternative of immunohistochemistry primary antibody against ND in poultry. Two adult male New Zealand White rabbits weighed 2.5 kg were vaccinated seven days after the adaptation using intraperitoneal injection of the ND live vaccine at multilevel doses weekly. The serum was collected inactivated, and purified in the sixth week. A total number of 31 chicken samples were collected and their samples of brain, lung, spleen, and intestine were tested using immunohistochemistry and Reverse Transcription Polymerase Chain reaction (RT-PCR). The result showed that 19/31 (61%) were positive against immunohistochemistry and RT-PCR and a total of 12/31 (39%) were negative. Based on the obtained results, immunohistochemistry using ND polyclonal antibody had a similar accuracy with RT-PCR. It can be concluded that ND polyclonal antibody produced by vaccination in the rabbit could be used as the alternative immunohistochemistry primary antibody for diagnosing ND in poultry.
Keywords: Immunohistochemistry, Newcastle disease, Polyclonal antibody, Poultry, RT-PCR

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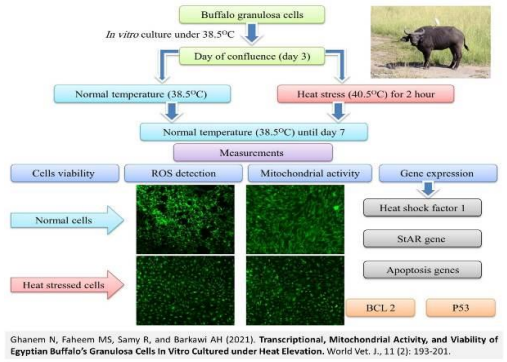
Research Paper

Transcriptional, Mitochondrial Activity, and Viability of Egyptian Buffalo's Granulosa Cells *In Vitro* Cultured under Heat Elevation

Ghanem N, Faheem MS, Samy R, and Barkawi AH.

World Vet. J. 11(2): 193-201, 2021; pii:S232245682100025-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj25



ABSTRACT: It is documented that heat stress caused impairment on the reproductive performance of dairy animals. However, there are few reports that have focused on the molecular and intracellular responses of *in vitro* cultured buffalo granulosa cells during heat elevation. The present study was conducted to investigate the effect of heat elevation during *in vitro* culture of buffalo granulosa cells on their viability, quality, mitochondrial activity, and transcriptional activity. Granulosa cells were harvested after aspiration of cumulus-oocytes complexes that were collected from abattoir ovaries. The granulosa cells were cultured *in vitro* either at a normal physiological temperature suitable for oocyte maturation and embryo development (38.5°C) or exposed to the elevated temperature of 40.5°C on day 3 of culture (the first two days were for confluence) for two hours of culture then continued at 38.5°C up to day 7 of culture. The viability of granulosa cells was measured using trypan blue and quality was estimated by measuring the level of intracellular reactive oxygen species (ROS) on day 7. Moreover, metabolic activity was performed by measuring the fluorescent intensity of mitochondria. Moreover, transcriptional activity was done by profiling four selected candidate genes using quantitative real-time PCR. The results indicated that the granulosa cells viability rate significantly decreased in the heat stress group (25.1 ± 3.7), compared to the control group (36.6 ± 5.3) on confluence day (day 3). In addition, the viability rate on the last day of culture (day 7) decreased in heat stress, compared to control (83.7 ± 4.5 and 97.4 ± 0.4, respectively). On the other hand, there was a nonsignificant difference in ROS profile between the control ($21.7*10^4$ ± 1.3) and the heat-stressed group (15.7 ± 0.7) on day 7 of culture. However, the mitochondrial fluorescent intensity was higher in the control (21.9 ± 1.9) than in the heat-stressed group (15.4 ± 0.8) on day 7 of culture. The expression of cellular defense (HSF1) and apoptosis-inducing gene (P53) were significantly up-regulated in granulosa cells exposed to heat elevation, compared to the control group. On the other hand, the steroidogenesis-regulating gene (StAR) was down-regulated in granulosa cells cultured under heat shock, compared to the control group. In conclusion, heat stress reduced the viability of granulosa cells by inducing the expression of an apoptosis-related gene (P53) and compromised expression of genes regulating the steroid biosynthesis, which resulted in up-regulation of cell defense gene (HSF1) in an attempt to ameliorate the deleterious effect of heat stress on the biological activity of the granulosa cells.
Keywords: Apoptosis, Granulosa, Heat stress, Gene expression

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Review

Supplementation of *Moringa oleifera* Leaf Meal in Layer Chickens' Feed: A Review

Gobezie E.

World Vet. J. 11(2): 202-207, 2021; pii:S232245682100026-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj26

ABSTRACT: As a dietary supplement for animals, *Moringa oleifera* is very useful because its leaves are very nutritious. *Moringa oleifera* leaves are rich in fats, proteins, vitamins, and minerals with antimicrobial effects. Leaf tea is used to treat ulcers in the stomach and diarrhea. Moringa leaves are considered healthy food sources and are recommended for anemia from malnutrition due to the high protein, fiber, and iron content of the leaves. *Moringa oleifera* leaves are primarily used for medicinal and human consumption purposes since they are abundant in antioxidants and other nutrients. Due to the low energy and digestibility of proteins, *Moringa oleifera* leaf meal supplementation increases feed intake and feed conversion ratio, as well as decreasing egg mass yield, percentage of egg production, and egg weight. More research in these areas is required to make full use of the potential advantages of the Moringa oleifera plant as layer feed.
Keywords: Layer chicken, Laying performance, Leaf meal, *Moringa oleifera*

Gobezie E (2021). Supplementation of *Moringa oleifera* Leaf Meal in Layer Chickens' Feed: A Review. *World Vet. J.*, 11 (2): 202-206.

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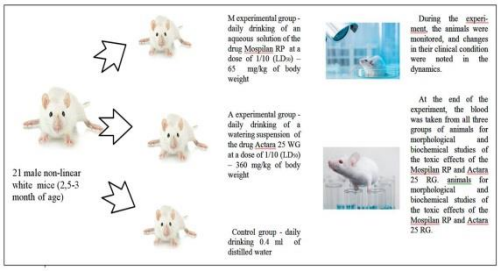
Research Paper

Assessing the Chronic Poisoning of White Mice Affected by Mospilan RP and Actara 25 WG.

Dukhnytskyi V, Sokolyuk V, Kozii N, Ligomina I, Karpyuk V, and Honcharenko V.

World Vet. J. 11(2): 208-214, 2021; pii:S232245682100027-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj27

ABSTRACT: Neonicotinoids are a relatively small group of organic compounds that are widely used in crop production as insecticides. They are highly toxic to insects, and much less toxic to mammals, including humans. Accordingly, the present study aimed to investigate the effects of chronic toxicity of insecticides from the group of neonicotinoids Mospilan RP (active substance acetamiprid) and Actara 25 WG (active substance thiamethoxam) on white mice. The chronic toxicity was induced by daily internal introduction of Mospilan RP and Actara 25 WG to mice for 30 days at the doses of 1/10 of Median Lethal Dose reported as 65 and 363 mg/kg of body weight, respectively. The affected mice showed thrombocytosis, neutrophilic leukocytosis, and lymphocytopenia. Blood plasma hyperproteinemia in mice treated with Mospilan RP and Actara 25 WG was characterized by an increase in globulins content by almost 30.0% in both groups. In Mospilan RP and Actara 25 WG treated groups, there was a reduction in urea content by 43.6% and 31.5%, respectively, an increase in aspartate aminotransferase activity by 80% and 60.0%, and γ-glutamyltranspeptidase by 80% and almost 400%, respectively. Compared to the control group, the activity of alanine aminotransferase increased to 23.0% only in mice that were given Mospilan RP but not in mice that were given Actara 25 WG.
Keywords: Actara 25 WG, Chronic Toxicity, Insecticides Toxicity, Mospilan PP, Neonicotinoids, White Mice



Dukhnytskyi V, Sokolyuk V, Kozii N, Ligomina I, Karpyuk V, and Honcharenko V (2021). **Assessing the Chronic Poisoning of White Mice Affected by Mospilan RP and Actara 25 WG.** *World Vet. J.*, 11 (2): 208-214.

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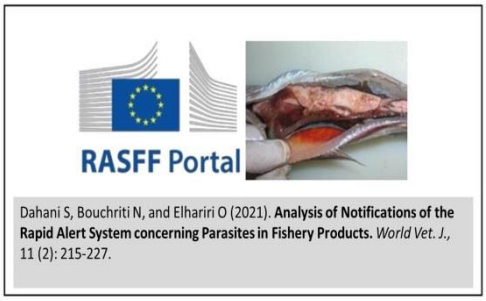
Research Paper

Analysis of Notifications of the Rapid Alert System concerning Parasites in Fishery Products.

Dahani S, Bouchriti N, and Elhariri O.

World Vet. J. 11(2): 215-227, 2021; pii:S232245682100028-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj28

ABSTRACT: Fish and fishery products are one of Morocco's most important export products. Fish parasitism is a natural worldwide phenomenon. Fish parasites have a very wide distribution and are found in both the northern and the southern hemispheres of the globe. The present study aimed to assess parasitic infestation in fishery products by



Dahani S, Bouchriti N, and Elhariri O (2021). **Analysis of Notifications of the Rapid Alert System concerning Parasites in Fishery Products.** *World Vet. J.*, 11 (2): 215-227.

analyzing notifications available in the European rapid alert system for food and feed. The analysis involved 663 notifications registered from 2001 to 2019 on the grounds of parasitic infestation. For Morocco, 651 notifications concerning the different exported food products were analyzed. Among the 663 notifications for the presence of parasites, 161 (24.3%) were border rejections. A total number of 20 countries have been detected with the presence of parasites in their exported fish and fish products. The main fish species concerned with this hazard were Hake (26%), Silver Scabbardfish (10.5%), and Angler (9.3%). In Morocco, among the 651 notifications, 373 concerned with seafood (57.2%). The number of border rejections of fishery products was 220 that is 33.8% of overall notifications. Fish and fish products category are the most concerned with 170 rejections (26.1%), with 64 notifications due to the presence of parasites (37.6%). The Silver Scabbardfish was the species most affected by parasite infestations (23.5%), followed by European Anchovy (12.5%) and Swordfish (10.9%). In conclusion, the nematode *Anisakis* is the most common parasite in fish infestation while the plerocercoïd larvae of the Cestoda *Gymnorhynchus gigas* seems to have a predilection to infest the Atlantic Pomfret (*Brama brama*).
Keywords: Fish, Morocco, Notification, Parasite, Rapid alert system for food and feed

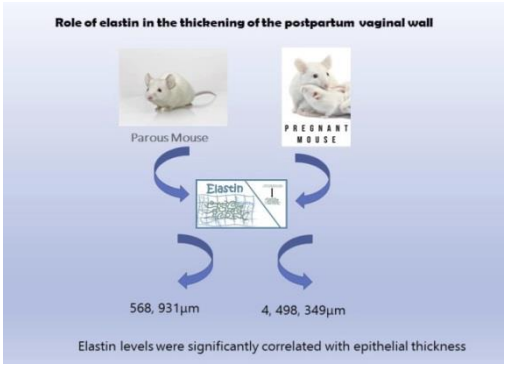
[Full text-PDF] [XML] [Google Scholar]

Research Paper

Role of Elastin Expression in Thickening the Postpartum Vaginal Wall in Virgin and Postpartum Rat Models

Setyaningrum T, Listiawan MY, Tjokroprawiro BA, Santoso B, Prakoeswa CRS, and Widjiati W.

World Vet. J. 11(2): 228-234, 2021; pii:S232245682100029-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj29



ABSTRACT: Childbirth induces a number of alterations, including ligament weakening and increased vaginal distensibility. The occurrence of vaginal laxity or distensibility is associated with the vaginal wall and introitus overstretching during vaginal parturition while the pathophysiology is due to increased levator dimension and trauma to the levator ani muscle through avulsion (macrotrauma) or overdistension (microtrauma). Elastin is an extracellular matrix protein that confers elastic properties to organs and tissues, particularly those requiring elasticity. Elastin plays a vital role in the functioning of numerous tissues, such as the lungs, blood vessels, heart valves, ligaments, tendons, and skin. It is also a component of the vaginal mucosa. The aim of the present was to evaluate the role of elastin in the thickening of the postpartum vaginal wall composed of epithelial mucosa, and to understand the mechanism underlying vaginal laxity or distensibility within parous and nulliparous animal models. A total of 32 female white rats (*Rattus norvegicus*) were used in the present study. They were divided into two groups, each group consisting of 16 rats. The control group (C0) consisted of virgin nulliparous rats, which were sacrificed on the second day after vaginal parturition. Pregnant rats (group C1) were sacrificed on the second day after vaginal parturition. The median elastin expression in group C1 was higher (3 ± 0.56) than group C0 (2.85 ± 0.75). The mean thickness of the vaginal mucosal epithelium in group C0 (56,8 931µm) was greater than group C1 (44,98 349µm). The comparison of vaginal mucosal epithelium thickness between the two groups indicated a significant difference between groups C0 and C1. Elastin levels were significantly correlated with epithelial thickness. The expression of elastin significantly affects the vaginal wall thickness, which further affects vaginal laxity or vaginal distensibility.
Keywords: Distensibility, Elastin, Vaginal wall, Animals

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

Research Paper

Molecular Characterization of Chicken Anaemia Virus Circulating in Commercial Poultry Flocks in Egypt during 2020

Abdelhalim A, Samir A and Yehia N.

World Vet. J. 11(2): 235-241, 2021; pii:S232245682100030-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj30

ABSTRACT: Chicken Anemia Virus (CAV) is an extremely contagious immunosuppressive disease causing high economic losses in poultry production. In the present study, tissue samples (bone marrow, thymus, and spleen) were collected from 86 different broiler chicken farms located in fourteen governorates in Egypt during 2020. They suffered from retard growth, weakness, and a drop in egg production with an observed mortality rate ranged 5-15%. A total of 26 samples were positive for CAV using PCR in six governorates in Lower Egypt with a 30% incidence rate, especially in Sharkia (78%), Ismailia (62.5%), and Alexandria (60%). The viral protein1 (VP1) gene of CAV was genetically characterized by sequencing of 10 selected viruses in six governorates. revealing that all Egyptian strains were clustered into two groups (A, B) that was distinct from vaccine strains (Del-Ros, Cux-1, and 26PA) which

were clustered in group C. The seven Egyptian viruses in this study (A-Egypt-AN1-2020 to A-Egypt-AN7-2020) were clustered with the viruses from Japan, Argentina, and Malaysia in group A, and the other three viruses (A-Egypt-AN8-2020, A-Egypt-AN9-2020, A-Egypt-AN10-2020) were clustered with the viruses from Nigeria, and India in group B. The Egyptian viruses in the current study acquired new specific mutations clustering them into new subgroups (2A, 2B). By mutation analysis comparing with Del-Rose reference strains, V75I, M97L, and K139Q, E144Q were recorded in all viruses in the group A and B. All Egyptian viruses in the current study had specific new mutations at Y13N, H22N. Moreover, mutation at G74E in Egyptian viruses recorded in the current study was related to sub group 2A, I83V in three strains (A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020), and S140A in the hypervariable region was found in four strains (A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and A/Egypt/AN5/2020) in subgroup 2A. Furthermore, Q139 and Q144 amino acid substitutions, which are important in viral replication, were observed in all viruses. The field viruses in the study were distinct from the vaccinal strains by phylogenetic analysis and A.A. identity. In conclusion, the CAV was continuously circulating in Egypt from different genotypes. It acquired new specific mutations clustering them in a new subgroup, and it was distinct from vaccinal strains. Therefore, it is important to conduct continuous monitoring on the genetic evolution of CAV and further studies on the pathogenicity of the virus and the vaccine efficacy.

Keywords: Chicken Anemia Virus, Egypt, Genetic evolution, Viral protein 1 gene

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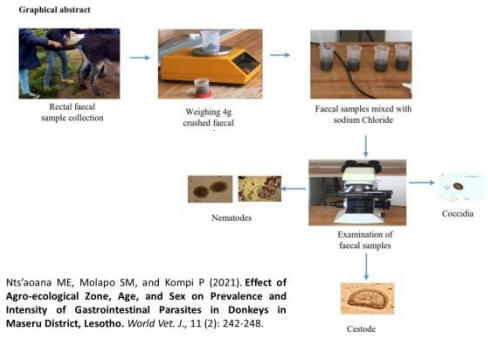
Research Paper

Effect of Agro-ecological Zone, Age, and Sex on Prevalence and Intensity of Gastrointestinal Parasites in Donkeys in Maseru District, Lesotho

Nts'aoana ME, Molapo SM, and Kompi P.

World Vet. J. 11(2): 242-248, 2021; pii:S232245682100031-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj31

ABSTRACT: Gastrointestinal parasites are considered to be silent killers of animals. The objective of the current study was to determine the effect of the agro-ecological zone, age, and sex on the parasite prevalence and fecal egg/oocyst count in donkeys residing in Lesotho. A total number of 720 fecal samples were collected rectally from 120 indigenous donkeys that were randomly selected from the highlands, foothills, and lowlands of Maseru district, Lesotho. The fecal samples were collected every two months for one year and examined using the floatation technique. The overall prevalence for nematodes, coccidia, and cestodes in donkeys were 87.78%, 4.31%, and 1.53%, respectively. The highest nematode prevalence and intensity were detected in the donkeys of highlands. The coccidian infection was lower in the lowlands while cestodes infection was more prevalent in the foothills. Donkey's age had an impact on the nematode fecal egg load but did not affect the prevalence of nematodes in donkeys. Age did not significantly affect the prevalence and fecal egg/oocyst count of cestodes and coccidia. Male donkeys had a higher prevalence and fecal egg count of cestodes. In conclusion, the nematodes were found to be the major gastrointestinal parasites of donkeys in the Maseru district. Therefore, there is a need to design a sustainable strategy aimed at controlling the gastrointestinal parasites in donkeys.



Keywords: Agro-ecological zone, Eimeria, Fecal egg count, Helminth, Prevalence

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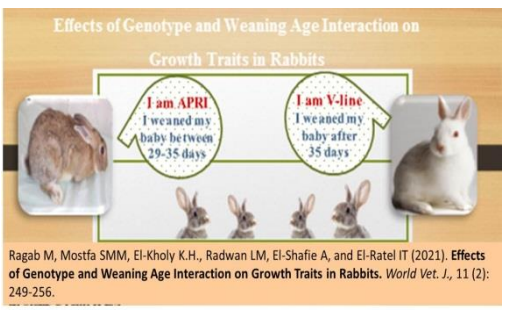
Research Paper

Effects of Genotype and Weaning Age Interaction on Growth Traits in Rabbits

Ragab M, Mostfa SMM, El-Kholy K.H., Radwan LM, El-Shafie A, and El-Ratel IT.

World Vet. J. 11(2): 249-256, 2021; pii:S232245682100032-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj32

ABSTRACT: Weaning age is an important factor that affects the growth and health of weaned animals. Therefore, the current experiment was conducted to study genotype (G) and weaning age (WA) interaction (G×WA) effects on growth traits of the animals belonged to two lines of rabbits (APRI and V line) reared under Egyptian conditions. Multiparous doe rabbits were serviced to obtain 225 litters with 1800 young rabbits at weaning. The weaning ages ranged from 26 to 43 days where the young rabbits were weaned at different ages (≥28 days, WA1; 28 < Treatment ≥ 35 days, WA2; 35 < Treatment ≥ 40 day, WA3 and 40< Treatment, WA4). Body weight (BW) from 4 to 16 weeks of age and corresponding average daily gain (ADGt1-t2) were measured. The BW significantly increased in APRI rabbits, compared to those in V line at the different ages where at the end of the fattening period, the difference was 105 g per animal with higher ADG. Regarding the weaning age effects, positive effects were observed where the highest BW was observed at the fattening period. The ADG of rabbits weaned in late weaning was higher than in early weaning with significant differences. The observed results suggest the existence of relevant G×WA interaction for the investigated traits. Therefore, the weaning age of 29-35 days is recommended for young APRI rabbits while it is suggested to wean the V rabbits after 35 days. The study confirmed that early weaning is not preferable for the rabbit



under Egyptian conditions and it is better to wean young rabbits at the minimum age of 30 days to achieve the best BW and growth rate.
Keywords: Fattening period, Genotype, Growth traits, Rabbit, Weaning age

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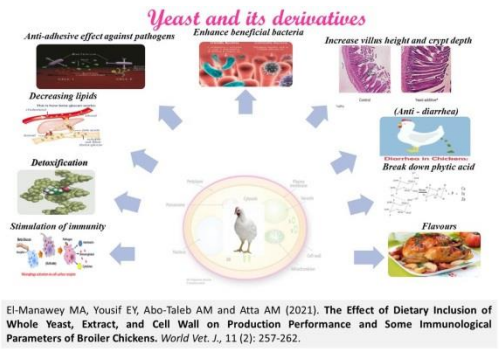
Research Paper

The Effect of Dietary Inclusion of Whole Yeast, Extract, and Cell Wall on Production Performance and Some Immunological Parameters of Broiler Chickens

El-Manawey MA, Yousif EY, Abo-Taleb AM and Atta AM.

World Vet. J. 11(2): 257-262, 2021; pii:S232245682100033-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj33

ABSTRACT: A total number of 192 male one-day-old broilers chickens were randomly divided into four treatment groups of 48 chickens. Chickens of group one fed a plain diet without any supplement (control), while the diets ib groups two, three, and four were supplemented with Whole Yeast (WY, Saccharomyces cerevisiae, 0.1%), Yeast Cell Wall (YCW, 0.3 %), and Yeast Extract (YE, 0.07 %), respectively. At the end of the experimental period (35 days), the bodyweight of chickens and the feed intake of each cage were measured, and then the feed conversation ratio was calculated. Blood samples were also collected to measure the serum components and relative spleen, bursa of Fabricius, and thymus gland. The results obtained indicated that all productive performance parameters improved in response to the feeding supplementation. Blood parameters indicated that the treated groups had a significantly higher level of serum total protein and albumin as well as significantly lower serum total lipids and cholesterol. The enzyme activities of ALT, AST, and ALP were significantly reduced by WY, YCW, and YE supplementation. The relative organ weights of the spleen, bursa of Fabricius, and thymus increased significantly in broilers fed with WY, YCW, and YE, and the highest values were observed in the chickens fed with WY. It can be demonstrated that the supplementation of WY or its derivatives in the diet of broiler chickens improves the production performance as well as the physiological and immunological parameters, and consequently produce a healthier chicken.
Keywords: Broilers, Immunity, Yeast, Yeast cell wall, Yeast extract



El-Manawey MA, Yousif EY, Abo-Taleb AM and Atta AM (2021). **The Effect of Dietary Inclusion of Whole Yeast, Extract, and Cell Wall on Production Performance and Some Immunological Parameters of Broiler Chickens.** *World Vet. J.*, 11 (2): 257-262.

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

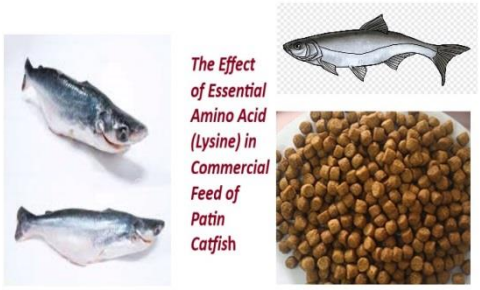
Short Communication

The Effect of Essential Amino Acid (Lysine) in Commercial Feed of Patin Catfish (*Pangasius sp.*)

Yaqin MA, Agustono, and Lokapimasari W.

World Vet. J. 11(2): 263-266, 2021; pii:S232245682100034-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj34

ABSTRACT: The Patin catfish (*Pangasius* sp.) is a species of fish that is widely cultivated both in quarantine and in ponds. The success of Patin catfish cultivation is influenced by several factors, one of them is the feed. Patin catfish need essential amino acids to meet their needs. The addition of the amino acid (lysine) in the commercial feed not only affects the metabolism of the fish but also the content of Omega-3 and Omega-6 would be found in the fish**.** This study was conducted to observe the influences of essential lysine on the content of Omega-3 and Omega-6 of Patin catfish. This was an experimental study with a completely randomized design method, consisting of four treatments and five replications. The treatment which was given to experimental fish was commercial feed with the addition of lysine at different doses. The result indicated that the addition of lysine in commercial feed for 30 days of this research had a significant effect ($p < 0.05$) on the increase in the content of Omega-3 and Omega-6 in Patin catfish meat (*Pangasius* sp.). Based on the results of the current study, Patin catfish can be a good source of Omega-3 and omega 6 if the feed that is used in the cultivation process, contains lysine as an amino acid source.
Keywords: Cultivation, Lysine, Omega-3, Omega-6, *Pangasius* sp.



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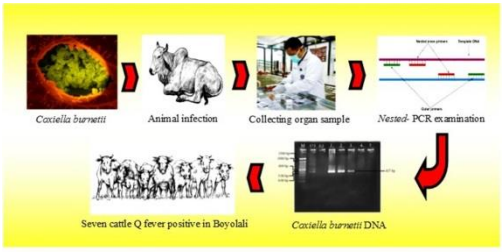
Research Paper

Detection of *Coxiella burnetii* (query fever) DNA by nested-PCR in beef cattle from Ampel slaughterhouse, Boyolali Regency, middle java, Indonesia.

Nugroho EP, Setiyono A, Hadi UK, Winarsih W, and Astuti D.

World Vet. J. 11(2): 267-272, 2021; pii:S232245682100035-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj35

ABSTRACT: *Coxiella burnetii* (*C. burnetii*) is a Gram-negative and obligate intracellular bacterium that causes Query fever (Q fever). The aim of the present study was to detect *C. burnetii* in beef cattle from Ampel slaughterhouse at Boyolali Regency, Middle Java, Indonesia. Spleen, heart, liver, lung, and kidney samples were collected from 100 cattle and used for Nested-PCR (nPCR) with four types of primers (OMP1, OMP2, OMP3, and OMP4). Five stages of pooling extraction were performed on 100 individual samples. The nPCR amplified a 437 bp DNA fragment from the fifth pool on the sampled heart, lung, and spleen. Furthermore, 10 individual samples from the fifth pool were re-tested by nPCR to find out the number of positive individual samples. Of 10 samples, the obtained result indicated the presence of *C. burnetii* DNA in 7 samples, 6 from Simmental cattle and 1 from Ongole cattle. Therefore, it can be strongly suspected that there are 7 out of 100 local breed beef cattle positive of Q fever at Boyolali Regency, Middle Java, Indonesia.



Nugroho EP, Setiyono A, Hadi UK, Winarsih W, and Astuti D (2021). **Detection of *Coxiella burnetii* (query fever) DNA by nested-PCR in beef cattle from Ampel slaughterhouse, Boyolali Regency, middle java, Indonesia**. *World Vet. J.*, 11 (2): 267-272.

Keywords: Beef cattle, Boyolali, *Coxiella burnetii*, Nested-PCR, Query fever case.

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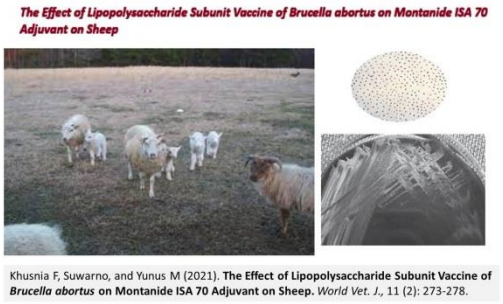
Short Communication

The Effect of Lipopolysaccharide Subunit Vaccine of Brucella abortus on Montanide ISA 70 Adjuvant on Sheep.

Khusnia F, Suwarno, and Yunus M.

World Vet. J. 11(2): 273-278, 2021; pii:S232245682100036-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj36

ABSTRACT: Brucellosis is one of the most important zoonotic diseases in the entire world. This disease results in serious economic loss and public health problems. The disease is caused by gram-negative bacteria of the genus *Brucella*. There is a need to perform control programs, such as conducting a vaccination program on livestock. One of the vaccine components that can be used is *B. abortus* lipopolysaccharide. The present study aimed to find out the effect of *B. abortus* lipopolysaccharide subunit vaccine in adjuvant Montanide ISA 70 against antibody titer and interferon-gamma (IFN- γ) level by administrating different doses and different post-vaccination sampling times. *B. abortus* lipopolysaccharide was used in the current study as an antigen and Montanide ISA 70 as an adjuvant. The samples were divided into three groups, each containing six sheep. In the control group (P0), the samples received no treatment. In the first treatment group (P1), the samples were subjected to the injection of *B. abortus* lipopolysaccharide subunit in Montanide ISA 70 adjuvant of 50 mg/ml. Regarding the second treatment group (P2), the samples had an injection of *B. abortus* lipopolysaccharide subunit vaccine in 100 mg/ml. The results showed that the administration of *B. abortus* lipopolysaccharide subunit vaccine in the Montanide ISA 70 adjuvant could influence the formation of antibodies and IFN-γ secretion on sheep. The administration of a dose of 100 µg/ml indicated a greater antibody titer, compared to the dose of 50 µg/ml. The administration of the vaccine at a dose of 50 µg/ml revealed a greater IFN-γ level value in comparison with the dose of 100 µg/ml. The result of the study on IFN-γ level indicated the control group had a greater IFN-γ level rather than the treatment group. In Conclusion, The administration of *B. abortus* lipopolysaccharide subunit vaccine in Montanide ISA 70 adjuvant could influence the formation of IFN-γ antibody and secretion on sheep.



Khusnia F, Suwarno, and Yunus M (2021). **The Effect of Lipopolysaccharide Subunit Vaccine of *Brucella abortus* on Montanide ISA 70 Adjuvant on Sheep.** *World Vet. J.*, 11 (2): 273-278.

Keywords: *B. abortus*, Lipopolysaccharide, Montanide ISA 70 Adjuvant, Vaccine

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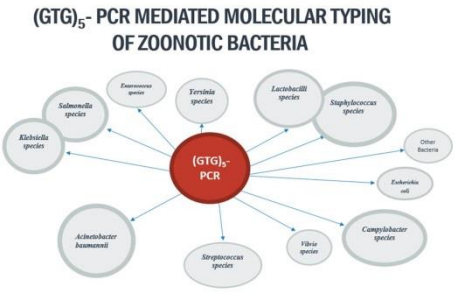
Review

$(GTG)_5$-PCR Mediated Molecular Typing of Zoonotic Bacteria.

Babazadeh D, and Ranjbar R.

World Vet. J. 11(2): 279-283, 2021; pii:S232245682100037-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj37

ABSTRACT: The present review aimed to reveal the role of $(GTG)_5$-PCR microbial typing in indicating the routes and source of infections, investigate the outbreaks and genotypes of clinical strains, as well as finding virulent strains and epidemiology of bacterial isolates. All available and published data in Google scholar, PubMed, ResearchGate, and Science Direct during the past two decades that used the $(GTG)_5$-PCR method for genotyping the bacterial isolates were included in the current study. The findings have indicated that



Babazadeh D, and Ranjbar R (2021). **$(GTG)_5$-PCR Mediated Molecular Typing of Zoonotic Bacteria.** *World Vet. J.*, 11 (2): 279-283.

$(GTG)_5$-PCR can be recommended as a possible, cost-effective, fast, and easy tool for molecular typing of bacterial isolates.
Keywords: Zoonotic bacteria, $(GTG)_5$-PCR, Molecular typing

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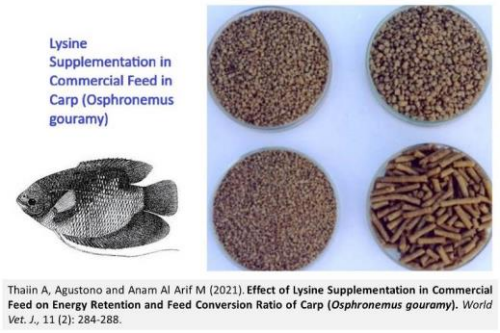
Short Communication

Effect of Lysine Supplementation in Commercial Feed on Energy Retention and Feed Conversion Ratio of Carp (*Osphronemus gouramy*).

Thaiin A, Agustono and Anam Al Arif M.

World Vet. J. 11(2):284-288, 2021; pii:S232245682100038-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj38



Thaiin A, Agustono and Anam Al Arif M (2021). **Effect of Lysine Supplementation in Commercial Feed on Energy Retention and Feed Conversion Ratio of Carp (*Osphronemus gouramy*).** *World Vet. J.*, 11 (2): 284-288.

ABSTRACT: The long period of raising carp *(Osphronemus gouramy)* causes the need for excessive feed. One way that can accelerate the growth of this fish in order to shorten the maintenance period is by the addition of essential amino acids, such as lysine. However, this certainly gives its own influence on energy retention. Therefore, the aim of this study was to determine the influences of addition of lysine in feed on energy retention and feed conversion ratio of carp. The research method used an experimental method with a completely randomized design consisting of five treatments and four replications. The treatments used were the addition of Lysine 0%, 1%, 1.5%, 2%, and 2.5% to the feed. The present experiment was conducted for a year. The results showed that the addition of lysine as much as 2% in commercial feed can increase the energy retention of carp *(Osphronemus gouramy).* Moreover, the addition of lysine by giving up to 2.5% cannot reduce the feed conversion ratio in carp *(Osphronemus gourami)* rearing. It can be concluded that the use of lysine has different effects related to the increase in retention and decrease conversion ratio in carp.
Keywords: Carp, Conversion ratio, Energy retention, Lysine

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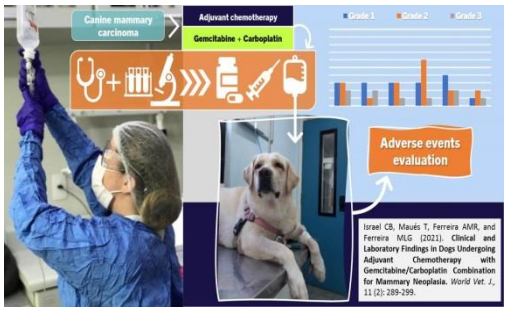
Research Paper

Clinical and Laboratory Findings in Dogs Undergoing Adjuvant Chemotherapy with Gemcitabine/Carboplatin Combination for Mammary Neoplasia.

Israel CB, Maués T, Ferreira AMR, and Ferreira MLG.

World Vet. J. 11(2): 289-299, 2021; pii:S232245682100039-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj39



Israel CB, Maués T, Ferreira AMR, and Ferreira MLG (2021). **Clinical and Laboratory Findings in Dogs Undergoing Adjuvant Chemotherapy with Gemcitabine/Carboplatin Combination for Mammary Neoplasia.** *World Vet. J.*, 11 (2): 289-299.

ABSTRACT: Adjuvant chemotherapy might be indicated in some canine mammary cancer cases due to metastatic potential. In this regard, studies to determine adverse events following chemotherapy protocols are valuable. The purpose of this prospective clinical trial was to evaluate the safety and tolerability of gemcitabine and carboplatin combination in dogs with malignant mammary tumors. For this prospective clinical trial, 21 female dogs mastectomized due to malignant mammary neoplasia underwent adjuvant chemotherapy with gemcitabine (3 mg/kg, 60-minute IV infusion) and carboplatin (10 mg/kg, 20-minute IV infusion) based protocol every 21 days for three cycles. They were monitored periodically for treatment-related adverse events by clinical and laboratory evaluations. A total of 17 (80.9%) dogs developed leukopenia, 10 (47.6%) neutropenia, and 15 (71.4%) thrombocytopenia at least once along with the three chemotherapy cycles. All these hematologic toxicities were grade 1, 2, or 3. Two (9.5%) animals had evidence of gastrointestinal toxicity; however, clinical signs were mild to moderate (grades 1 and 2). No dog had life-threatening adverse events (grade 4) or even died (grade 5) of treatment-related complications. The adjuvant chemotherapy protocol with gemcitabine and carboplatin was well-tolerated and safe in female dogs for mammary cancer treatment with self-limiting hematological and gastrointestinal adverse events.
Keywords: Adverse event, Canine, Mastectomy, Toxicity, Tumor

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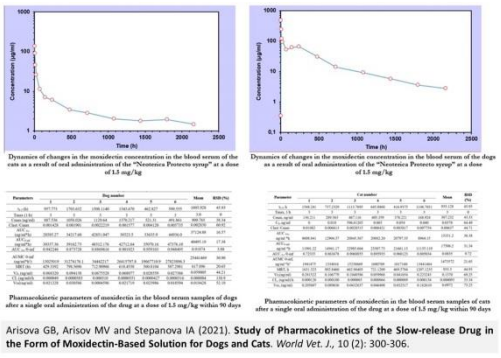
Research Paper

Study of Pharmacokinetics of the Slow-release Drug in the Form of Moxidectin-Based Solution for Dogs and Cats.

Arisova GB, Arisov MV and Stepanova IA (2021).

World Vet. J. 11(2): 300-306, 2021; pii:S232245682100040-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj40

ABSTRACT: The pharmacokinetic characteristics of the moxidectin-based drugs have been studied in the blood serum of animals after a single oral administration of the drug at the therapeutic dose in form of syrup. The drug is intended to control parasitic diseases of cats and dogs. The present studies on cats and dogs (drug administration and blood sampling) were conducted in the experimental farm of Kurilovo, Russia, for three months. The study involved six dogs and six cats, half breed, aged one to four years. The samples included six dogs (four male and two female) and six cats (three male and three female), and groups were formed according to the principle of analog groups. The drug, moxidectin, was orally administered once at the dose of 1.5 mg per one kg of animal's weight. The active substance of the drug was identified in the blood serum of animals by High-Performance Liquid Chromatography (HPLC) with fluorescence detection. The result of the current study showed that based on the pharmacokinetics of moxidectin, the concentration of the active substance in the blood serum after three hours reached 134.80-498.09 ng/ml in cats and 479.07-1459.40 ng/ml in dogs. The obtained results indicated that a single administration of the drug at the recommended therapeutic dose could ensure the maintenance of therapeutic concentrations of moxidectin in the blood, and accordingly, the protection of animals from parasites for up to 90 days.



Arisova GB, Arisov MV and Stepanova IA (2021). **Study of Pharmacokinetics of the Slow-release Drug in the Form of Moxidectin-Based Solution for Dogs and Cats**. *World Vet. J.*, 10 (2): 300-306.

Keywords: Cats, Dogs, Moxidectin, Pharmacokinetics, Solution

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

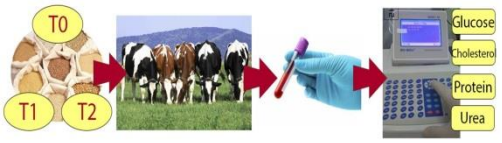
Research Paper

The Effect of Different Dietary Energy and Protein Sources on Blood Profile of Crossbreed Holstein Dairy Cows Raised in Small Stake Holder Farms.

Hudaya MF, Sitaresmi PI, and Widayati DT.

World Vet. J. 11(2): 307-312, 2021; pii:S232245682100041-11; DOI: https://dx.doi.org/10.54203/scil.2021.wvj41

ABSTRACT: The study aimed to evaluate the effect of protein and energy supplementation on the biochemical blood parameters in Holstein cows. The effect of energy and protein supplementation used corn and soybean meal was evaluated on biochemical blood profile in three groups of Holstein cows raised in small stakeholder farmers in Yogyakarta from February to May 2020. Thirty multiparous Holstein cows were allocated to three treatment groups, namely T0 in which the cows fed by the basal diet from the local farmer as well as the T1 (3.5% energy and protein supplementation) and T2 (5% energy and protein supplementation), in which the cows were fed by added energy and protein supplementation. The diets designed for the treatment groups were different from the basal diet by adding two additional ingredients which were soybean meal and corn meal in purpose to depress the stress from adaptive feeding. The results showed that the treated cows (T1 and T2) had significantly higher serum concentrations of glucose (T1 = 2.12 ± 0.49 mmol/L, T2 = 1.86 ± 0.40 mmol/L) rather than T0 (0.98 ± 0.48 mmol/L). The total concentration of serum protein and urea in treated cows was significantly lower than those with the basal diet. Total serum protein and urea in T1 were 0.69 ± 1.37 mmol/L and 7.21 ± 1.99 mmol/L, respectively; which they were 0.63 ± 0.06 mmol/L and 7.69 ± 3.07 mmol/L in T2, compared to the T0 which were 0.82 ±0.05 mmol/L and 7.69 ± 3.07 mmol/L, respectively. There was no significant difference in blood cholesterol among all treatment groups. In conclusion, the supplementations that varied in the proportion of energy and protein intake affected some biochemical blood profiles, such as glucose, protein, and blood urea nitrogen.



Hudaya MF, Sitaresmi PI, and Widayati DT (2021). **The Effect of Different Dietary Energy and Protein Sources on Blood Profile of Crossbreed Holstein Dairy Cows Raised in Small Stake Holder Farms.** *World Vet. J.*, 11 (2): 307-312.

Keywords: Biochemical blood parameters, Crossbreed Holstein cows, Energy supplementation, Protein supplementation, Traditional farmers

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Editorial

The Grass Was Greener - Climate Change, One Health, and the High Hopes to Mitigate COVID-19, Avian Influenza, and other Zoonotic Emerging Diseases.

Bonilla-Aldana DK, Faccini-Martínez ÁA, Vallejo-Timaran DA, Bocanegra-Viteri FdeM, Ruiz-Saenz J, Paniz-Mondolfi AE, Rodriguez-Morales AJ, and Suárez JA.

World Vet. J. 11(2): 313-316, 2021; pii:S232245682100042-11
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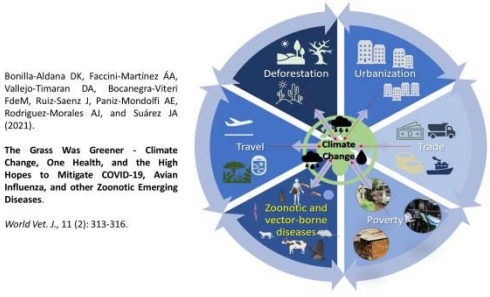

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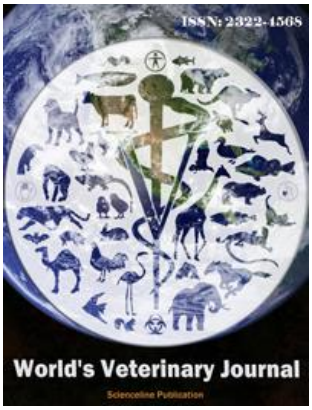

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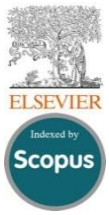

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Prevalence of Avian Influenza H5N6 in Birds: A Systematic Review and Meta-analysis of Other Viral Zoonosis

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SYSTEMATIC REVIEW
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ABSTRACT

Avian influenza viruses (AIV) are zoonotic pathogens that can potentially affect humans and potentially be epidemic in a region. Birds (such as poultry and wild birds) serve as potential reservoirs for these viruses, highlighting the importance of determining AIV prevalence in the avian population. No systematic reviews have been published on this issue in the world so far. The present systematic literature review following the PRISMA standard, with meta-analysis, used three databases to globally assess the Influenza H5N6 infection in birds (including poultry and wild birds). A model of random-effects meta-analysis was performed to calculate the pooled prevalence and 95% Confidence Interval (95% CI) for the prevalence of Influenza H5N6 infection in birds. A total number of 14,605 articles published from 2015 to 2020 were retrieved. After screening the abstract/title, 37 articles were selected for full-text assessment, and 15 were included for qualitative and quantitative analyses. Of the total number of birds (n = 13,416 birds), the pool prevalence by RT-PCR was 3.5% (95% CI: 2.8-4.3%). From the total, 39.67% of the birds assessed were ducks (family Anatidae), in which pool prevalence was 7.7% (95% CI: 4.4-11.0). In chickens (*Gallus gallus domesticus*), the pool prevalence was 3.3% (95% CI 1.9-4.8). Vietnam was the country with the highest pool prevalence; 7.9% (95% CI 4.0-11.7%). Bangladesh was the country with the lowest pool prevalence of 0.4% (95% CI 0.2-0.7%). A considerable proportion of infected birds tested positive highlighted the relevance of individual animals as reservoirs of H5N6. Ducks and chickens were found to be positive by RT-PCR in over 3% of the cases. These data suggest their relevance in maintaining zoonotic transmission and their potential implications for epidemics and even pandemics in the near future.

Keywords: H5N6, Influenza, Meta-Analysis, Molecular diagnosis, RT-PCR, Systematic Review

INTRODUCTION

Avian influenza viruses (AIV) belong to the Alphainfluenzavirus genus in the Orthomyxoviridae family (Lefkowitz et al., 2018). They can be classified into Low-Pathogenic Avian Influenza Viruses (LPAIVs) with water birds as primary host reservoirs and Highly Pathogenic Avian Influenza Viruses (HPAIVs) with poultry as the main host reservoirs (Dhingra et al., 2018). These viruses can be brought long distances by aquatic birds, transmitted at chicken farms, and infect naive poultry (Bi et al., 2016). Highly Pathogenic Avian Influenza Viruses (HPAIVs) remain an underlying threat to global health and the economy. Some of these viruses carry potential pandemic risks (Swayne et al., 2017; Shin et al., 2020; Shittu et al., 2020).

H5N6, an AIV subtype, was first isolated from mallards in 1975 (Garcia et al., 1997). This virus has continuously evolved and reasserted to generate novel HPAIVs that have led to several epidemics incidents; in 2014, Laos and Vietnam reported an Influenza H5N6 outbreak that killed hundreds of birds (Shen et al., 2015), possibly imported from live poultry from China (Wong et al., 2015). In the same year, China reported the first fatal case of Influenza H5N6

To cite this paper: Bonilla-Aldana DK, Holguin-Rivera Y, Cortes-Bonilla I, Cardona-Trujillo M.C., García-Barco A, Bedoya-Arias HA, Patiño-Cadavid LJ, Aguirre-Florez M, Balbin-Ramon GJ, Erazo-Arana DC, Zambrano LI, Perez-Garcia L, Rodriguez-Morales AJ, and Paniz-Mondolfi A (2021). Prevalence of Avian Influenza H5N6 in Birds: A Systematic Review and Meta-analysis of Other Viral Zoonosis. *World Vet. J.*, 11 (2): 146-156.

among its people (Pan et al., 2016). To date, 24 confirmed cases of human infection with influenza A (H5N6) virus have been reported to the World Health Organization (WHO) from China since 2014 resulting in seven deaths (World Health Organization, 2020).

Since birds play a pivotal role as natural hosts and reservoirs for this virus, a clearer understanding of bird-to-human transmission dynamics across wild, urban, and suburban settings is essential. Thus, a systematic review and meta-analysis of AIV were set to synthesize previously published data that assessed H1N6 infection in birds using the reverse-transcriptase polymerase chain reaction (RT-PCR). Then, this systematic review's main objective was to summarize the frequency of Influenza H5N6 infection in birds reported in currently available observational studies. Also, it was to examine the differences among the pool prevalence of H5N6 infections by animal, sample, and year.

METHODS

Protocol

The present systematic review followed the PRISMA statement's recommendations (Preferred Reporting Items for Systematic Review and Meta-Analysis, Moher et al., 2009).

Eligibility criteria

Published peer-reviewed articles that reported H5N6 infection in birds with serological or molecular confirmation by RT-PCR were included. The articles' language was not limited to English, and the publications were considered from January 1, 2002, to April 1, 2020, when the search ended. The exclusion criteria included review articles, opinion articles, correspondence articles or letters not presenting original data, and reports with incomplete information.

Information sources and search strategy

Medline/PubMed, Scopus, and Web of Sciences were used in the present systematic review. The search procedure was accomplished using the following terms "influenza", "avian influenza", "H5N6", "birds", and "influenza A". These words were used in combination while searching. Searching for this review ended on the date April 1, 2020, and a group of four different researchers evaluated the results yielded independently.

Study selection

The initial search strategy was first screened by title and abstract, as used in other systematic reviews (Rodriguez-Morales et al., 2020). The full text of relevant articles was examined for inclusion and exclusion criteria (Figure 1). When an article reported the same information from the same patient, both reports' information was combined to obtain complementary data, counted as a single case. Observational studies that reported the frequency of H5N6 infection in birds were included for quantitative synthesis (metanalysis).

Data collection and data items

Data extraction questionnaires, including information on the type of articles, publishing institution, country, year, and date of publication, as well as the number of infected animals assessed by RT-PCR, were filled independently by four researchers. An additional researcher checked the article list and data extractions to ensure no duplicate articles or duplicate information of the same study and resolved discrepancies about study inclusion. Regarding countries, the review found studies from China, Bangladesh, Myanmar, and Vietnam.

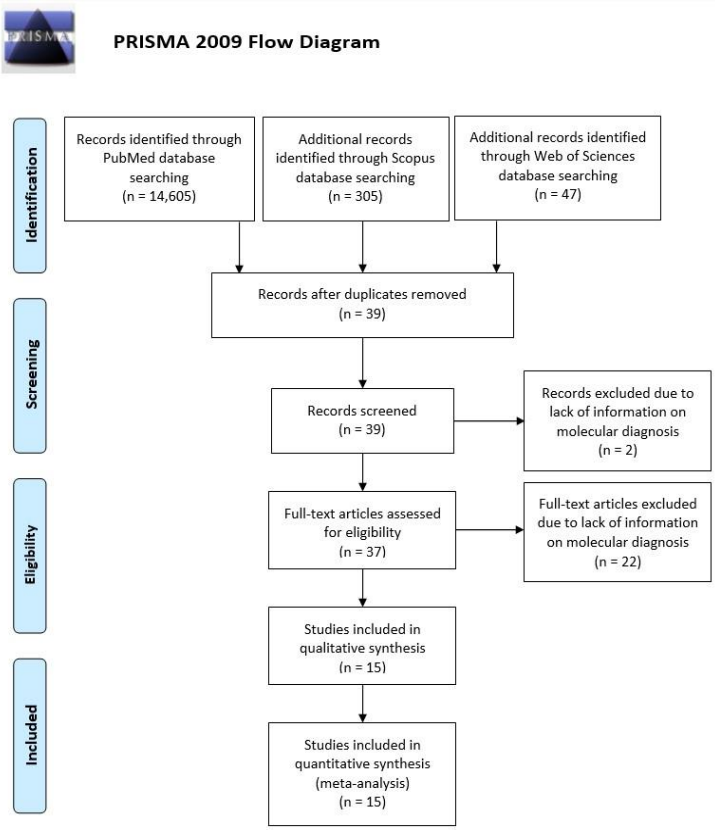

Figure 1. Study selection and characteristics of the articles included and considered for the qualitative and quantitative synthesis of data

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Assessment of methodological quality and risk of bias

To evaluate the quality of cross-sectional studies (AXIS), the critical appraisal tool from the Quality Appraisal of Case Series Studies Checklist of the Institute of Health Economics (IHE) was used (IHE, 2014; Downes et al., 2016). Publication bias was measured using a funnel plot. A model of random-effects was used to calculate the pooled prevalence and 95% CI, given variable degrees of data heterogeneity. The intrinsic heterogeneity in any systematic review of studies from published literature should be considered, then, also Egger's test was applied.

Statistical approach

Unit discordance for variables was resolved by converting all units into a standard measurement. The baseline data were analyzed using Stata version 14.0, licensed by Universidad Tecnológica de Pereira.

The meta-analysis was performed using Stata, Open Meta (Analyst) Software, and Comprehensive Meta-Analysis ve.3.3® licensed by Universidad Tecnológica de Pereira, Colombia. Pooled prevalences and their 95% confidence intervals (95% CIs) were used to summarize the weighted effect size for each study grouping variable using the binary random-effects model, considering the sample size of individual studies. For median ages, a continuous random-effect model was applied (DerSimonian-Laird procedure). A model of random-effects meta-analysis presumes that the effects being estimated in the different studies are not identical but follow some distribution. For random-effects analyses, the pooled estimate and 95% CIs refer to the center of the pooled prevalence distribution but do not describe the width of the distribution. Often the pooled estimate and its 95% CI are quoted in isolation as an alternative estimate of the quantity evaluated in a fixed-effect meta-analysis, which is inappropriate. The 95% CI from a random-effects meta-analysis describes uncertainty in the mean of systematically different prevalence in different studies.

Measures of heterogeneity, including Cochran's Q statistic, the I^2 index, and the tau-squared test, were estimated and reported, as elsewhere (Rodriguez-Morales et al., 2020). The subgroup analyses (sub-meta-analyses) were performed by diagnostic technique, animals, and countries.

RESULTS

Study selection and characteristics

A total of 14,605 articles were retrieved using the mentioned search strategy; 37 articles were selected for full-text assessment after screening by abstract and title. The rest were excluded as not containing relevant information and data for the systematic review. Twenty-two articles were excluded due to lack of information on molecular diagnosis, and 15 articles were finally included for the final qualitative and quantitative meta-analysis (Figure 1). Table 1 presents the main characteristics of the included studies.

The present review included 15 cross-sectional prevalence studies published from January 1, 2015, to April 1, 2020, which most of them were from China (81%), Vietnam (9%), Myanmar (9%), and Bangladesh (3%, tables 1 and 2), with a total of 13,416 birds assessed by RT-PCR. Three main variables (bird grouping, countries, and years) for the meta-analyses were analyzed (Table 2). Publication bias was reviewed with a funnel plot for the standard error by logit event, with no evidence of bias (Figure 2). Additionally, the Egger test suggested no substantial evidence of publication bias ($p = 0.568$).

Individual study characteristics

The mean number of included animals for RT-PCR per study was 407, with positive rates ranging from 0 to 53.8% (Tables 1-2).

Main findings

The RT-PCR pool prevalence for H5N6 was 3.5% (95% CI: 2.8-4.3%, Figure 3), 39.67% corresponded to ducks, with a pool prevalence of 7.7% (95% CI: 4.4-11.0, Figure 4), 35.63% corresponded to chickens, with a pool prevalence of 3.3% (95% CI: 1.9-4.8), and 19.15% of them were non-specified poultry birds, with the pool prevalence of 5.1% (95% CI: 0.0-14.8%, Table 2 and Figure 4).

Among the countries with the highest prevalence, Vietnam and China showed no significant differences. Vietnam with 538 animals and China with 4212 animals yielded a pool prevalence of 7.9% (95%CI: 4.0-11.7%) and 6.0% (95%CI: 4.3-7.8%). Myanmar and Bangladesh yielded a prevalence of 1.3% and 0.4%, respectively (Figure 5 and Table 2). The year 2018 had the highest prevalence (21.2%), followed by 2019 (8.3%) and 2020 (5.0%, Figure 6 and Table 2).

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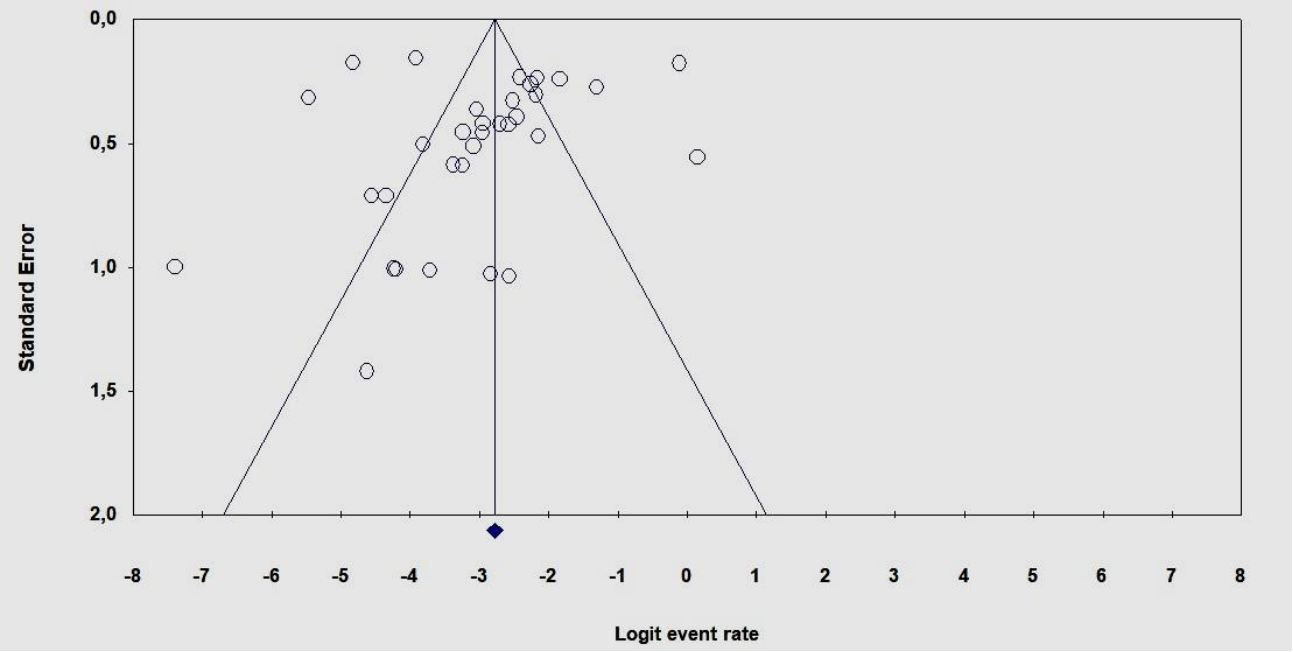

Figure 2. Funnel-plot for the standard error by logit event rate to assess the publication bias

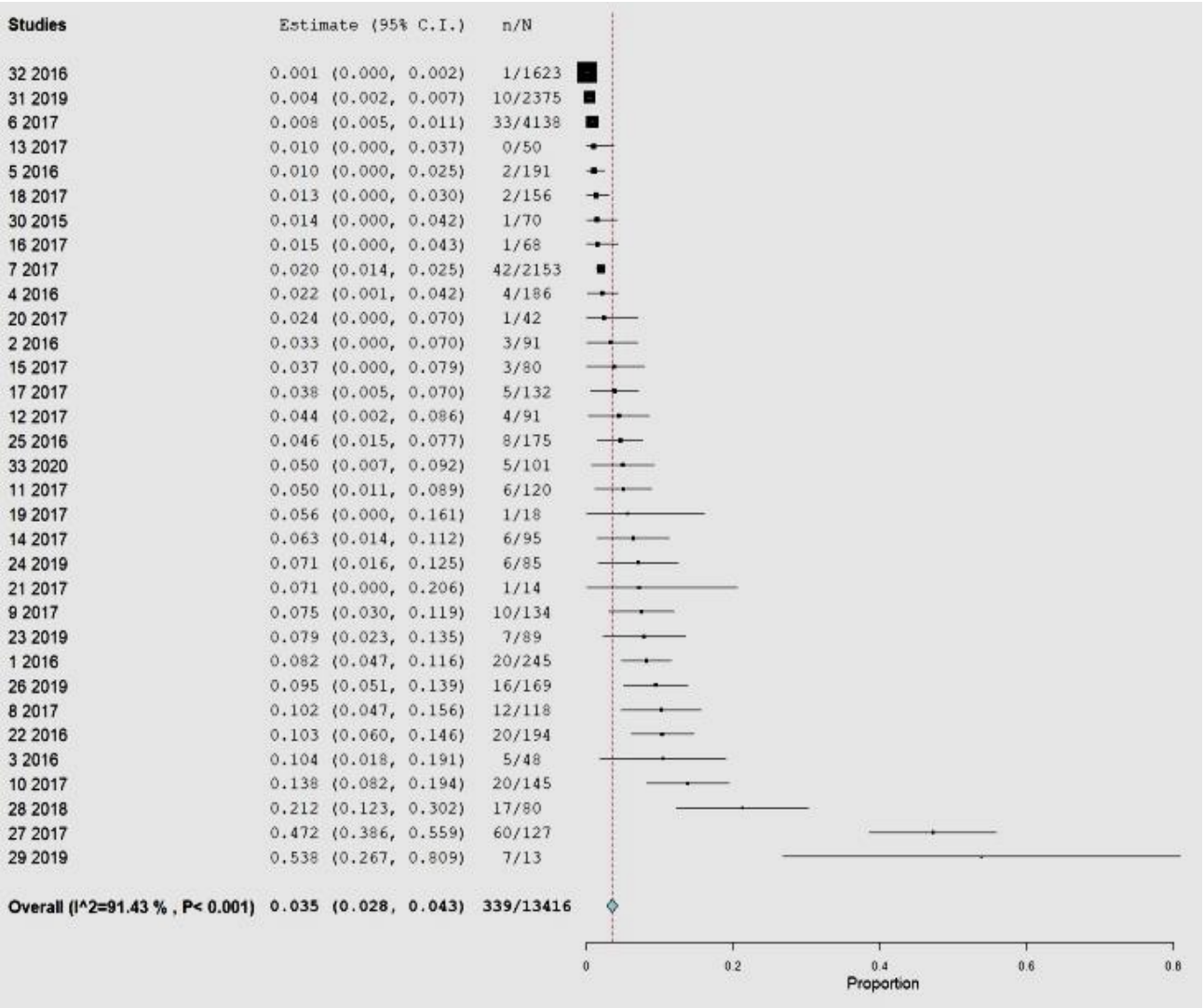

Figure 3. Forrest plot of the pooled prevalence meta-analysis of H5N6 infection in birds

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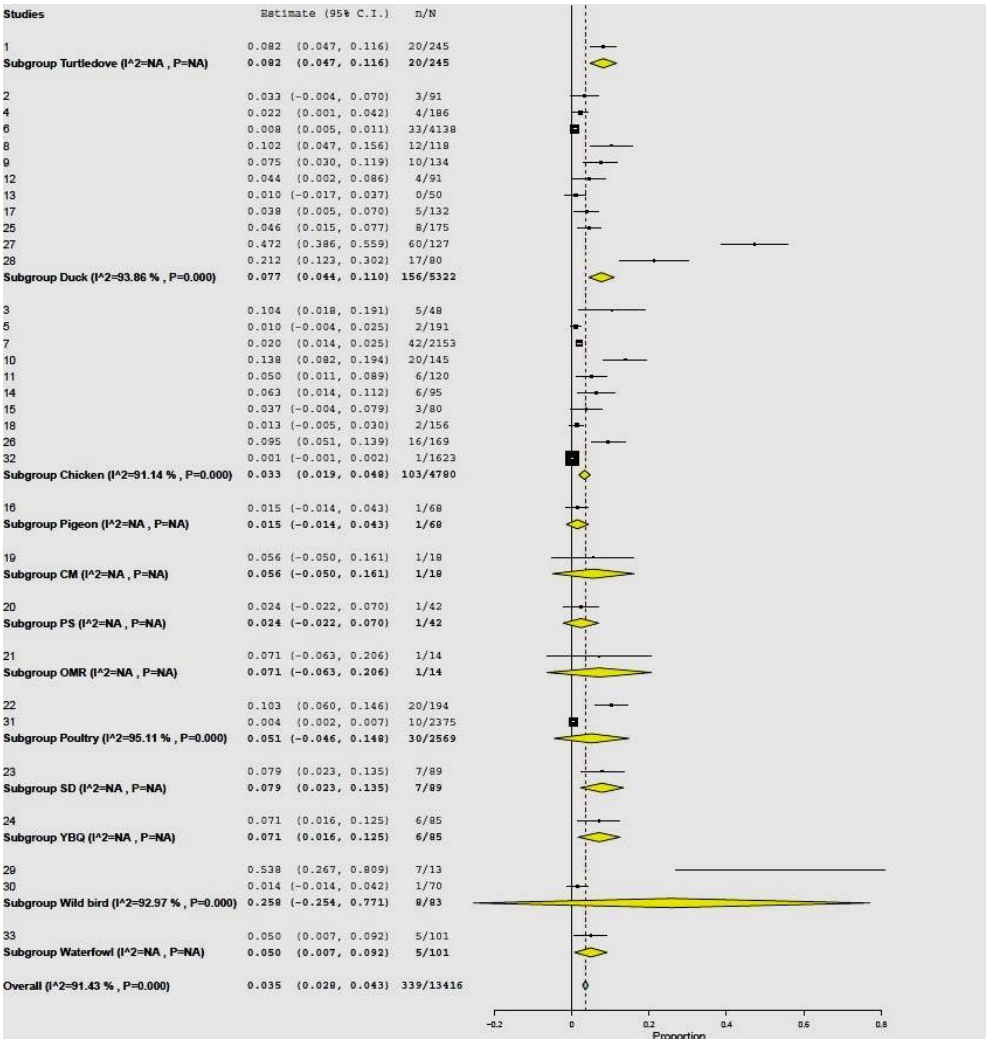

Figure 4. Forrest plot of the pooled prevalence meta-analysis of H5N6 infection in birds, by families or bird groups

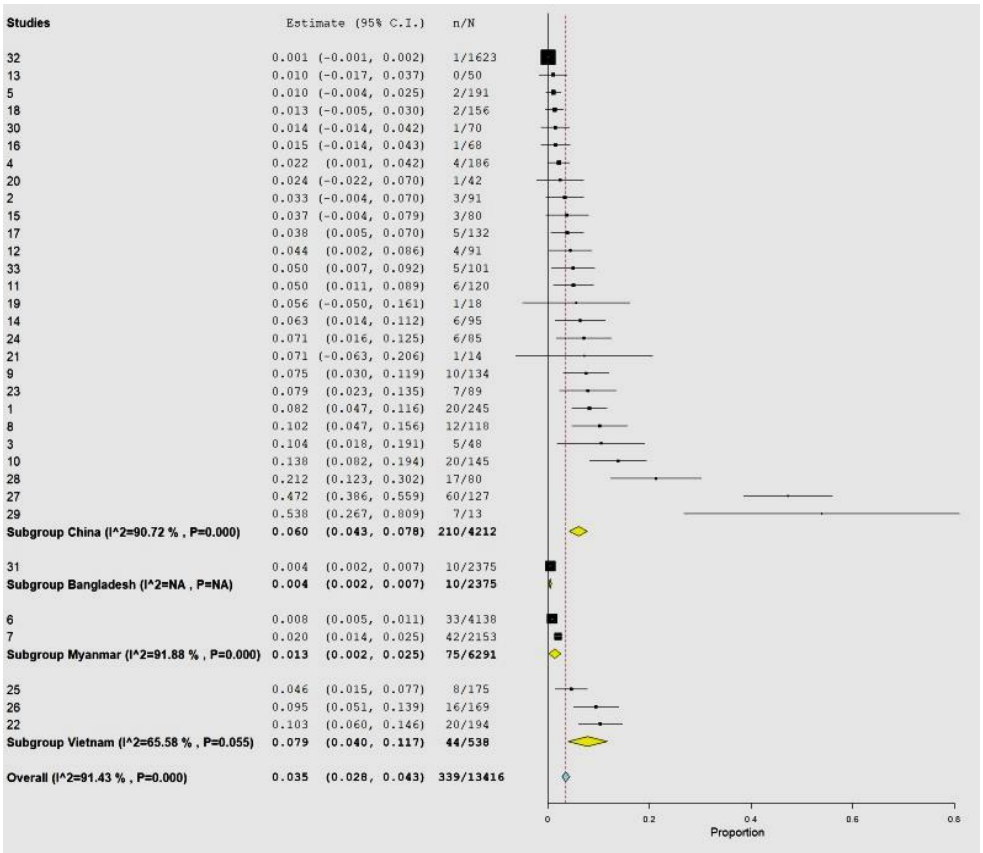

Figure 5. Forrest plot of the pooled prevalence meta-analysis of H5N6 infection in birds in terms of countries

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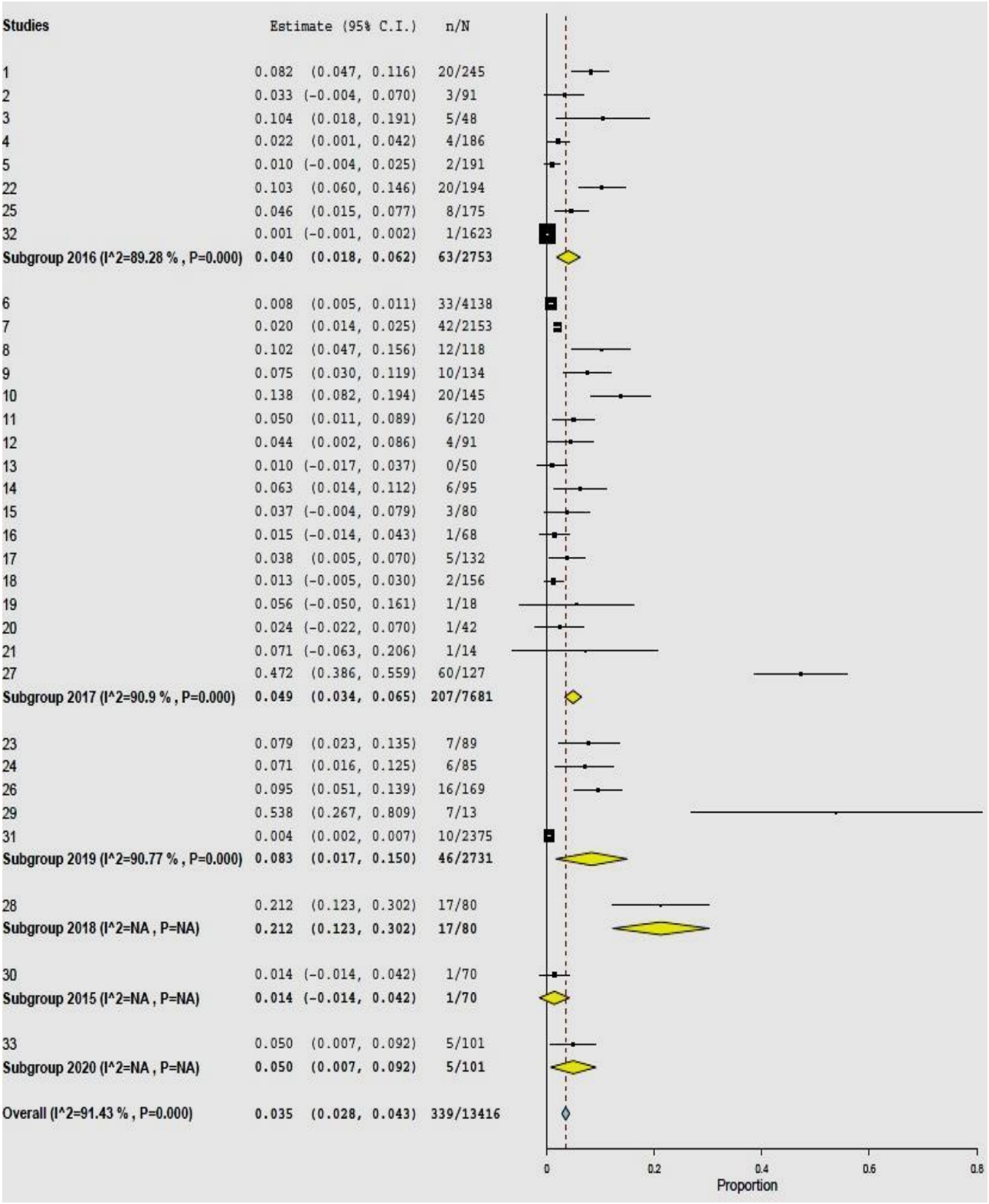

Figure 6. Forrest plot of the pooled prevalence meta-analysis of H5N6 infection in birds regarding years

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Table 1. Characteristics of the included studies on avian Influenza H5N1 in birds

Title of the study	Publication Year	Study Years	Country	Place	Birds Assessed	Sample	N	n (+)	Positive (%)	Reference
Diversity and evolution of avian influenza viruses in live poultry markets, free-range poultry, and wild wetland birds in China	2016	2014-2015	China	Hubei	Turtledove	Fecal	245	20	8.2	(Chen et al., 2016)
	2016	2014-2015	China	Hubei	Duck	Fecal	91	3	3.3	
	2016	2014-2015	China	Hubei	Chicken	Fecal	48	5	10.4	
	2016	2014-2015	China	Zhejiang	Duck	Fecal	186	4	2.2	
	2016	2014-2015	China	Zhejiang	Chicken	Fecal	191	2	1.0	
Emerging Zoonotic Influenza A Virus Detection in Myanmar: Surveillance Practices and Findings	2017	2014-2016	Myanmar	N/A	Duck	Oropharyngeal	4138	33	0.8	(Tun Win et al., 2017)
	2017	2014-2016	Myanmar	N/A	Chicken	Oropharyngeal	2153	42	2.0	
Diversity, evolution and population dynamics of avian influenza viruses circulating in the live poultry markets in China	2017	2014-2015	China	Hubei	Duck	Fecal	118	12	10.2	(Chen et al., 2017)
	2017	2014-2015	China	Hubei	Duck	Cloacal	134	10	7.5	
	2017	2014-2015	China	Hubei	Chicken	Fecal	145	20	13.8	
	2017	2014-2015	China	Hubei	Chicken	Cloacal	120	6	5.0	
	2017	2014-2015	China	Zhejiang	Duck	Fecal	91	4	4.4	
	2017	2014-2015	China	Zhejiang	Duck	Cloacal	50	0	0.0	
	2017	2014-2015	China	Zhejiang	Chicken	Fecal	95	6	6.3	
	2017	2013-2015	China	Zhejiang	Chicken	Cloacal	80	3	3.8	
	2017	2014-2015	China	Zhejiang	Pigeon	Cloacal	68	1	1.5	
	2017	2014-2015	China	Jiangxi	Duck	Fecal	132	5	3.8	
	2017	2014-2015	China	Jiangxi	Chicken	Fecal	156	2	1.3	
Highly pathogenic H5N6 influenza A viruses recovered from wild birds in Guangdong, southern China, 2014–2015	2017	2014-2015	China	Guandong	CM	Fecal	18	1	5.6	(Kang et al., 2017)
	2017	2014-2015	China	Guandong	PS	Fecal	42	1	2.4	
	2017	2014-2015	China	Guandong	OMR	Fecal	14	1	7.1	
Shifting Clade Distribution, Reassortment, and Emergence of New Subtypes of Highly Pathogenic Avian Influenza A(H5) Viruses Collected from Vietnamese poultry from 2012 to 2015	2016	2012-2015	Vietnam	Vietnam	Poultry	Oropharyngeal	194	20	10.3	(Nguyen et al., 2017)
First Detection of a Novel Reassortant Avian Influenza A(H5N6) Clade 2.3.2.1c Virus, Isolated from a Wild Bird in China	2019	2016	China	N/A	SD	Fecal	89	7	7.9	(Zhang et al., 2019)
	2019	2016	China	N/A	YBQ	Fecal	85	6	7.1	
Genetic and antigenic characterization of H5, H6, and H9 avian influenza viruses circulating in live bird markets with intervention in the center part of Vietnam	2016	2014	Vietnam	Thua Thien Hue	Duck	Fecal	175	8	4.6	(Chu et al., 2016)

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Title of the study	Publication Year	Study Years	Country	Place	Birds Assessed	Sample	N	n (+)	Positive (%)	Reference
Poultry trading behaviors in Vietnamese live bird markets as risk factors for avian influenza infection in chickens	2019	2017	Vietnam	northern Vietnam	Chicken	Oropharyngeal	169	16	9.5	(Sealy et al., 2019)
Identification of two novel avian influenzas a (H5N6) viruses in wild birds, Shanghai, in 2016	2017	2016	China	Shanghai: Chongming Dongtan, Nanhui Dongtan, Jiuduansha	Duck	CTS	127	60	47.2	(He et al., 2017)
Genetics, pathogenicity, and transmissibility of novel reassortant H5N6 highly pathogenic avian influenza viruses first isolated from migratory birds in western China	2018	2015	China	Changshantou	Duck	OCS	80	17	21.3	(Lu et al., 2018)
First Detection of a Novel Reassortant Avian Influenza A(H5N6) Clade 2.3.2.1c Virus, Isolated from a Wild Bird in China	2019	2016	China	Suichuan County	Wild bird	TCS	13	7	53.8	(Zhang et al., 2019)
Fatal H5N6 Avian Influenza Virus Infection in a Domestic Cat and Wild Birds in China	2015	2014	China	Sichuan province	Wild bird	Feces	70	1	1.4	(Yu et al., 2015)
Detection of highly pathogenic avian influenza A(H5N6) viruses in waterfowl in Bangladesh	2019	2016-2017	Bangladesh		Poultry	TCS	2375	10	0.4	(Yang et al., 2019)
Novel H7N2 and H5N6 Avian influenza A viruses in sentinel chickens: A sentinel chicken surveillance study	2016	2014	China	Jiangsu Province	Chicken	CTS	1623	1	0.1	(Zhao et al., 2016)
Novel H5N6 Avian Influenza Virus Reassortants with European H5N8 Isolated in Migratory Birds, China	2020	2017	China	Ningxia Hui Autonomous Region	Waterfowl	OCS	101	5	5.0	(Sun et al., 2020)

N: Total number assessed, n: number of positive, CM: Common moorhen, PS: Pallas's sandgrouse, OMR: oriental magpie-robin, SD: Streptopelia decaocto, YBQ: Yellow-legged button quail, CTS: Cloacal and tracheal swab, OCS: Oropharyngeal and cloacal swabs, TCS: Tracheal and cloacal swab, N/A: Not available or reported.

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Table 2. Meta-analysis outcomes (random-effects model) (prevalences of influenza H5N6, overall and subanalyses)*

Subgroups	Number of Studies	Pool Prevalence (%)	95% CI	n	Q†	I^{2} ‡	t^{2} §	p value
All the studies	15	3.5	2.8-4.3	13,416	373.460	91.431	0.001	< 0.001
Prevalence by Bird grouping								
Ducks	11	7.7	4.4-11.0	5,322	126.786	93.86	0.001	< 0.001
Chickens	10	3.3	1.9-4.8	4,780	101.601	91.14	0.001	< 0.001
Non-specified poultry	2	5.1	0.0-14.8	2,569	20.439	95.11	0.001	< 0.001
Prevalence by Countries								
Vietnam	3	7.9	4.0-11.7	538	5.811	65.58	0.100	0.05
China	28	6.0	4.3-7.8	4,212	298.457	90.72	0.001	< 0.001
Myanmar	2	1.3	0.2-2.5	6,291	12.320	91.88	0.001	< 0.001
Prevalence by Years								
2019	5	8.3	1.7-15.0	2,731	43.323	90.77	0.001	< 0.001
2017	17	4.9	3.4-6.5	7,681	175.786	90.9	0.001	< 0.001
2016	8	4.0	1.8-6.2	2,753	65.279	89.28	0.001	< 0.001

* 95% CI: 95% confidence interval. † Cochran's Q statistic for heterogeneity. ‡ I^{2} index for the degree of heterogeneity. § Tau-squared measure of heterogeneity.

DISCUSSION

Recent studies suggest that HPAI outbreaks from 2016 to 2018 caused by novel reassortant clade 2.3.4.4 H5N6 viruses resulted in the death of one billion birds in South Korea (Shin et al., 2020). In 2020, the clade 2.3.4.4B was reported in Iran after complete-genome sequencing of 28 H5Nx viruses circulating in the country from 2016 to 2018 (Abdollahi et al., 2020). In the same year, a study reported the first African case of HPAI (H5N6) virus (clade 2.3.4.4b) in a duck identified at a live-poultry market (LPM) in Nigeria whose genome was nearly linked to the European H5N6 viruses (2017-2018) (Shittu et al., 2020). However, as observed in the current meta-analysis, a tremendous prevalence burden exists within the Asian continent.

The prevalence rates of H5N6 infection using RT-PCR were reported up to 7.7%, with an upper limit of the confidence level of 11.0% in ducks and 3.3% in chickens. Both birds (ducks and chickens) share close contact with human beings, especially in the Asian live-poultry market (Fang et al., 2016). Ducks play a critical role in viral preservation and dissemination throughout different settings and environments. Thus, the control of H5N6 within LMPs is pivotal to eradicate influenza from poultry (Chen et al., 2019; de Vries et al., 2018). The continued interaction between humans and poultry in these settings poses a significant risk for human spillover infection and potential emerging health threats of epidemic or pandemic proportions. The increased H5N6 prevalence in LPMs has shifted public health efforts towards sustained LPM surveillance to retrieve relevant epidemiological information and provide early warnings of human infection with AIV. Interventions, such as live-poultry market surveillance and closings, should be implemented to mitigate the potential risk of infection in humans when these viruses are detected widely (Fang et al., 2016).

H5N6 Influenza is just one example of a current systematic phenomenon. The complete focus of public health towards Coronavirus disease 2019 (COVID-19) detracts nearly all attention away from other latent but relevant infectious diseases. As observed in the present meta-analysis, from 2015 to 2020, there has been consistent evidence of approximately 3% prevalence rates by RT-PCR for H5N6 Influenza in birds.

In 2020, a study reported a patient infected with the avian influenza A (H5N6) virus by aerosol exposure in China (Li et al., 2020). That case had no history of exposure to LMPs but had a record of exposure to live poultry placed in a car with closed doors and windows. The samples collected from the patient's lower respiratory tract and remaining frozen chicken meat were positive for the influenza A (H5N6) virus (Li et al., 2020). Earlier that year, a fatal case of H5N6 in an obese 9-year-old Chinese girl was reported. She was initially presented with fever and coughing, and then pneumonia, Acute Respiratory Distress Syndrome, and respiratory failure were developed. Aspirates from the lower respiratory tract and anal swabs were taken serially in that patient up to the death. A novel reassortant H5N6 virus was isolated, and genome sequencing and phylogenetic analysis were performed. Except for the polymerase acid protein (PA) gene, all the other seven genes of the virus belonged to H5N6 genotype A (S4-like virus) (Chen et al., 2020). Given these alarming cases in China, extraordinary measures should be implemented to mitigate or avoid future outbreaks of avian influenza.

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Although the current meta-analysis found a pool prevalence of 3.5%, some selected studies reached more than 21% (Figure 3). Considering the number of assessed birds, these findings should be considered relevant. A significant concern is raised, even more considering that there are no effective vaccines to prevent human H5N6 Influenza infection, although some candidates have been recently tested (Chen et al., 2019; de Vries et al., 2018). One of them, the rDEVus78HA vaccine, efficiently protected ducks against challenges with isolated heterologous H5N6 and H5N8 viruses (Chen et al., 2019). Another vaccine candidate, rMVA-H5 (Clinical trials registration: NTR3401), seemed to be effective against antigenically distinct H5 viruses (de Vries et al., 2018).

Primary prevention is critical in diseases with a high case fatality rate, typical for many HPAI infection cases (Bi et al., 2016). H5N6 virus induces large economic losses to poultry breeding industries in developing regions worldwide, especially in Asia. HPAI H5 clade 2.3.4.4 viruses were introduced in Europe in late 2014 and re-introduced in late 2016, following detections in Asia and Russia (Poen et al., 2019). Recent outbreaks reported in captive *Pavo cristatus* in Jiangxi Province, China, suggest avian influenza as a critical latent threat to public health (Li et al., 2019).

The present results highlighted the relevance of individual birds as reservoirs for H5N6. Ducks and chickens were found positive by RT-PCR in over 3% of the cases, showcasing their relevance in maintaining zoonotic transmission with the consequent risk of disease outbreaks. Additional research and enhanced LPM in China and other countries worldwide should be promptly considered to prevent more subsequent outbreaks.

DECLARATIONS

Present study was a part of the thesis of Veterinary Medicine and Zootechnics of D.C. Erazo-Arana, at Universidad Tecnológica de Pereira, Pereira, Risaralda, Colombia, under the supervision of D.K. Bonilla-Aldana and A.J. Rodriguez-Morales.

Authors' contributions

D. Katterine Bonilla-Aldana conceived the idea of the study. Yeimer Holguin-Rivera, Isabella Cortes-Bonilla, María C. Cardona-Trujillo, Alejandra García-Barco, Hugo A. Bedoya-Arias, Leidy Jhoana Patiño-Cadavid, Mateo Aguirre-Florez, Graciela J. Balbin-Ramon, Delcy C. Erazo-Arana collected data. Alfonso J. Rodriguez-Morales and D. Katterine Bonilla-Aldana analyzed the data. Alfonso J. Rodriguez-Morales wrote the first draft. All authors wrote and revised the subsequent drafts. All authors approved the final submitted version and the data analysis.

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Competing interests

All authors declare no competing interests to be reported.

REFERENCES

Abdollahi H, Ali ASM, Monne I, Milani A, Habibi M, Zamperin G, Ghafouri SA, Maghsoudloo H, Mohammadpoor B, Esmaeilzadeh S et al. (2020). Spatial spread and emergence of reassortant h5 highly pathogenic avian influenza viruses in Iran reassortant h5 highly pathogenic avian influenza virus in Iran. Infection Genetics and Evolution, 83: Article number 104342. DOI: https://www.doi.org/10.1016/j.meegid.2020.104342

Bi Y, Chen Q, Wang Q, Chen J, Jin T, Wong G, Quan C, Liu J, Wu J, Yin R et al. (2016). Genesis, evolution and prevalence of h5n6 avian influenza viruses in China. Cell Host and Microbe, 20(6): 810-821. DOI: https://www.doi.org/10.1016/j.chom.2016.10.022

Chen LJ, Lin XD, Guo WP, Tian JH, Wang W, Ying XH, Wang MR, Yu B, Yang ZQ, Shi M et al. (2016). Diversity and evolution of avian influenza viruses in live poultry markets, free-range poultry and wild wetland birds in China. Journal of General Virology, 97(4): 844-854. DOI: https://www.doi.org/10.1099/jgv.0.000399

Chen LJ, Lin XD, Tian JH, Liao Y, Ying XH, Shao JW, Yu B, Guo JJ, Wang MR, Peng Y et al. (2017). Diversity, evolution and population dynamics of avian influenza viruses circulating in the live poultry markets in China. Virology, 505: 33-41. DOI: https://www.doi.org/10.1016/j.virol.2017.02.009

Chen LL, Huo X, Qi X, Liu C, Huang H, Yu H, Dong Z, Deng F, Peng J, Hang H et al. (2020). A fatal paediatric case infected with reassortant avian influenza a(h5n6) virus in eastern China. Transboundary and Emerging Diseases, In press. DOI: https://www.doi.org/10.1111/tbed.13561

Chen P, Ding L, Jiang Y, Zeng X, Deng G, Shi J, Li Y, Liu L, Zhao Y, Hu Y et al. (2019). Protective efficacy in farmed ducks of a duck enteritis virus-vectored vaccine against h5n1, h5n6, and h5n8 avian influenza viruses. Vaccine, 37(40): 5925-5929. DOI: https://www.doi.org/10.1016/j.vaccine.2019.08.026

Chu DH, Okamatsu M, Matsuno K, Hiono T, Ogasawara K, Nguyen LT, Van Nguyen L, Nguyen TN, Nguyen TT, Van Pham D et al. (2016). Genetic and antigenic characterization of h5, h6 and h9 avian influenza viruses circulating in live bird markets with intervention in the center part of Vietnam. Veterinary Microbiology, 192: 194-203. DOI: https://www.doi.org/10.1016/j.vetmic.2016.07.016

de Vries RD, Altenburg AF, Nieuwkoop NJ, de Bruin E, van Trierum SE, Pronk MR, Lamers MM, Richard M, Nieuwenhuijse DF, Koopmans MPG et al. (2018). Induction of cross-clade antibody and t-cell responses by a modified vaccinia virus Ankara-based influenza a(h5n1) vaccine in a randomized phase 1/2a clinical trial. The Journal of Infectious Diseases, 218(4): 614-623. DOI: https://www.doi.org/10.1093/infdis/jiy214

Dhingra MS, Artois J, Dellicour S, Lemey P, Dauphin G, Von Dobschuetz S, Van Boeckel TP, Castellan DM, Morzaria S, and Gilbert M (2018). Geographical and historical patterns in the emergences of novel highly pathogenic avian influenza (hpai) h5 and h7 viruses in poultry. Frontiers in Veterinary Science, 5: Article number 84. DOI: https://www.doi.org/10.3389/fvets.2018.00084

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Downes MJ, Brennan ML, Williams HC, and Dean RS (2016). Development of a critical appraisal tool to assess the quality of cross-sectional studies (axis). BMJ Open, 6(12): e011458. DOI: https://www.doi.org/10.1136/bmjopen-2016-011458

Fang S, Bai T, Yang L, Wang X, Peng B, Liu H, Geng Y, Zhang R, Ma H, Zhu W et al. (2016). Sustained live poultry market surveillance contributes to early warnings for human infection with avian influenza viruses. Emerging Microbes and Infections, 5(8): e79. DOI: https://www.doi.org/10.1038/emi.2016.75

Garcia M, Suarez DL, Crawford JM, Latimer JW, Slemons RD, Swayne DE, and Perdue ML (1997). Evolution of h5 subtype avian influenza a viruses in North America. Virus Research, 51(2): 115-124. DOI: https://www.doi.org/10.1016/s0168-1702(97)00087-7

He G, Zhou L, Zhu C, Shi H, Li X, Wu D, Liu J, Lv J, Hu C, Li Z et al. (2017). Identification of two novel avian influenza a(h5n6) viruses in wild birds, shanghai, in 2016. Veterinary Microbiology, 208: 53-57. DOI: https://www.doi.org/10.1016/j.vetmic.2017.07.017

Institute of Health Economics (IHE) (2014). Quality appraisal of case series studies checklist. Available at: http://www.ihe.ca/research-programs/rmd/cssqac/cssqac-about

Kang Y, Liu L, Feng M, Yuan R, Huang C, Tan Y, Gao P, Xiang D, Zhao X, Li Y et al. (2017). Highly pathogenic h5n6 influenza a viruses recovered from wild birds in Guangdong, southern China, 2014-2015. Scientific Reports, 7: Article number 44410. DOI: https://www.doi.org/10.1038/srep44410

Lefkowitz EJ, Dempsey DM, Hendrickson RC, Orton RJ, Siddell SG, and Smith DB (2018). Virus taxonomy: The database of the international committee on taxonomy of viruses (ictv). Nucleic Acids Research, 46: 708-717. DOI: https://www.doi.org/10.1093/nar/gkx932

Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, Pan P, Wang W, Hu D, Liu X et al. (2020). Coronavirus infections and immune responses. Journal of Medical Virology, 92(4): 424-432. DOI: https://www.doi.org/10.1002/jmv.25685

Li M, Feng S, Lv S, Luo J, Guo J, Sun J, and He H (2019). Highly pathogenic h5n6 avian influenza virus outbreak in Pavo cristatus in Jiangxi province, China. Emerging Microbes and Infections, 8(1): 377-380. DOI: https://www.doi.org/10.1080/22221751.2019.1586411

Lu S, Zhao Z, Zhang J, Wang W, He X, Yu M, Zhang C, Li X, Guo Z, Yang X et al. (2018). Genetics, pathogenicity and transmissibility of novel reassortant h5n6 highly pathogenic avian influenza viruses first isolated from migratory birds in western China. Emerging Microbes and Infections, 7(1): Article number 6. DOI: https://www.doi.org/10.1038/s41426-017-0001-1

Moher D, Liberati A, Tetzlaff J, Altman DG, and Group P (2009). Preferred reporting items for systematic reviews and meta-analyses: The Prisma statement. PLOS Medicine, 6(7): e1000097. DOI: https://www.doi.org/10.1371/journal.pmed.1000097

Nguyen DT, Jang Y, Nguyen TD, Jones J, Shepard SS, Yang H, Gerloff N, Fabrizio T, Nguyen LV, Inui K et al. (2017). Shifting clade distribution, reassortment, and emergence of new subtypes of highly pathogenic avian influenza a(h5) viruses collected from Vietnamese poultry from 2012 to 2015. Journal of Virology, 91(5): 1-18. DOI: https://www.doi.org/10.1128/JVI.01708-16

Pan M, Gao R, Lv Q, Huang S, Zhou Z, Yang L, Li X, Zhao X, Zou X, Tong W et al. (2016). Human infection with a novel, highly pathogenic avian influenza a(h5n6) virus: Virological and clinical findings. Journal of Infection, 72(1): 52-59. DOI: https://www.doi.org/10.1016/j.jinf.2015.06.009

Poen MJ, Venkatesh D, Bestebroer TM, Vuong O, Scheuer RD, Oude Munnink BB, de Meulder D, Richard M, Kuiken T, Koopmans MPG et al. (2019). Co-circulation of genetically distinct highly pathogenic avian influenza a clade 2.3.4.4 (h5n6) viruses in wild waterfowl and poultry in Europe and East Asia, 2017-18. Virus Evolution, 5(1): Artice number 4. DOI: https://www.doi.org/10.1093/ve/vez004

Rodriguez-Morales AJ, Cardona-Ospina JA, Gutierrez-Ocampo E, Villamizar-Pena R, Holguin-Rivera Y, Escalera-Antezana JP, Alvarado-Arnez LE, Bonilla-Aldana DK, Franco-Paredes C, Henao-Martinez AF et al. (2020). Clinical, laboratory and imaging features of covid-19: A systematic review and meta-analysis. Travel Medicine and Infectious Disease, 34: Article number 101623. DOI: https://www.doi.org/10.1016/j.tmaid.2020.101623

Sealy JE, Fournie G, Trang PH, Dang NH, Sadeyen JR, Thanh TL, van Doorn HR, Bryant JE, and Iqbal M (2019). Poultry trading behaviours in Vietnamese live bird markets as risk factors for avian influenza infection in chickens. Transboundary and Emerging Diseases, 66(6): 2507-2516. DOI: https://www.doi.org/10.1111/tbed.13308

Shen H, Wu B, Chen Y, Bi Y, and Xie Q (2015). Influenza a(h5n6) virus reassortant, southern China, 2014. Emerging Infectious Diseases, 21(7): 1261-1262. DOI: https://www.doi.org/10.3201/eid2107.140838

Shin J, Kang S, Byeon H, Cho SM, Kim SY, Chung YJ, and Jung SH (2020). Highly pathogenic h5n6 avian influenza virus subtype clade 2.3.4.4 indigenous in South Korea. Scientific Reports, 10(1): Article number 7241. DOI: https://www.doi.org/10.1038/s41598-020-64125-x

Shittu I, Bianco A, Gado D, Mkpuma N, Sulaiman L, Laleye A, Gobbo F, Bortolami A, Bonfante F, Vakuru C et al. (2020). First detection of highly pathogenic h5n6 avian influenza virus on the African continent. Emerging Microbes and Infections, pp. 1-14. DOI: https://www.doi.org/10.1080/22221751.2020.1757999

Sun J, Zhao L, Li X, Meng W, Chu D, Yang X, Peng P, Zhi M, Qin S, Fu T et al. (2020). Novel h5n6 avian influenza virus reassortants with European h5n8 isolated in migratory birds, China. Transboundary and Emerging Diseases, 67(2): 648-660. DOI: https://www.doi.org/10.1111/tbed.13380

Swayne DE, Hill RE, and Clifford J (2017). Safe application of regionalization for trade in poultry and poultry products during highly pathogenic avian influenza outbreaks in the USA. Avian Pathology, 46(2): 125-130. DOI: https://www.doi.org/10.1080/03079457.2016.1257775

Tun Win Y, Gardner E, Hadrill D, Su Mon CC, Kyin MM, Maw MT, Claes F, von Dobschuetz S, Kalpravidh W, Wongsathapornchai K et al. (2017). Emerging zoonotic influenza a virus detection in myanmar: Surveillance practices and findings. Health Security, 15(5): 483-493. DOI: https://www.doi.org/10.1089/hs.2016.0131

Wong FY, Phommachanh P, Kalpravidh W, Chanthavisouk C, Gilbert J, Bingham J, Davies KR, Cooke J, Eagles D, Phiphakhavong S et al. (2015). Reassortant highly pathogenic influenza a(h5n6) virus in Laos. Emerging Infectious Diseases, 21(3): 511-516. DOI: https://www.doi.org/10.3201/eid2103.141488

World Health Organization (WHO) (2020). Human infection with avian influenza a(h5) viruses. *Human infection with avian influenza A(H5N6) virus* Retrieved 03/23/2020, 2020. Available at: https://www.who.int/docs/default-source/wpro---documents/emergency/surveillance/avian-influenza/ai-20200313.pdf?sfvrsn=223ca73f_42

Yang G, Chowdury S, Hodges E, Rahman MZ, Jang Y, Hossain ME, Jones J, Stark TJ, Di H, Cook PW et al. (2019). Detection of highly pathogenic avian influenza a(h5n6) viruses in waterfowl in Bangladesh. Virology, 534: 36-44. DOI: https://www.doi.org/10.1016/j.virol.2019.05.011

Yu Z, Gao X, Wang T, Li Y, Li Y, Xu Y, Chu D, Sun H, Wu C, Li S et al. (2015). Fatal h5n6 avian influenza virus infection in a domestic cat and wild birds in China. Scientific Reports, 5: Article number 10704. DOI: https://www.doi.org/10.1038/srep10704

Zhang T, Li R, Zhu G, Chang J, and Xu B (2019). First detection of a novel reassortant avian influenza a(h5n6) clade 2.3.2.1c virus, isolated from a wild bird in china. Microbiology Resource Announcements, 8(36): e00797-19. DOI: https://www.doi.org/10.1128/MRA.00797-19

Zhao T, Qian YH, Chen SH, Wang GL, Wu MN, Huang Y, Ma GY, Fang LQ, Gray GC, Lu B et al. (2016). Novel h7n2 and h5n6 avian influenza a viruses in sentinel chickens: A sentinel chicken surveillance study. Frontiers in Microbiology, 7: Article number 1766. DOI: https://www.doi.org/10.3389/fmicb.2016.01766

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Reviewing Effective Factors of Alimentary Deficiency in Animals Reproductive Functions

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ABSTRACT

Animal reproduction is one of the main factors limiting the efficiency of livestock production. Its optimal level is possibly achieved when certain conditions are created for animals. As reproduction is a complex reflex process depending on neuroendocrine regulatory mechanisms, the character and strength of stimuli, which, in turn, is due to a number of factors. Under normal conditions, the body of animals is affected by many different factors, which are appropriately transformed and specified by positive or negative reactions. Inhibitory factors include air pool, saturated with harmful substances and gases, ionizing radiation, poor water quality along with altered redox properties, hypokinesia combined with poor unbalanced feeding, systematic chronic stress, presence of toxic substances in feed, and the deficiency of vitamins and other bioantioxidants in feed or their excessive spending. Of the wide range of genetic and paratypic factors of negative impacts on reproductive capacity, the most common one is alimentary, which causes impaired reproductive function due to deficiencies in the rules, regulations, and feeding regime of animals. In particular, the alimentary can be associated with both general malnutrition (starvation) and overfeeding (obesity). However, the alimentary form of infertility mostly occurs due to low-quality diets when the diet lacks vital components (mainly vitamins, macro-, and micronutrients) or the quantitative ratios of the ingredients are violated. This is possible even if the total nutritional value of the diet meets the established requirements for the physiological needs of the body. Vitamins, micro-, and macronutrients are ecologically deficient factors of disturbance of animal reproductive function, the influence of which is observed on all processes of reproduction, from fertilization to the postpartum period and the preservation of young animals. The pathogenesis of their insufficiency is associated with the violation of steroido-, gameto-, and embryogenesis and the emergence of ante-, intra-, neo- and postnatal pathologies, respectively. Therefore, treatments and prevention measures should be aimed at providing animals with biologically complete balanced feeding and replenishment of the body with vitamins and minerals. However, all these issues remain incompletely studied and need further research.

Keywords: Alimentary deficiency, Animals, Reproductive function.

INTRODUCTION

The optimal level of procreation is possible only if certain conditions are created for animals because reproduction is an extremely important function with a complex reflex process that depends on neuroendocrine regulatory mechanisms and the character and strength of stimuli acting on the body (Koshovyj, 2004; Iolchiev et al., 2014; Kagermazov and Taov, 2018). Under normal conditions, the body of animals is affected by many different factors, which are appropriately transformed and manifested by positive or negative reactions. Inhibitory factors include air pool saturated with harmful substances and gases, ionizing radiation, poor water quality along with altered redox properties, hypokinesia combined with poor and unbalanced feeding, systematic chronic stress, presence of toxic substances in feed, and the deficiency of vitamins and other bioantioxidants in feed or their excessive spending (Skljarov, 2017).

Currently, there is a rapid development of important sections of veterinary science, including vitaminology and microelementology, as the main areas in the study of etiopathogenesis, as well as structural and functional changes in the organs of alimentary-deficient nutrition. This also applies to veterinary reproductive medicine, in particular, perinatology, mammology, gynecology, and andrology (Beleckaja and Onul, 2014; Skljarov and Koshevoj, 2016).

RESULTS AND DISCUSSION

Of the wide range of genetic and paratypic factors of negative impacts on reproductive capacity, the most common one is alimentary, which causes reproductive dysfunction due to deficiencies in norms, rules, and feeding regimes (Bindari et

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al., 2013; Amin, 2014; Ibtisham et al., 2018). In particular, it can be associated with both general malnutrition (starvation) and overfeeding (obesity). However, the alimentary form of infertility commonly occurs based on low-quality diets, which means when the diet lacks vital components (mainly vitamins, macro- and micronutrients), or the quantitative ratio among them is disturbed (Mahan and Vallet, 1997; Hovdenak and Haram, 2012; Omur et al., 2016). This is possible even if the total nutritional value of the diet meets the established requirements for the physiological needs of the organism (Vizner, 1976; Perevozchikov et al., 2014).

The most sensitive animals to high-quality defective feeding are the heifers and highly productive animals, especially in the winter-spring period of stall keeping. The absence or lack of vitamins and minerals in the diet, even with satisfactory fattening of animals, can cause violation of sexual cyclicity, disorders of fertilization and implantation, various pathologies of pregnancy, labor, and the postpartum period (Pasynkova, 2013; Shatohin et al., 2017).

These disorders have a wide range, which can be temporary or permanent, and affect all aspects of the reproductive process, including morphological and functional state of the self-regulatory system (hypothalamus, pituitary, gonads, uterus), readiness and ability to develop an egg cell from maturing to physiologically determined follicles, ability and readiness for fertilization. Which is possible during regular sexual cycles without violating the internal and external signs of their manifestation; absence of disorders of implantation (nidation) of the embryo in the mucous membrane of the uterus; functioning of the fetoplacental complex, fetal development, pregnancy, childbirth; the birth of a full-fledged offspring, which itself, after directed growth, can reach sexual maturity promptly; the course of the postpartum period; and timing and fullness of the manifestation of sexual hunting after childbirth (Koshovyj, 2004; Skliarov et al., 2020a).

Vitamins

Vitamins are one of the most important factors influencing sexual function. Vitamins are substances of high biological activity that are involved in all vital processes (for example, metabolic processes, immunity, hormonal genesis and others) in the body. To date, more than 20 vitamins and vitamin-like substances have been studied, the deficiency or absence of which leads to disorders in the body. Although animals need small quantities of vitamins, their constant lack in the diet can lead to metabolic disorders, specific diseases, reduced productivity and product quality, as well as impaired reproducibility (Plemjashov, 2010; Matte and Lauridsen, 2013). Vitamins play both direct and indirect roles in the process of reproduction, especially endocrine and genital systems. Histochemistry has shown an increase in the content of some vitamins in many endocrine organs. From this, it can be concluded that the organs associated with the reproductive system need more of these vitamins (Vizner, 1976).

Not only the amount but also the ratio of vitamins entering the body is important for reproduction. The vitamin requirements depend on the composition of feed, animal productivity, and depot of vitamins in the body, moreover, vitamin requirements increase with pathological conditions and the use of drugs (antibiotics and sulfonamides) (Sidorkin et al., 2007; Savinova et al., 2015). Hypovitaminosis is more often reported in some animals due to vitamin deficiency in the diet or mineral imbalance in the feed affecting their metabolism and absorption (Umahanov, 2017).

Fat-soluble vitamins

Vitamin A (retinol) is the most important component for the functioning of the reproductive system, compared to other vitamins. Therefore, retinol is rightly called the vitamin of reproduction (Hurley and Doane, 1989; Koshovyj, 2004). In recent years, the causes of insufficient supply of Vitamin A in animals and prevention measurements have grown into a problem that is of paramount importance in herd reproduction. This issue became critical especially during the winter stall keeping of animals because the precursor of retinol is carotene, which is easily destroyed by environmental factors, such as temperature and humidity (Hurley and Doane, 1989; Blaner et al., 2016; Kuz'minova et al., 2017). Vitamin A is important in maintaining the body's resistance to adverse external and internal factors, ensuring the normal state of the epithelium of the genital mucosa, follicular epithelium of the ovary, and secretory activity of the uterine glands. Retinol is required for the formation of steroid and pituitary hormones, and also plays an important role in cell differentiation during embryogenesis, and in particular the formation of the fetal immune system (Skliarov et al., 2020b). Its deficiency causes disorders in spermatogenesis. In fact, sexual cycles are defective, implantation is disrupted, embryonic mortality occurs, pregnancy, childbirth, and the postpartum period are complicated, and the offspring is not very viable (Hogarth and Griswold, 2010; Gromova et al., 2019; Maia et al., 2019).

Vitamins of group D are active regulators of phosphorus calcium metabolism, providing their normal ratio in the blood of animals (Bourdeau et al., 1986). Vitamin D deficiency is usually observed during the winter when it is difficult to walk and isolate animals. Vitamin D becomes important for reproduction when the level of calcium and phosphorus supply deviates significantly from the optimal rate (Brommage et al., 1990).

Information addressing the specific effects of Vitamin D on reproductive function is limited (Hurley and Doane, 1989). It is known that the level of reproduction depends on the provision of the body with Vitamin D (Luk et al., 2012). As Gromova et al. (2017) mentioned, Vitamin D levels have a direct effect on the genitals, and an indirect effect on stimulation of the synthesis of steroid hormones (estrogen, progesterone, testosterone) in both males and females.

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Moreover, there are convincing data on the effect of Vitamin D on many gynecological diseases (Kalinchenko et al., 2016). Expressed D-hypovitaminosis in animals during the active growth period inhibits the development of the genitals (Vizner, 1976), and also affects the physiology of the ovary (Irani and Merhi, 2014).

In all these cases, the inclusion of Vitamin D in the diet or its use in doses that would meet the needs of the body can prevent possible disorders of the reproductive capacity of animals to some extent, including the duration of the placenta, the period from calving to first insemination, and fertilization index (Hurley and Doane, 1989; Umahanov, 2017).

Vitamin E is found in almost all tissues, but especially in the uterus, testicles, adrenal glands, and pituitary gland, it is much more than in other organs indicating the specificity of the functions of this vitamin in these organs (Vizner, 1976; Koshovyj, 2004). Vitamin E acts in the body as an antioxidant regulating the body's absorption of Vitamin A, and fat metabolism (Hurley and Doane, 1989). In addition, it affects the activity of the anterior pituitary gland and stimulates the production of gonadotropins (Vizner, 1976).

With E-hypovitaminosis in pregnant females, toxic products of fat metabolism are accumulated, which are harmful to the embryo. Accordingly, the incidence of embryonic mortality, stillbirths, and non-viable fetuses are increased (Vizner, 1976). In males, there are degenerative processes in sperm, epithelium of seminiferous tubules, reduced sexual activity, and also various pathologies of adnexal gonads can arise (Evans and Bishop, 1922; Gullickson et al., 1949; Hurley and Doane, 1989). In experiments on pigs, an increase in the size of the placenta and a decrease in the mortality of piglets with the use of Vitamin E have been described, but this positive result remained unclear (Pinelli-Saavedra, 2003).

Regarding Vitamin K, there are few reports of the effects of Vitamin K on the reproductive functions in animals (Potter, 1945; Metta and Johnson, 1960; Jacob et al., 2012). Thus, studies on rodents have reported that the concentration of Vitamin K in the ovaries was higher than other organs, indicating its role in female reproduction (Huber et al., 1999). Moore et al. (1942) described the cases of abortion in rabbits fed a diet deficient in Vitamin K. Vizner (1976), citing data from Italian authors, pointed to the positive effect of Vitamin K on the reproductive capacity, which is thought to be due to its stimulation of the production of anterior pituitary hormones. Vitamin K deficiency in breeding chickens can cause increased embryonic mortality. However, both of these provisions are controversial. New roles of Vitamin K have been studied, including the role of estrogen in its metabolism, which requires further research (Truong and Booth, 2011). Baldoceda-Baldeon et al. (2014) found a positive effect of Vitamin K on the embryonic development of cattle in vitro, particularly its addition contributed to the improvement of their morphological quality.

Water soluble vitamins

Vitamins of group B takes an active part as coenzymes in many enzymatic processes, its deficiency inhibits specific metabolic processes. Their deficiency reveals significant disorders of reproductive function, which is consistent with the well-known theory of the participation of enzymes in the process of reproduction (Vizner, 1976). All types of Vitamins of group B are essential for fetal growth and development, and their deficiency causes abortion (Lewis and Everson, 1952; Hurley and Doane, 1989).

Hertz (1946) reported the effect of Vitamin B on endocrinological aspects of reproduction. Although Vitamin B deficiency can affect the reproductive capacity in animals, it is less potent than other vitamins. As the lack of other vitamins, B-avitaminosis or hypovitaminosis B in practice may be relevant only for pigs and domestic fowl since they are synthesized in the forestomach of ruminants. The uncontrolled use of antibiotics can lead to Vitamin B deficiency in ruminants.

In severe cases, thiamine deficiency leads to impaired development of the embryonic epithelium and reduced fertility. In roosters, there is an inhibition of testicular development and degeneration, and in chickens, it leads to ovarian atrophy. In pigs, premature births, high infant mortality, and increased ugliness can be observed (Vizner, 1976).

Dysfunction of reproduction due to lack of riboflavin is described mainly in pigs and domestic fowl. In sows, there may be a decrease in fertility as well as infertility due to the resorption of embryos or fetal death and the birth of low-viability piglets. Skeletal anomalies in the fetus were also reported (shortening of various bones and fusion of ribs). In the hens, egg-laying was disturbed, incubation qualities of eggs was deteriorated especially in the second week of incubation (period of high embryonic activity), low-viability dwarf chickens hatched with widespread edema and characteristic down curl leading to a high mortality rate at an early age (Vizner, 1976).

The mere violation of the synthesis of sterols and steroids due to the lack of pantothenic acid indicated its connection with the processes of reproduction. Young pigs with pantothenic acid deficiency are useless due to underdevelopment of the genitals or atrophy and inactivity of the ovaries. Adult females pigs are normally fertilized, but the fetuses often die. Infertility occurs due to the resorption of embryos, and later the loss of lactation. Piglets are born dead, underdeveloped, or poorly viable. In poultry, a decrease in the content of pantothenic acid in eggs due to its lack in the diet is associated with a decrease in hatchability. Chickens are unable to hatch from their own eggs and remain in the shell. Egg-laying is also disturbed (Vizner, 1976).

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Sows that do not receive or receive little choline from feed, give birth to low-viability piglets, most of which have gonadal abnormalities and low live weight before weaning. Regarding breeding chickens and laying hens, reduced egg production, often the expulsion of some yolks and increased mortality of chickens were often observed (Vizner, 1976). Vitamin B_{12} deficiency is associated with adverse effects on mothers and newborns, including developmental abnormalities, miscarriages, preeclampsia, and low birth weight (Reznikoff-Etiévant et al., 2002; Finkelstein et al., 2015).

In the absence of cyanocobalamin, pigs and domestic fowl are particularly affected by reproductive disorders, in which their own synthesis of Vitamin B_{12} does not meet the requirements. At the same time, Vitamin B_{12} supplements can reduce embryonic mortality, and increase the vitality of newborn piglets. In domestic fowl with Vitamin B_{12} deficiency, egg production was disturbed with a decrease in the incubation qualities of eggs, and there was a report of increased embryonic and post-embryonic mortality as well as a decrease in the ability to hatch (Luck et al., 1995). Hatched chickens had partial disorders in bone development. Instead, ruminants with sufficient intake of cobalt in the feed do not need Vitamin B_{12} supplements due to their ability to synthesize it in the required amount (Vizner, 1976).

Vitamin C (ascorbic acid) is an active stimulant of metabolism, participates in blood clotting, stimulates the phagocytic activity of leukocytes, reticulocyte system, and the formation of antibodies, and increases the body's defenses against infection (Hurley and Doane, 1989). Ascorbic acid is found in all tissues of the body, but in the pituitary gland, adrenal glands, and ovaries, it is much more than in the blood. This suggests that Vitamin C is required for the functioning of these incretory glands associated with animal reproduction. Ascorbic acid has long been associated with fertility, but any consistent study of the mechanism of its action on the reproductive organs has not been conducted (Briggs, 1973; Millar, 1992).

Three main functions of ascorbic acid that may explain its reproductive effects are considered, namely its promotion of collagen synthesis, its role in hormone production, and its ability to protect cells from free radicals. It can be concluded that ascorbic acid is a key compound in the physiology of the gonads, which requires further research, and that a reassessment of its potential clinical significance in the treatment of various types of male and female infertility will be timely (Luck et al., 1995). It is believed low blood ascorbic acid can lead to some functional disorders in the genitals of cows, especially the ovaries. Although some researchers claim that Vitamin C supplements can increase the fertility of ruminants, there is no experimental evidence (Koshovyj, 2004). Other reports have indicated the effects of ascorbic acid on gonadotropic hormone activity (Di Cio and Schteingart, 1942) and male fertility (Dawson et al., 1990). With a normal supply of Vitamin C to the body, a positive effect on the activity of gonadotropic hormones and male fertility is expected. In the case of insufficiency, these processes reduce their activity.

Ascorbic acid is one of the vitamins of the so-called antioxidant complex, similar to Vitamins A and E. With such a combined vitamin deficiency, it disrupts not only sexual cycling but also the emerging dysbiotic processes in the biotopes of the reproductive tract, which are known to be predictors of inflammatory diseases of the internal genitals, and can threaten the loss of reproductive function (Golovanova and Strokova, 2011). In this regard, there are reports of beneficial effects of parenterally administered ascorbic acid in various forms of infertility in cattle. Thus, the introduction of this vitamin 1-2 times a week at a dosage of 1-2 g per 500 kg of live weight could increase the sexual activity of young bulls. In the pituitary gland, adrenal glands, as well as in the testicles and ovaries, Vitamin C is much more than in the circulating blood. The content of Vitamin C in semen clearly correlates with its quality. Its presence in the above endocrine glands leads to the conclusion that ascorbic acid is necessary for the normal functioning of the most important incretory organs associated with the reproductive system (Vizner, 1976).

Although all vitamins affect reproduction, Vitamins A, D, and E usually play a dominant role. Thus, the use of vitamin supplements in the dry and postpartum periods of cows had a positive effect on their reproductive function and viability of obtained calves. Administration of Vitamin A, trivitamin, or tetravit oil for dry cows in the second half of the winter stall period significantly reduced the number of postpartum diseases, shortened the period of placenta separation, increased the fertility from the first insemination, contributed to the reduction of early embryonic mortality, reduced the service periods and the periods between calving, and reduced the percentage of the diseases in newborn calves (Manalili and Bernardo, 1996; Weiss, 1998).

Research by Umahanov (2017) indicated the possibility of preventing early embryonic losses and improving the results of insemination of cows by injection of Vitamins A, D, and E in the first days after calving. Adding a complex of mentioned fat-soluble vitamins to the feed for 1.5-2 months before calving and one to two months after calving provided a reduction in the incidence of placental retention and the occurrence of postpartum genital diseases, accelerated the onset of hunting, increased the effectiveness of insemination of cows, reduced the period between calving, and sharply decreased the morbidity rate of calves.

Minerals

The influence of minerals (macro- and microelements) on metabolic processes can be judged from the consequences that arise from insufficient or excessive intake by animals. These effects are multifaceted and

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characterized by varying degrees of intensity (Christian, 2003). Among them, it is necessary to allocate the disturbance of reproductive ability of animals, reduced viability of newborns, dysfunction of organs and systems and the occurrence of nutritional diseases, reduced animal productivity, insufficiently nutrient diets, and increased feed costs for product formation (Bohstedt, 1942; Malysheva, 2007).

Animals are in need of minerals. In addition to sufficient intake, their certain ratio among themselves is also required. The value of individual inorganic substances for reproduction varies, but most of them are clearly defined. Usually the lack of not one, but several elements is registered (Vizner, 1976; Ramakrishnan et al., 1999; Christian, 2003).

About 70 chemical elements were found in the tissues and organs of animals. Minerals according to Koshovyj (2004), made 4-6% of the live weight of animals. Calcium and phosphorus are so closely associated with a metabolism, and have many common interactions that need to be considered in conjunction (Ruan et al., 2007). In addition, the symptoms of calcium deficiency are partially similar to the symptoms of phosphorus deficiency (Vizner, 1976). To date, there is no evidence that calcium and phosphorus imbalances have a significant effect on reproductive function, but at the same time, changes in the Ca/P ratio may affect ovarian function (Yasothai, 2014).

If the reserves of calcium and phosphorus are scarce or already depleted, the activity of the gonads is disrupted, as the breast has an advantage in the order to supply minerals. This causes violations of the sexual cycle and fertilization, due to which the period between calving is prolonged, and the animal remains temporarily infertile. The period of rest of sexual functions is maintained until the lack of minerals is compensated, accordingly, the conditions for the physiological course of sexual functions for fertilization and subsequent formation of the zygote in the uterus will not be created. Another situation is observed until the end of lactation. When milk production decreases and the body's mineral balance is restored by consuming foods with adequate calcium and phosphorus, the required amounts of these elements are used to, and thus, re-create the conditions for normal sexual activity (Vizner, 1976).

The decrease in reproductive capacity observed in phosphorus deficiency should be considered as a consequence of general metabolic disorders, especially lack of energy, as acquired by the neuroendocrine system, the syndrome of adaptation to poor feeding conditions. Thus, phosphorus deficiency significantly reduces the body's resistance to infectious diseases, fatness, feed intake, and the ability to transform carotene into Vitamin A, which can cause infertility (Vizner, 1976). Calcium and phosphorus deficiency or their impaired ratio leads to anaphrodisia or defective sexual cycles and other reproductive disorders (Hurley and Doane, 1989; Seyfi et al., 2005; Ruginosu et al., 2011). Their connection with Vitamin D is especially important for reproductive processes (Vizner, 1976; Sun et al., 2015).

Although none of the mineral elements has a strictly specific effect on the function of reproduction, phosphorus, based on its outstanding role in the intermediate metabolism, is still effective in the reproduction process. When comparing the mineral status of an animal with normal and reduced reproductive capacity, phosphorus deficiency is always found in the diets of the latter (Koshovyj, 2004). It has been reported that low phosphorus consumption caused a decrease in ovarian function, delayed puberty, and low fertility rates (Yasothai, 2014).

During sexual hunting and heat in the midbrain, pituitary gland, and ovaries, the organs that control sexual function and phosphate metabolism increase (Hurley et al., 1982; Tallam et al., 2005). Sperm also has a lot of phosphorus-containing proteins. The assumption of the existence of diseases associated with phosphorus deficiency is made. In particular, the connection between phosphorus deficiency and cattle disease, accompanied by ataxia, spastic paralysis, and severe reproductive disorders was noted by Vizner (1976).

Vizner (1976) summarized the results and generalizes the negative impact of phosphorus deficiency in cows on the manifestations of their reproductive capacity after a general analysis of literature reports. Infertility caused by phosphorus deficiency can be eliminated by providing the body with this element – with food or by introducing phosphorus-containing drugs (Koshovyj, 2004).

However, some other observations and research results have indicated that reducing the amount of feed by 30% leads to a sharp decline in reproductive capacity in the absence of a difference in the level of phosphorus (Vizner, 1976). Pregnant were those animals that later died from phosphorus deficiency or showed clinically pronounced bone changes. Prolonged keeping of animals on deficient diets for phosphorus has led to a marked inhibition of their development and growth, but the rhythm of sexual cycles has not changed. The study of the uterus and ovaries, conducted after the experiments, has not indicated an adverse effect of phosphorus deficiency on genital function. This indicates an indirect relationship between phosphorus levels and reproductive dysfunction.

Calcium is essential in mammalian breeding conditions (Baczyk et al., 2011; Correia et al., 2015; Kornbluth and Fissore, 2015). First, it promotes critical processes of individual development, such as skeletal formation and mineralization, lungs, kidneys, and neural circuits (Leclerc et al., 2011; Riccardi et al., 2013; Kovacs, 2015). Similar to phosphorus, this mineral is most often associated with reduced fertility in highly productive dairy cows. Calcium violation (deficiency) is usually common during childbirth or for several days after it. Negative calcium balance in the first third of lactation often occurs due to the use of its reserve during pregnancy. This can cause a sharp decrease in serum calcium, which will create conditions for postpartum paresis (Yasothai, 2014). Catt et al. (1985) found that the

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processes of activation of gonadotropin secretion receptors strongly depend on the level of calcium supply. The connection between the level of calcium metabolism and reproduction in poultry was revealed (Simkiss, 1961; Luck and Scanes, 1979). Vizner (1976) concluded that some forms of infertility in cows can be eliminated by introducing carbon dioxide into the diet of animals, and by injecting calcium gluconate.

The biological role of magnesium is multifaceted, as it is an essential element of many biochemical processes. Magnesium plays an important role as an enzyme activator, and usually has no direct effect on the reproductive status of animals (Linlin et al., 2013). However, its deficiency is associated with some gynecological pathologies and often leads to serious complications of pregnancy (Gromova et al., 2008). Sufficient provision of the mother's body with this important element creates the basis for full fetal development and the birth of healthy offspring. The need of a pregnant females' organism for magnesium often exceeds its supply, and this fact allows researchers to consider the pregnancy as a physiological model of hypomagnesemia. Therefore, magnesium deficiency during pregnancy can cause adverse effects on both mother and fetus, impaired embryo implantation, placental pathology, threatened abortion, premature birth, and weakness of labor were taken into consideration (Dadak, 2013; Rosner et al., 2016).

Jeong et al. (2018) found a positive relationship among serum magnesium concentration, peri and postpartum disorders, and reproductive performance in dairy cows. There are increasingly recent data on the regulatory role of magnesium on hormonal, namely on the reproductive function of the body (Strizhakov et al., 2009). Thus, Chandra et al. (2013) described the apparent beneficial effects of magnesium on the male gonadal system, including testicular histology, steroidogenesis, and spermatogenesis. Maggio et al. (2011) found a significant direct relationship between magnesium levels and serum testosterone levels. However, information about the mechanism of this dependence in the scientific literature is not enough. In the body, magnesium remains antagonistic to calcium, and any disturbances in the homeostasis of calcium-phosphorus-magnesium may have some effect on the reproduction (Yasothai, 2014).

Potassium plays an important role in the excitation of nerve and muscle tissue and is involved in carbohydrate metabolism. It maintains normal osmotic pressure and is of great importance in the processes of water metabolism. In addition, potassium specifically affects the activity of many enzymes (Koshovyj, 2004). Potassium iodide has a positive effect on the functional activity of the reproductive system. Potassium iodide preparations have a protective effect for the prevention of pathologies of pregnancy and the treatment of breast diseases (Pan'kiv, 2016).

Pavlova et al. (2014) established the undoubted positive effect of potassium humate on the reproductive system and determined that even exposure to it generally leads to positive biological consequences. This was manifested in males in an increase in the mass ratio of testes; reduction of pathological forms of sperm, and in females - in the reduction of atretic ovarian bodies, and an increase in the number of mature follicles. Karow et al. (1992) noted the positive effect of adding K^+ to media for sperm survival. Brainard et al. (2007) indicated the role of potassium ion channels in the regulation of myometrial tone during pregnancy. In particular, during gestation, potassium channels support the uterus at rest, promoting membrane resting potential and counteracting contractile stimuli.

Concerning the effect of potassium on reproductive function, Vizner (1976) reported that during heat or after estrogen administration to spayed animals, the potassium content in the endometrium increases, and the sodium concentration decreases. The introduction of progesterone causes the opposite effect meaning that a decrease in potassium levels and an increase in sodium content were noted. Although no direct effect has been identified, it should be assumed that these sex-related changes affect animal reproduction.

Sodium is needed to build new body tissues and plays an important role in water metabolism. An important function of sodium in the body is to maintain normal osmotic pressure in body fluids. This element is the main cation that determines the amount of reserve alkalinity of blood plasma and acid-base state of the body (Koshovyj, 2004).

Sodium deficiency can cause placental retention in cows and sexual rhythm disorders (Vizner, 1976). Beljaev et al. (2007) showed that the use of sodium selenite helps to improve the reproductive functions of sows. The results of the research by Posohova (2004) indicated a beneficial effect of sodium humate on the condition of animals, particularly in pregnant individuals, which is manifested in the improvement of their reproductive function, the development of more complete offspring.

The sodium humate has a positive effect on the reproductive function of cows in the postpartum period, in particular, its use can reduce the level of prenatal and postpartum complications, facilitate the course of labor, and increase the safety of offspring (Ushkalov, 2001; Bezuglova and Zinchenko, 2016). An important role in metabolism belongs to micronutrients, the deficiency of which differently affects the function of individual organs, including the genital organs (Smith and Akinbamijo, 2000; Ballantine et al., 2002; Ojha, et al., 2018). In biogeochemical provinces where soil, water, and feed are poor in iodine, manganese, copper, zinc, cobalt, and selenium, animal infertility is often observed (Corah and Ives, 1991; Ermakov et al., 2012).

Iodine is actively involved in the functions of all female genitals. The process of reproduction is significant metabolic stress in the genital organs, which can lead to acute iodine deficiency (Abdul-Fadle and Ayoub, 1970; Koshovyj, 2004). Iodine affects the synthesis of thyroid hormones, the activity of which during estrus is higher than in other phenomena of the sexual cycle (Yasothai, 2014). Therefore, signs of iodine deficiency are delayed puberty,

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suppressed or irregular estrus (Puls, 1994). Iodine is also important for fetal development (Yasothai, 2014). Infertility, abortion, placental delay, stillbirth, or low viability have been reported in animals with iodine deficiency (Keen and Hurley, 1989). However, by adding iodine to the diet, these pathological processes can be eliminated relatively quickly (Vizner, 1976; Koshovyj, 2004).

Cobalt is a trace element that the body needs in such small quantities, but its functional impact is significant. The physiological role of cobalt is diverse, and infertility due to hypocobaltosis is likely to occur as a secondary consequence of impaired overall metabolism (Yasothai, 2014). Hypocobaltosis disrupts the synthesis of nucleic acids, muscle proteins, the activity of hydrolytic enzymes which causes delayed puberty, reduced fertility, abortion, hypofunction and subinvolution of the uterus, retention of placenta, and the birth of animals with reduced vital activity (Puls, 1988; Hurley and Doane, 1989).

Copper is also considered an important mineral for reproduction (Yasothai, 2014). It is involved in several enzyme structures, and is an integral component of redox processes and carbohydrate metabolism, the level of which affects the reproductive function and activity of sex hormones (Hurley and Doane, 1989). The activity of some endocrine glands is associated with the presence of copper, in particular, the formation of hormones in the pituitary gland that stimulate the function of the gonads (Koshovyj, 2004). Copper deficiency is manifested mainly in reproductive disorders of females, particularly in the embryonic and neonatal periods of development. Reviewing the works of numerous authors has indicated that copper deficiency in cows has a negative effect on reproductive function, such as defective sexual cycles, arrhythmia, reduced fertility, embryo resorption, delayed placenta, the birth of calves with low vital activity (Vizner, 1976; O'Dell, 1990). Furthermore, the addition of copper improves sperm quality (Puls, 1988; Yasothai, 2014).

Manganese is a part of many enzymes promoting the action of various vitamins. Manganese is necessary for the development and functioning of the reproductive system of animals since it accumulates in large quantities in the adrenal glands, ovaries, and uterus, especially during pregnancy (Vizner, 1976). Manganese deficiency inhibits the puberty of animals, the growth, development, and maturation of follicles in females, as well as irregular sexual cycles or prolonged periods of anestrus and abortion (Brown and Casillas, 1986; Corah, 1996; Yasothai, 2014).

Manganese deficiency was observed mainly in ruminants, birds, and less often in pigs. In ruminants, they are characterized by low fertility, abortion, and other disorders of reproductive function. With manganese deficiency in the body, there is a slowdown in sexual development in females, and the birth of calves with various abnormalities (Koshovyj, 2004). Manganese is important in the synthesis of cholesterol (Keen and Zidenberg-Cheer, 1990), which in turn is required for the synthesis of steroids such as progesterone, estrogen, and testosterone. Decreased concentrations of these steroids in the circulation due to manganese deficiency can lead to related reproductive pathology (Yasothai, 2014). Summarizing the above, it can be maintained that manganese plays a significant role in the body and that the deficiency of this element in the diet leads to significant violations of all parameters of reproduction (Plumlee et al., 1956; Hurley and Doane, 1989). Molybdenum belongs only to the micronutrients that are conditionally necessary for animals (Wirth and Mijal, 2010). Zhai et al. (2013) found a positive effect of molybdenum on human sperm quality, Zhang et al. (2013) reported the good quality of oocytes, and Bi et al. (2013) revealed findings about the impact of molybdenum on the development of preimplantation embryos. Reproductive disorders due to molybdenum deficiency are described, in males as decreased libido, impaired spermatogenesis, and sterility, and there were reports of delayed puberty, reduced fertility, and anestrus in females (Yasothai, 2014). However, molybdenum deficiency is a rare phenomenon, and its excess with toxic effects is more often registered (Corah and Ives, 1991).

Selenium is involved in aerobic oxidation by regulating the rate of redox reactions and is considered an essential biologically active microelement. It is effective in treating many diseases and infertility in animals (Mehdi and Dufrasne, 2016). In addition, it regulates the absorption and consumption of Vitamins A, C, K, and, especially Vitamin E, in the body, enhancing their effect. In terms of its effect on the body, this microelement is similar in its effect on Vitamin E. It is believed that the mechanism of action of Vitamin E and selenium on the fertility and reproductive health of animals is based on the detection of antioxidant properties of these compounds. By protecting cells from reactive oxygen species, Vitamin E and selenium prevent damage to sperm, ovicells, and embryos, and are involved in supporting the immune function of animals (Hurley and Doane, 1989; Gabryszuk and Klewiec, 2002; Albanes et al., 2014).

The safety margin (difference between a normal requirement and toxic dose) for selenium is so narrow that its deficiency in farm animals is quite rare than its toxicity, but causes weak, latent, or irregular estrus, early embryonic mortality, birth of weak offspring, and abortions in females, inhibition of spermatogenesis and deterioration of sperm quality in males (Hurley and Doane, 1989; Yasothai, 2014; Ojha et al., 2018). Selenium deficiency in diets leads to resorption of embryos, delayed placenta, infertility, and mastitis. There is evidence indicating that selenium deficiency increases the frequency of abortions (Hedstrom et al., 1986). The use of selenium drugs reliably prevents these pathological processes (Giadinis et al., 2016; Khalili et al., 2020).

Among the essential microelements, a special place is occupied by zinc, which is present in all cells of the body, and participates in various metabolic processes in the active centers of more than 200 enzymes (Prasad, 2003; Beleckaja and Onul, 2014). Zinc is necessary for the body for the respiratory process, activating the function of many enzymes,

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affecting the metabolism and activity of sex and gonadotropic hormones of the pituitary gland, and playing an important role in optimizing fertilization and reproduction of animals (Koshovyj, 2004). Zinc is known to be important in puberty (development of secondary sexual characteristics), reproductive capacity in males (gonadal cell development and sperm quality), and all reproductive events (heat, pregnancy, and lactation) in females (Maas, 1987). In addition, zinc plays a crucial role in the restoration and maintenance of the uterine mucosa after childbirth and the early period of return to normal reproductive function and estrus (Greene et al., 1998; Yasothai, 2014). A large number of studies have described the effect of zinc deficiency in males, in particular, its importance for normal spermatogenesis and steroidogenesis (Keen and Hurley, 1989). Zinc is also involved in the gestational health of the newborn and mother, and its deficiency is a factor in the increased risk of placental abruption, premature rupture of membranes, premature birth, and weakness of labor (Jameson, 1993; Scholl et al., 1993; Christian, 2003). In addition, its insufficiency leads to suppression of gonadal function and estrogenic activity. This condition in animals causes a decrease in reproductive activity although it has been reported infrequently (Jameson, 1976; Keen and Hurley, 1989).

The value of cadmium for reproduction should be sought primarily in its antagonistic action on zinc. An even stronger antagonism is attributed to cadmium relative to copper, a deficiency of which is noted for cadmium overdose (Vishnjakov and Lebedev, 2011; Zhegalova et al., 2018). However, as theory and practice show, cadmium toxicosis of alimentary origin in animals is unlikely (Vizner, 1976). In addition, there is a piece of evidence to negate the negative effects of cadmium on reproductive function (Lafuente et al., 2002). Moreover, the results of research by Thomas (1993) suggested that cadmium can have a direct effect on the level of the pituitary gland by altering gonadotropins. The data of Lafuente et al. (2003) declared that cadmium differently affects the secretory mechanisms of the studied pituitary hormones depending on the dose. Keck et al. (1995) found no significant correlation between sperm cadmium concentration and normal sperm parameters or between cadmium concentration and fertility status.

Iron deficiency anemia is a well-known risk factor for pregnancy complications, such as intrauterine growth retardation and fetal development, preterm birth, low birth weight, postpartum hemorrhage, maternal and infant mortality (Baker, 2000; Georgieff, 2020). In the first months of life, the main source of iron for a neonatal animal is the iron transferred via the utero of the mother to the fetus, so a pregnant female must receive a sufficient amount of this microelement (Cetin et al., 2010; Berger et al., 2011; Caserta et al., 2013). Data from Murray-Kolb and Beard (2009) suggested that iron deficiency in the mother adversely affects the mother-child interaction, and iron supplementation protects them against these adverse effects.

Saliy et al. (2013) noted the importance of iron deficiency in the formation of reproductive disorders in hypothalamic dysfunction of puberty, the extreme expression of which is the hypothalamic syndrome of puberty, leading to infertility and miscarriage in the reproductive age. Saliy et al. (2013) noted that this nature of the changes may have clinical and diagnostic value in assessing the severity of the reproductive dysfunction in this pathology. Siemons and Mahler (1987) reported the role of iron deficiency in the development of hypogonadotropic hypogonadism, manifested by a lack of libido and aspermia. The use of iron supplements helped to restore reproductive function.

Chromium is one of the "young" microelements, as its functions in the animal body are still not fully disclosed, but the undeniable importance of this element for health has been proved (Kisljakova and Lomaeva, 2017). Chromium is a vital microelement that is a constant component of cells of all organs and tissues, one of the functions of which is to participate in the regulation of fat synthesis and carbohydrate metabolism. Increased costs are observed during pregnancy, and one of the main manifestations of its deficit is a violation of the reproductive function in males (Reutina, 2009). In particular, Anderson and Polansky (1981) reported that male chromium-deficient rats had reduced sperm and sperm cell counts and reduced fertility.

Chromium has a significant effect on follicle maturation and the release of luteinizing hormone (Sheela and Ajay, 2004). In addition, chromium enhances the effects of insulin by increasing the absorption of glucose and amino acids through the body's cells, and thereby, improving energy balance, which leads to improved reproductive function (Stoecker, 1990). It is confirmed that the addition of chromium propionate to the basic diet of cows can improve the reproductive functions of animals, in particular, to reduce the percentage of abnormal births, cases of the delayed placenta, and the duration of the service period (Kisljakova and Lomaeva, 2017).

The data obtained by Serjakov and Judina (2014) indicated that the introduction of sows into the diet could increase fertility, litter weight at birth, average live weight of piglets at birth, milk yield, weight of piglets, and litter during weaning and storage of piglets during suckling.

CONCLUSION

Thus, vitamins, micro-, and macroelements are alimentary-deficient factors of disturbance of reproductive function of animals, the influence of which is observed in all reproduction processes, from fertilization to the postpartum period and the preservation of young animals. The pathogenesis of their insufficiency is associated with a violation of steroidogenesis, gametogenesis, and embryogenesis and the emergence of ante, intra, neo, and postnatal pathologies.

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Therefore, treatment and prevention measures should be aimed at providing animals with biologically complete balanced feeding and replenishment of the body with vitamins and minerals. However, all these issues remained incompletely studied and further research of their impact on the manifestation of animal reproductive functions are needed.

DECLARATIONS

Authors' contributions

P. Skliarov designed the main idea and wrote the review. S. Fedorenko, S. Naumenko, O. Onyshchenko, A. Pasternak, L. Roman, M. Lieshchova, D. Bilyi, and O. Bobrytska equally involved in the searching of literature review, writing up the paper, and critically analyzing the core idea of the paper, and reviewing the manuscript. All authors approved the final submitted version and the data analysis.

Consent to publish

The authors approved and agreed to publish the manuscript.

Competing interests

The authors have declared no conflict of interest. Ethical issues (including plagiarism, double publication and/or submission, and redundancy) have been checked by the authors.

REFERENCES

Abdul-Fadle W, and Ayoub LA (1970). Iodine metabolism in relation to reproductive status in cows. Zentralblatt fur Veterinarmedizin, 17(1): 62-67. DOI: https://www.doi.org/10.1111/j.1439-0442.1970.tb00775.x

Albanes D, Till C, Klein EA, Goodman PJ, Mondul AM, Weinstein S, Taylor PR, Parnes HL, Gaziano JM, Song X et al. (2014). Plasma tocopherols and risk of prostate cancer in the selenium and Vitamin E cancer prevention trial (SELECT). Cancer Prevention Research, 7(9): 886-895. DOI: https://www.doi.org/10.1158/19406207.CAPR-14-0058

Amin Rooh Ul (2014). Nutrition: Its role in reproductive functioning of cattle – a review. Veterinary Clinical Science, 2(1): 1-9. Available at: http://jakraya.com/journal/pdf/2-vcsArticle_1.pdf

Anderson RA, and Polansky MM (1981). Dietary chromium deficiency effect on sperm count and fertility in rats. Biological Trace Element Research, 3(1): 1-5. DOI: https://www.doi.org/10.1007/bf02789119

Baczyk D, Kingdom JC, and Uhlén P (2011). Calcium signaling in placenta. Cell Calcium, 49: 350-356. DOI: https://www.doi.org/10.1016/j.ceca.2010.12.003

Baker WF Jr (2000). Iron deficiency in pregnancy, obstetrics, and gynecology. Hematology/Oncology Clinics of North America, 14(5): 1061-1077. DOI: https://www.doi.org/10.1016/s0889-8588(05)70171-4

Baldoceda-Baldeon LM, Gagné D, Vigneault C, Blondin P, and Robert C (2014). Improvement of bovine in vitro embryo production by Vitamin K (2) supplementation. Reproduction, 148(5): 489-497. DOI: https://www.doi.org/10.1530/rep-14-0324

Ballantine HT, Socha MT, Tomlinson DAD, Johnson AB, Fielding AS, Shearer JK, and Van Amstel SR (2002). Effects of feeding complexed zinc, manganese, copper, and cobalt to late gestation and lactating dairy cows on claw integrity, reproduction, and lactation performance. The Professional Animal Scientist, 18(3): 211-218. DOI: https://www.doi.org/10.15232/S1080-7446(15)31524-2

Beleckaja JN, and Onul NM (2014). The effect of zinc on the reproductive function of experimental animals. Mikrojelementy v Medicine, 15(2): 22-28. Available at: https://elibrary.ru/item.asp?id=21692639

Beljaev VI, Shabunin SV, and Balym JP (2007). The influence of selenium preparations on the productivity and reproductive functions of sows. Veterinarnyj Vrach, 2: 38-40. Available at: https://elibrary.ru/item.asp?id=11732988

Berger J, Wieringa FT, Lacroux A, and Dijkhuizen MA (2011). Strategies to prevent iron deficiency and improve reproductive health. Nutrition Reviews, 69(1): 78-86. DOI: https://www.doi.org/10.1111/j.1753-4887.2011.00436.x

Bezuglova OS, and Zinchenko VE (2016). The use of humic preparations in animal husbandry (review). Dostizhenija Nauki i Tehniki APK, 30(2): 89-93. Available at: https://cyberleninka.ru/article/n/primenenie-guminovyh-preparatov-v-zhivotnovodstve-obzor/viewer

Bindari YR, Shrestha S, Shrestha N, and Gaire TN (2013). Effects of nutrition on reproduction – A review. Advances in Applied Science Research, 4(1): 421-429. Available at: https://www.imedpub.com/articles/effects-of-nutrition-on-reproduction-a-review.pdf

Blaner WS, Li Y, Brun PJ, Yuen JJ, Lee SA, and Clugston RD (2016). Vitamin A absorption, storage and mobilization. The Biochemistry of Retinoid Signaling. Springer, Dordrecht, pp. 95-125. DOI: https://www.doi.org/10.1007/978-94-024-0945-1_4

Bohstedt G (1942). Minerals in dairy cattle nutrition: A review. Journal of Dairy Science, 25(5): 441-458. DOI: https://www.doi.org/10.3168/jds.s0022-0302(42)95312-8

Bourdeau JE, Schwer-Dymerski DA, Stern PH, and Langman CB (1986). Calcium and phosphorus metabolism in chronically vitamin D-deficient laboratory rabbits. Mineral and Electrolyte Metabolism, 12(3): 176-185. Available at: https://pubmed.ncbi.nlm.nih.gov/3755216/

Brainard AM, Korovkina VP, and England SK (2007). Potassium channels and uterine function. Seminars in Cell and Developmental Biology, pp. 332-339. DOI: https://www.doi.org/10.1016/j.semcdb.2007.05.008.

Briggs MH (1973). Vitamin C and infertility. Lancet, 2: 677-678. DOI: https://www.doi.org/10.1016/s0140-6736(73)92513-0

Brommage R, Baxter DC, and Gierke LW (1990). Vitamin D-independent intestinal calcium and phosphorus absorption during reproduction.American Journal of Physiology-Gastrointestinal and Liver Physiology, 259(4): 631-638. DOI: https://www.doi.org/10.1152/ajpgi.1990.259.4.g631

Brown MA, and Casillas ER (1986). Manganese and manganese-ATP interactions with bovine sperm adenylate cyclase. Archives of Biochemistry and Biophysics, 244(2): 719-726. DOI: https://www.doi.org/10.1016/0003-9861(86)90641-7

Caserta D, Graziano A, Monte GL, Bordi G, and Moscarini M (2013). Heavy metals and placental fetal–maternal barrier: a mini–review on the major concerns. European Review for Medical and Pharmacological Sciences, 17(16): 2198-2206. Available at: https://pubmed.ncbi.nlm.nih.gov/23893187/

Catt KJ, Loumaye E, Wynn PC, Iwashita M, Hirota K, Morgan RO, and Chang JP (1985). GnRH receptors and actions in the control of reproductive function. Journal of Steroid Biochemistry, 23(5): 677-689. DOI: https://www.doi.org/10.1016/s0022-4731(85)80003-0

Cetin I, Berti C, and Calabrese S (2010). Role of micronutrients in the periconceptional period. Human Reproduction Update, 16(1): 80-95. DOI: https://www.doi.org/10.1093/humupd/dmp025

Chandra AK, Sengupta P, Goswami H, and Sarkar M (2013). Effects of dietary magnesium on testicular histology, steroidogenesis, spermatogenesis and oxidative stress markers in adult rats. Indian Journal of Experimental Biology, 51: 37-47. Available at: https://pubmed.ncbi.nlm.nih.gov/23441478/

Christian P (2003). Micronutrients and reproductive health issues: An international perspective. The Journal of Nutrition, 133(6): 1969-1973. DOI: https://www.doi.org/10.1093/jn/133.6.1969s

Corah L (1996). Trace mineral requirements of grazing cattle. Animal Feed Science and Technology, 59: 61-70. DOI: https://www.doi.org/10.1016/0377-8401(95)00887-x

Corah LR, and Ives S (1991). The effects of essential trace minerals on reproduction in beef cattle. Veterinary Clinics of North America: Food Animal Practice, 7(1): 41-57. DOI: https://www.doi.org/10.1016/S0749-0720(15)30809-4

Correia J, Michelangeli F, and Publicover S (2015). Regulation and roles of Ca^{2+} stores in human sperm. Reproduction, 150: 65-76. DOI: https://www.doi.org/10.1530/REP-15-0102

Dadak K (2013). Magnesium deficiency in obstetrics and gynecology. Akusherstvo, ginekologija i reprodukcija, 7(2): 6-14. Available at: https://www.gynecology.su/jour/article/view/13?locale=en_US

Dawson EB, Harris WA, and Powell LC (1990). Relationship between ascorbic acid and male fertility. World Review of Nutrition and Dietetics, 62: 1-26. DOI: https://www.doi.org/10.1159/000417532

Di Cio AV, and Schteingart M (1942). The influence of ascorbic acid on the activity of gonadotrophic hormones. Endocrinology, 30: 263-264. DOI: https://www.doi.org/10.1210/endo-30-2-263

Ermakov VV, Karpova EA, Korzh VD, and Ostroumov SA (2012). Innovative aspects of biogeochemistry. Moskva, p. 340. Available at: https://5bio5.blogspot.com/2019/08/23-1.html

Evans HM, and Bishop KS (1922). On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science, 56: 650-651. DOI: https://www.doi.org/10.1126/science.56.1458.650

Finkelstein JL, Layden AJ, and Stover PJ (2015). Vitamin B-12 and perinatal health. Advances in Nutrition, 6(5): 552-563. DOI: https://www.doi.org/10.3945/an.115.008201

Gabryszuk M, and Klewiec J (2002). Effect of injecting 2- and 3-year-old ewes with selenium and selenium-vitamin E on reproduction and rearing of lambs. Small Ruminant Research, 43(2): 127-132. DOI: https://www.doi.org/10.1016/S0921-4488(02)00005-6

Georgieff MK (2020). Iron deficiency in pregnancy. American Journal of Obstetrics and Gynecology, 223(4): 516-523. DOI: https://www.doi.org/10.1016/j.ajog.2020.03.006

Giadinis ND, Loukopoulos P, Petridou EJ, Panousis N, Konstantoudaki K, Filioussis G, Tsousis G, Brozos C, Koutsoumpas AT, Chaintoutis SC et al. (2016). Abortions in three beef cattle herds attributed to selenium deficiency. Pakistan Veterinary Journal, 36: 145-148. Available at: https://researchoutput.csu.edu.au/en/publications/abortions-in-three-beef-cattle-herds-attributed-to-selenium-defic

Golovanova VA, and Strokova OA (2011). The functional state of the reproductive system of young women in conditions of a deficiency of antioxidant complex vitamins. Vestnik Orenburgskogo gosudarstvennogo universiteta, 16(135): 261-263. Available at: https://cyberleninka.ru/article/n/funktsionalnoe-sostoyanie-reproduktivnoy-sistemy-molodyh-zhenschin-v-usloviyah-defitsita-vitaminov-antioksidantnogo-kompleksa

Greene LW, Johnson AB, Paterson J, and Ansotegui R (1998). Role of trace minerals in cow–calf cycle examined. Feedstuffs, 70(27): 12-17. Available at: https://agris.fao.org/agris-search/search.do?recordID=US1997078392

Gromova OA, Serov VN, and Torshin IJ (2008). Magnesium in obstetrics and gynecology: A history of application and modern views. Trudnyj Pacient, 6(8): 10-15. Available at: https://cyberleninka.ru/article/n/magniy-v-akusherstve-i-ginekologii-istoriya-primeneniya-i-sovremennye-vzglyady/viewer

Gromova OA, Torshin IJ, Tetruashvili NK, Kodencova VM, and Rudakov KV (2019). Vitamin A in obstetrics: basic and clinical research. Medicinskij Alfavit, 1(1): 59-69. DOI: https://www.doi.org/10.33667/2078-5631-2019-1-1(376)-59-69

Gromova OA, Torshin IY, Grishina TR, and Malyavskaya SI (2017). On the role of vitamin D in the prevention and treatment of male infertility. Kachestvennaya Klinicheskaya Praktika, 3: 61-71. https://cyberleninka.ru/article/n/o-rolyah-vitamina-d-v-profilaktike-i-terapii-muzhskogo-besplodiya

Gullickson TW, Palmer LS, Boyd WL, Nelson JW, Olson P, Calverley CE, and Boyer PD (1949). Vitamin E in the nutrition of cattle. 1. Effect of feeding Vitamin E poor rations on reproduction, health, milk production, and growth. Journal of Dairy Science, 32: 495-508. DOI: https://www.doi.org/10.3168/jds.S0022-0302(49)92071-8

Hedstrom OR, Maas JP, Hultgren BD, Lassen ED, Wallner-Pendleton EA, and Snyder SP (1986). Selenium deficiency in bovine, equine, and ovine with emphasis on its association with chronic diseases. In: 29 Annual Proceedings American Association of Veterinary Laboratory Diagnosticians (USA), pp. 101-121. Available at: https://agris.fao.org/agris-search/search.do?recordID=US8735979

Hertz R (1946). Effect of B vitamins on the endocrinological aspects of reproduction. In: Vitamins and Hormones. Academic Press, pp. 135-146. DOI: https://www.doi.org/10.1016/S0083-6729(08)61081-X

Hogarth CA, and Griswold MD (2010). The key role of vitamin A in spermatogenesis. The Journal of Clinical Investigation, 120(4): 956-962. DOI: https://www.doi.org/10.1172/jci41303

Hovdenak N, and Haram K (2012). Influence of mineral and vitamin supplements on pregnancy outcome. European Journal of Obstetrics and Gynecology and Reproductive Biology, 164(2): 127-132. DOI: https://www.doi.org/10.1016/j.ejogrb.2012.06.020

Huber AM, Davidson KW, O'Brien-Morse ME, and Sadowski JA (1999). Tissue phylloquinone and menaquinones in rats are affected by age and gender. The Journal of Nutrition, 129(5): 1039-1044. DOI: https://www.doi.org/10.1093/jn/129.5.1039

Hurley WL, and Doane RM (1989). Recent developments in the roles of Vitamins and minerals in reproduction. Journal of Dairy Science, 72(3): 784-804. DOI: https://www.doi.org/10.3168/jds.s0022-0302(89)79170-0

Hurley WL, Edgerton LA, Olds D, and Hemken RW (1982). Estrous behavior and endocrine status of dairy heifers with varied intakes of phosphorus. Journal of Dairy Science, 65(10): 1979-1986. DOI: https://www.doi.org/10.3168/jds.s0022-0302(82)82447-8

Ibtisham F, Nawab AAMIR, Li G, Xiao M, An L, and Nasee G (2018). Effect of nutrition on reproductive efficiency of dairy animals. Medycyna Weterynaryjna, 74(6): 356-361. DOI: https://www.doi.org/10.21521/mw.6025

Iolchiev BS, Kononov VP, Tadzhieva AV, Bagirov VA, D'jakevich ON, and Mambetaliev MA (2014). Factors affecting the level of reproduction of cows in modern dairy cattle breeding. Agrarnaja Rossija, (12): 18-21. DOI: https://www.doi.org/10.30906/1999-5636-2014-12-18-21

Irani M, and Merhi Z (2014). Role of Vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertility and Sterility, 102(2): 460-468. DOI: https://www.doi.org/10.1016/j.fertnstert.2014.04.046

Jacob J, Endepols S, Pelz HJ, Kampling E, Cooper TG, Yeung CH, Redmann K, and Schlatt S (2012). Vitamin K requirement and reproduction in bromadiolone-resistant Norway rats. Pest Management Science, 68(3): 378-385. DOI: https://www.doi.org/10.1002/ps.2273

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Jameson S (1976). Effects of zinc deficiency in human reproduction. Acta Medica Scandinavica, 593: 1-89. Available at: https://pubmed.ncbi.nlm.nih.gov/1067745/

Jameson S (1993). Zinc status in pregnancy: the effect of zinc therapy on perinatal mortality, prematurity, and placental ablation. Annals of the New York Academy of Sciences, 678(1): 178-192. DOI: https://www.doi.org/10.1111/j.1749-6632.1993.tb26121.x

Jeong JK, Choi IS, Moon SH, Kang HG, and Kim IH (2018). Relationship between serum magnesium concentration during the transition period, peri- and postpartum disorders, and reproductive performance in dairy cows. Livestock Science, 213: 1-6. DOI: https://www.doi.org/10.1016/j.livsci.2018.04.013

Kagermazov CB, and Taov IH (2018). The influence of external environmental conditions on the reproductive function of cows in the conditions of the Kabardino-Balkarian Republic. Agrarnaja Rossija, 10: 37-40. DOI: https://www.doi.org/10.30906/1999-5636-2018-10-37-40

Kalinchenko SJ, Zhilenko MI, Gusakova DA, Tjuzikov R, Mshalaja GZ, Sablin KS, and Dymova AV (2016). Vitamin D and Women's Reproductive Health. Problemy Reprodukcii, 22(4): 28-36. DOI: https://www.doi.org/10.17116/repro201622428-36

Karow AM, Gilbert WB, and Black JB (1992). Effects of temperature, potassium concentration, and sugar on human spermatozoa motility: A cell preservation model from reproductive medicine. Cryobiology, 29(2): 250-254. DOI: https://www.doi.org/10.1016/0011-2240(92)90023-U

Keck C, Bramkamp G, Behre HM, Müller C, Jockenhövel F, and Nieschlag E (1995). Lack of correlation between cadmium in seminal plasma and fertility status of nonexposed individuals and two cadmium-exposed patients. Reproductive Toxicology, 9(1): 35-40. DOI: https://www.doi.org/10.1016/0890-6238(94)00053-Y

Keen CL and Hurley LS (1989). Zinc and reproduction: effects of deficiency on foetal and postnatal development. In: Zinc in human biology. Springer, London, pp. 183-220. DOI: https://www.doi.org/10.1007/978-1-4471-3879-2_12

Keen CL, and Zidenberg-Cheer S (1990). Manganese. In: Present knowledge in nutrition. ML Brown (Ed). International life Science Institute. Washington DC, Nutrition Foundation, pp. 279-288. Available at: https://ci.nii.ac.jp/naid/10011118264/

Khalili M, Chamani M, Amanlou H, Nikkhah A, Sadeghi AA, Dehkordi FK, Rafiei M, and Shirani V (2020). The effect of feeding inorganic and organic selenium sources on the hematological blood parameters, reproduction and health of dairy cows in the transition period. Acta Scientiarum. Animal Sciences, 42: e45371. DOI: https://www.doi.org/10.4025/actascianimsci.v42i1.45371

Kisljakova EM, and Lomaeva AA (2017). The effect of organic chromium additives on the productive and reproductive performance of black and white cows. Uchenye zapiski Kazanskoj gosudarstvennoj akademii veterinarnoj mediciny im. Baumana, 232(4): 76-80. Available at: https://cyberleninka.ru/article/n/vliyanie-dobavok-organicheskogo-hroma-na-produktivnye-i-reproduktivnye-pokazateli-korov-cherno-pestroy-porody/viewer

Kornbluth S, and Fissore R (2015). Vertebrate reproduction. Cold Spring Harbor Perspectives in Biology, 7: a006064. DOI: https://www.doi.org/10.1101/cshperspect.a006064

Koshovyj VP (2004). Obstetric–gynecologic pathology in cows. Harkiv, pp. 5-70. Available at: http://www.irbis-nbuv.gov.ua/cgi-bin/irbis_nbuv/cgiirbis_64.exe

Koshovyj VP (2008). Veterinary perinatology. Harkiv, pp. 9-459. Available at: https://dspace.dsau.dp.ua/jspui/handle/123456789/749

Kovacs CS (2015). Calcium, phosphorus, and bone metabolism in the fetus and newborn. Early Human Development, 91: 623-628. DOI: https://www.doi.org/10.1016/j.earlhumdev.2015.08.007

Kuz'minova EV, Semenenko MP, Koshhaev AG, and Troshin AN (2017). Biological functions of carotenoids in cattle reproduction. Politematicheskij Setevoj Jelektronnyj Nauchnyj Zhurnal Kubanskogo Gosudarstvennogo Agrarnogo Universiteta, 129: 1124-1136. DOI: https://www.doi.org/10.21515/1990-4665-129-080

Lafuente A, Cano P, and Esquifino AI (2003). Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose–dependent? Biometals, 16(2): 243-250. DOI: https://www.doi.org/10.1023/a:1020658128413

Lafuente A, González-Carracedo A, Márquez N, Pazo D, and Esquifino AI (2002). Oral cadmium exposure throughout puberty does not inhibit secretion of prolactin, GH and ACTH through dopamine metabolism changes in male rat. Journal of Trace Elements in Medicine and Biology, 16(4): 249-254. DOI: https://www.doi.org/10.1016/S0946-672X(02)80053-0

Leclerc C, Néant I, and Moreau M (2011). Early neural development in vertebrates is also a matter of calcium. Biochimie, 93: 2102-2111. DOI: https://www.doi.org/10.1016/j.biochi.2011.06.032

Lewis H, and Everson G (1952). Deposition of B vitamins in normally developing fetuses as evidence for increased vitamin needs of the rat for reproduction: II. pantothenic acid and biotin, two figures. The Journal of Nutrition, 46(1): 27-35. DOI: https://www.doi.org/10.1093/jn/46.1.27

Linlin G, Hailong J, and Guixin Q (2013). Effect and possible mechanism of magnesium on reproductive performance of sows. Feed Industry, p. 5. Available at: https://en.cnki.com.cn/Article_en/CJFDTotal-FEED201305006.htm

Luck MR, and Scanes CG (1979). The relationship between reproductive activity and blood calcium in the calcium-deficient hen. British Poultry science, 20(6): 559-564. DOI: https://www.doi.org/10.1080/00071667908416621

Luck MR, Jeyaseelan I, and Scholes RA (1995). Ascorbic acid and fertility. Biology of Reproduction, 52(2): 262-266. DOI https://www.doi.org/10.1095/biolreprod52.2.262

Luk J, Torrealday S, Neal Perry G, and Pal L (2012). Relevance of Vitamin D in reproduction. Human Reproduction, 27(10): 3015-3027. DOI: https://www.doi.org/10.1093/humrep/des248

Maas J (1987). Relationship between nutrition and reproduction in beef cattle. The Veterinary Clinics of North America. Food Animal Practice, 3(3): 633-646. DOI: https://www.doi.org/10.1016/s0749-0720(15)31135-x

Maggio M, Ceda GP, Lauretani F, Cattabiani C, Avantaggiato E, Morganti S, Ablondi F, Bandinelli S, Dominguez LJ, Barbagallo M et al. (2011). Magnesium and anabolic hormones in older men. International Journal of Andrology, 34: 594-600. DOI: https://www.doi.org/10.1111/j.1365-2605.2011.01193.x

Mahan DC, and Vallet JL (1997). Vitamin and mineral transfer during fetal development and the early postnatal period in pigs. Journal of Animal Science, 75(10): 2731-2738. Available at: https://www.doi.org/10.2527/1997.75102731x

Maia SB, Souza ASR, de Caminha MFC, da Silva SL, de Cruz R, Sá BLC, dos Santos CC, and Filho MB (2019). Vitamin A and Pregnancy. A Narrative Review. Nutrients, 11(3): Article number 681. DOI: https://www.doi.org/10.3390/nu11030681

Malysheva NV (2007). The influence of environmental and geochemical factors on the formation of reproductive function (PhD Thesis). GOU VPO «Orenburgskij gosudarstvennyj universitet», Moskva. Available at: https://www.dissercat.com/content/vliyanie-ekologo-geokhimicheskikh-faktorov-na-stanovlenie-reproduktivnoi-funktsii

Manalili SC, and Bernardo JC (1996). Reproductive performance of sows and breeding gilts induced with Apocrine S-oil with different levels of vitamin ADE supplementations. Araneta Research Journal, 34: 6-10. Available at: https://agris.fao.org/agris-search/search.do?recordID=PH1998101052

Matte JJ, and Lauridsen C (2013). Vitamins and vitamin utilization in swine. In: Sustainable Swine Nutrition. Wiley-Blackwell, pp. 139-172. DOI: https://www.doi.org/10.1002/9781118491454.ch6

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Mehdi Y, and Dufrasne I (2016). Selenium in cattle: A review. Molecules, 21(4): 1-14. DOI: https://www.doi.org/10.1186/1751-0147-46-229.10.3390/molecules21040545

Metta VC, and Johnson BC (1960). Effect of feeding vitamin K–deficient diets to female rats. The Journal of Nutrition, 72(4): 455-458. DOI: https://www.doi.org/10.1093/jn/72.4.455

Millar J (1992). Vitamin C – the primate fertility factor? Medical Hypotheses, 38: 292-295. DOI: https://www.doi.org/10.1016/0306-9877(92)90019-9

Moore RA, Bittenger I, Miller ML, and Hellman LM (1942). Abortion in rabbits fed a Vitamin K deficient diet. American Journal of Obstetrics and Gynecology, 43: 1007-1012. DOI: https://www.doi.org/10.1016/s0002-9378(42)91023-8

Murray-Kolb LE, and Beard JL (2009). Iron deficiency and child and maternal health. The American Journal of Clinical Nutrition, 89(3): 946-950. DOI: https://www.doi.org/10.3945/ajcn.2008.26692D

O'Dell L (1990). In: Present knowledge in nutrition. ML Brown, Ed, International life Sciences Institute Foundation. Washington DC, pp. 261-267. Available at: https://ci.nii.ac.jp/naid/10011118264/

Ojha L, Grewal S, Singh AK, Pal RP, and Mir SH (2018). Trace minerals and its role on reproductive performance of farm animals. Journal of Entomology and Zoology Studies, 6(4): 1406-1409. Available at: https://www.entomoljournal.com/archives/2018/vol6issue4/PartX/6-3-330-318.pdf

Omur A, Kirbas A, Aksu E, Kandemir F, Dorman E, Kaynar O, and Ucar O (2016). Effects of antioxidant vitamins (A, D, E) and trace elements (Cu, Mn, Se, Zn) on some metabolic and reproductive profiles in dairy cows during transition period. Polish Journal of Veterinary Sciences, 19(4): 697-706. DOI: https://www.doi.org/10.1515/pjvs-2016-0088

Pan'kiv VI (2016). The influence of thyroid disorders upon the female reproductive function. modern possibilities of phytotherapy. Reproductive Endocrinology, (27): 52-57. DOI: https://www.doi.org/10.18370/2309-4117.2016.27.52-57

Pasynkova TS (2013). The effect of feeding on the reproductive function of cows. Vestnik Izhevskoj Gosudarstvennoj Sel'skohozjajstvennoj Akademii, 2: 17-18. Available at: https://elibrary.ru/item.asp?id=21018838

Pavlova ON, Pinaeva ON, Leonov VV, and Garipov TV (2014). The effect of potassium humate on the reproductive system of rats. Vestnik medicinskogo instituta «Reaviz»: Reabilitacija, Vrach i Zdorov'e, 3(15): 50-53. Available at: https://cyberleninka.ru/article/n/vliyanie-gumata-kaliya-na-reproduktivnuyu-sistemu-krys/viewer

Perevozchikov AL, Batanov SD, Lopatina SA, and Mysik AT (2014). Intensification of the level of reproduction by rationed feeding of sows. Svynarstvo, (65): 244-249. Available at: http://www.irbis-nbuv.gov.ua/cgi-bin/irbis_nbuv/cgiirbis_64.exe?C21COM=2&I21DBN=UJRN&P21DBN=UJRN&IMAGE_FILE_DOWNLOAD=1&Image_file_name=PDF/svun_2014_65_45.pdf

Pinelli-Saavedra A (2003). Vitamin E in immunity and reproductive performance in pigs. Reproduction Nutrition Development, 43(5): 397-408. DOI: https://www.doi.org/10.1051/rnd:2003034

Plemjashov KV (2010). The value of vitamins for animal reproduction. Sankt-Peterburg, p. 68. Available at: https://elibrary.ru/item.asp?id=45840392

Plumlee MP, Thrasher DM, Beeson WM, Andrews FN, and Parker HE (1956). The effects of a manganese deficiency upon the growth, development, and reproduction of swine. Journal of Animal Science, 15(2): 352-367. DOI: https://www.doi.org/10.2527/jas1956.152352x

Posohova AV (2004). Experimental biomedical study of the nutritional use of sodium humate (Abstract of doctoral dissertation). Vladivostok: Vladivostokskij Gosudarstvennyj Medicinskij Universitet, p. 25. Available at: http://medical-diss.com/medicina/eksperimentalnoe-mediko-biologicheskoe-obosnovanie-pischevogo-ispolzovaniya-gumata-natriya

Potter EL (1945). The effect on infant mortality of Vitamin K administered during labor. American Journal of Obstetrics and Gynecology, 50: 235-247. DOI: https://www.doi.org/10.1097/00006254-194602000-00008

Prasad AS (2003). Zinc deficiency: Has been known of for 40 years but ignored by global health organisations. British Medical Journal, 326(7386): 409-410. DOI: https://www.doi.org/10.1136/bmj.326.7386.409

Puls R (1988). Mineral levels in animal health. Diagnostic data. Sherpa international, Clearbrook. British Columbia, Canada, p. 240. Available at: https://www.cabdirect.org/cabdirect/abstract/19892283865

Ramakrishnan U, Manjrekar R, Rivera J, Gonzáles-Cossío T, and Martorell R (1999). Micronutrients and pregnancy outcome: A review of the literature. Nutrition Research, 19(1): 103-159. DOI: https://www.doi.org/10.1016/s0271-5317(98)00178-x

Reutina SV (2009). The role of chromium in the human body. Vestnik Rossijskogo universiteta druzhby narodov, 4: 50-55. Available at: https://cyberleninka.ru/article/n/rol-hroma-v-organizme-cheloveka/viewer

Reznikoff-Etiévant MF, Zittoun J, Vaylet C, Pernet P, and Milliez J (2002). Low vitamin B12 level as a risk factor for very early recurrent abortion. European Journal of Obstetrics and Gynecology and Reproductive Biology, 104(2): 156-159. DOI: https://www.doi.org/10.1016/S0301-2115(02)00100-8

Riccardi D, Brennan SC, and Chang W (2013). The extracellular calcium-sensing receptor, CaSR, in fetal development. Best Practice & Research Clinical Endocrinology, 27: 443-453. DOI: https://www.doi.org/10.1016/j.beem.2013.02.010

Rosner JY, Gupta M, McGill M, Xue X, Chatterjee PK, Yoshida-Hay M, Robeson W, and Metz CN (2016). Magnesium deficiency during pregnancy in mice impairs placental size and function. Placenta, 39: 87-93. DOI: https://www.doi.org/10.1016/j.placenta.2016.01.009

Ruan Z, Zhang YG, Yin YL, Li TJ, Huang RL, Kim SW, Wu GY, and Deng ZY (2007). Dietary requirement of true digestible phosphorus and total calcium for growing pigs. Asian–Australasian Journal of Animal Sciences, 20(8): 1236-1242. DOI: https://www.doi.org/10.5713/ajas.2007.1236

Ruginosu E, Creangă Ş, Sofronie M, Mălăncuş R, Boghian V, and Solcan G (2011). The biochemical profile in cows with reproductive disorders. Cercetari Agronomice in Moldova, 44(2): 75-86. DOI: https://www.doi.org/10.2478/v10298-012-0035-4

Saliy MG, Tkachenko LV, Yakupova IH, and Polyakov VK (2013). The importance of iron deficiency in the formation of reproductive disorders in hypothalamic puberty dysfunction in adolescent girls. Astrahanskij medicinskij zhurnal, 8(4): 89-93. https://cyberleninka.ru/article/n/znachenie-defitsita-zheleza-v-formirovanii-reproduktivnyh-narusheniy-pri-gipotalamicheskoy-disfunktsii-pubertatnogo-perioda-u/viewer

Savinova AA, Semenchenko SV, and Falynskova NP (2015). Vitamins in livestock and veterinary medicine. Scientific magazine, Kontsep, pp. 3-56. Available at: https://elibrary.ru/item.asp?id=24018139

Scholl TO, Hediger ML, Schall JI, Fischer RL, and Khoo CS (1993). Low zinc intake during pregnancy: its association with preterm and very preterm delivery. American Journal of Epidemiology, 137(10): 1115-1124. DOI: https://www.doi.org/10.1093/oxfordjournals.aje.a116615

Serjakov IS, and Judina TA (2014). Reproductive qualities of sows when chromium is introduced into their diet. Razrabotka i vnedrenie novyh tehnologij poluchenija i pererabotki produkcii zhivotnovodstva. Troick: UGAVM, pp. 168-175. Available at: https://agris.fao.org/agris-search/search.do?recordID=BY2012000945

Seyfi H, Farzaneh N, and Mohri M (2005). Relationships between fertility, serum calcium, and inorganic phosphorus in dairy cows. Iranian Journal of Veterinary Research, 6(2): 74-78. Available at: https://www.sid.ir/en/journal/ViewPaper.aspx?id=41053

Shatohin PP, Suprunenko KV, Karysheva LP, Derenchuk JI, and Krylevec' JV (2017). Korektsiya at the time of Vitamin A and zinc at the blood of the blood of the remaining trimester of foals for nutritional deficiencies. Visnyk Poltavs'koi' Derzhavnoi' Agrarnoi' Akademii', 3: 96-100. DOI: https://www.doi.org/10.31210/visnyk2017.03.21

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Sheela C, and Ajay S (2004). Role of nutrition in reproduction: A review. Intas Polivet, 5(2): 229-234. Available at: https://www.cabdirect.org/cabdirect/abstract/20053131305

Sidorkin V, Polutov D, and Komarov A (2007). The role of vitamins in the prevention of diseases of the reproductive system in cows. Molochnoe i Mjasnoe Skotovodstvo, 5: 31-32. Available at: https://elibrary.ru/item.asp?id=9538621

Siemons LJ, and Mahler CH (1987). Hypogonadotropic hypogonadism in hemochromatosis: recovery of reproductive function after iron depletion. The Journal of Clinical Endocrinology and Metabolism, 65(3): 585-587. DOI: https://www.doi.org/10.1210/jcem-65-3-585

Simkiss K (1961). Calcium metabolism and avian reproduction. Biological Reviews, 36(3): 321-359. DOI: https://www.doi.org/10.1111/j.1469-185X.1961.tb01292.x

Skliarov PM, Fedorenko SJ, Naumenko SV, Onyshhenko OV, Pasternak AM, and Holda KO (2020a). Separate indices of homeostasis and the balance of the prooxidant–oxidant system in sheep for fetoplacental insufficiency. Ukrainian Journal of Ecology, 10(2): 440-448. DOI: https://www.doi.org/10.15421/2020_121

Skliarov PM, Fedorenko SY, Naumenko SV, Onischenko OV, and Holda KO (2020b). Retinol deficiency in animals: Etiopathogenesis and consequences. Regulatory Mechanisms in Biosystems, 11(2): 162-169. DOI: https://www.doi.org/10.15421/022024

Skljarov P (2017). Reproductive function in sheep and goats with Vitamin A deficiency. LAP LAMBERT Academic Publishing, p. 516. Available at: http://dspace.dsau.dp.ua/jspui/handle/123456789/709

Skljarov PM, and Koshevoj VP (2016). Complex preparations based on nanobiomaterials, prospects for use in reproduction of sheep and goats. Naukovyj visnyk L'vivs'kogo nacional'nogo universytetu veterynarnoi' medycyny ta biotehnologij imeni S.Z. G'zhyc'kogo, 18(2): 162-165. Available at: http://nbuv.gov.ua/UJRN/nvlnuvmbvn_2016_18_2_35

Smith OB, and Akinbamijo OO (2000). Micronutrients and reproduction in farm animals. Animal Reproduction Science, 60: 549-560. DOI: https://www.doi.org/10.1016/s0378-4320(00)00114-7

Stoecker BJ (1990). Chromium: Present knowledge in nutrition. International life sciences institute nutrition foundation, Washington, DC, pp. 287-293. Available at: https://ci.nii.ac.jp/naid/10011118264/

Strizhakov A, Davydov A, Lebedev V, Ignatko I, Makatsariya A, Mezhevitinova E, and Pshenichnikova T (2009). The biological role of magnesium in obstetrics and gynecology: Research data and clinical studies. Voprosy ginekologii, Akusherstva i Perinatologii, 3(8): 5-18. Available at: https://elibrary.ru/item.asp?id=12591288

Sun W, Chen L, Zhang W, Wang R, Goltzman D, and Miao D (2015). Active Vitamin D deficiency mediated by extracellular calcium and phosphorus results in male infertility in young mice. American Journal of Physiology-Endocrinology and Metabolism, 308(1): 51-62. DOI: https://www.doi.org/10.1152/ajpendo.00076.2014

Tallam SK, Ealy AD, Bryan KA, and Wu Z (2005). Ovarian activity and reproductive performance of dairy cows fed different amounts of phosphorus. Journal of Dairy Science, 88(10): 3609-3618. DOI: https://www.doi.org/10.3168/jds.s0022-0302(05)73046-0

Thomas P (1993). Effects of cadmium on gonadotropin secretion from Atlantic croaker pituitaries incubated *in vitro*. Marine Environmental Research, 35(1-2): 141-145. DOI: https://www.doi.org/10.1016/0141-1136(93)90028-X

Truong JT, and Booth SL (2011). Emerging issues in Vitamin K research. Journal of Evidence - Based Complementary and Alternative Medicine, 16(1): 73-79. DOI: https://www.doi.org/10.1177/1533210110392953

Umahanov MA (2017). The main factors affecting the reproductive functions of cattle. Gornoe sel'skoe Hozjajstvo, 1: 148-152. Available at: https://elibrary.ru/item.asp?id=29025471

Ushkalov VA (2001). The use of sodium humate to restore reproductive function in cows after childbirth. Veterinarija. Referativnyj Zhurnal, 4: 1135-1135. Available at: https://elibrary.ru/item.asp?id=6568826

Vishnjakov AI, and Lebedev SV (2011). Features of the influence of cadmium on mineral metabolism and morphological state of the reproductive system of mammals and birds. Fundamental'nye issledovanija, 1(9): 135-138. Available at: https://www.fundamental-research.ru/ru/article/view?id=28110

Vizner Z (1976). Feeding and fertility of farm animals. Moskva, p. 160. Available at: https://it.2lib.org/dl/2831963/b22513

Weiss WP (1998). Requirements of fat–soluble vitamins for dairy cows: A review. Journal of Dairy Science, 81(9): 2493-2501. DOI: https://www.doi.org/10.3168/jds.s0022-0302(98)70141-9

Wirth JJ, and Mijal RS (2010). Adverse effects of low level heavy metal exposure on male reproductive function. Systems Biology in Reproductive Medicine, 56(2): 147-167. DOI: https://www.doi.org/10.3109/19396360903582216

Yasothai R (2014). Importance of minerals on reproduction in dairy cattle. International Journal of Science, Environment and Technology, 3(6): 2051-2057. Available at: https://www.ijset.net/journal/446.pdf

Zhai XW, Zhang YL, Qi Q, Bai Y, Chen XL, Jin LJ, Ma XG, Shu RZ, Yang ZJ, and Liu FJ (2013). Effects of molybdenum on sperm quality and testis oxidative stress. Systems Biology in Reproductive Medicine, 59(5): 251-255. DOI: https://www.doi.org/10.3109/19396368.2013.791347

Zhang YL, Liu FJ, Chen XL, Zhang ZQ, Shu RZ, Yu XL, Zhai XW, Jin LJ, Ma XG, Qi Q et al. (2013). Dual effects of molybdenum on mouse oocyte quality and ovarian oxidative stress. Systems Biology in Reproductive Medicine, 59(6): 312-318. DOI: https://www.doi.org/10.3109/19396368.2013.826296

Zhegalova IV, Chumakova ZV, and Jurasov VV (2018). Cadmium and male reproductive health. Mikrojelementy v Medicine, 19(1): 24-34. Available at: http://www.journal.microelements.ru/trace_elements_in_medicine/2018_1/24_19(1)_2018.pdf

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The Broad Range of Coronaviruses Co-existing in Chiropteran: Implications for One Health

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ABSTRACT

Bats are a group of mammals that harbor the most significant number of coronaviruses. The aim of present review article was to analyze the broad spectrum of the coronavirus coexisting in Chiropterans hosts. Bats have certain types of cell receptors that allow them to be the potential hosts of a large number of viruses without the presence of any clinical manifestations, and to be a source of contagion infections for other animals and human species. Emphasis can be placed on five coronaviruses, such as Porcine Epidemic Diarrhea Disease, Severe Acute Diarrhea Syndrome, Middle East Respiratory Syndrome, Severe Acute Respiratory Syndrome, and Severe Acute Respiratory Syndrome 2, which have had significant impacts causing epidemic outbreaks in different parts of the world, and generating implications for both human and animal health. In conclusion, recent research indicated the importance of bats as potential hosts of multiple coroaviruses leading to some zoonotic diseases.

Keywords: Bats, Coronaviruses, Cross-species, Evolution, Spillover, Transmission

INTRODUCTION

Bats are a group of mammals that harbor the most significant number of coronaviruses. Coronaviruses are an extensive family of enveloped RNA viruses, having the ability to infect many species of animals and human beings (Bonilla-Aldana et al., 2020a; Bonilla-Aldana et al., 2020b; Bonilla-Aldana et al., 2021). The types of coronaviruses with high pathogenicity include Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), Middle East Respiratory Syndrome (MERS-CoV), and the most recent one named SARS-CoV-2 (Bonilla-Aldana et al., 2020a; Bonilla-Aldana et al., 2020b; Bonilla-Aldana et al., 2020f). One of the existing hypotheses given by the co-evolutionary history of bats and virus classes is a bat-virus relationship, which allows both to exist in constant equilibrium, managing to survive together (Banerjee et al., 2018; Wong et al., 2019; Basu-Ray et al., 2020). Bats have diversified cell types and receptors. Therefore, the bats can be potential hosts of many viruses, and thus the spread of viruses increases the possibility of transmission (Bonilla-Aldana et al., 2020c; Bonilla-Aldana et al., 2020d).

Interactions among bats with other animal species and humans can be critical factors for the transmission of coronaviruses, leading to devastating pandemics that affect the whole world (Bonilla-Aldana et al., 2020e; Dhama et al., 2020a; Dhama et al., 2020b). A great example of bat-human interactions occurred in China and Vietnam's restaurants and wildlife markets where zoonotic transmission of viruses has occurred (Wong et al., 2019; Huong et al., 2020).

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BIOLOGY OF BATS

Taxonomy

Bats are among the most diverse mammal order, second after rodents, with a complex taxonomy that is still under study because of conflicting phylogenetic relationships in light of new genetic data, especially at lower taxonomy levels (Amador et al., 2018). These mammals from the order Chiroptera with more than 1400 species share the evolutive characteristic of forelimbs adapted as wings. They were traditionally divided into macro and micro Chiroptera, assuming an independent evolution. However, recent DNA sequence data suggested that megabats (Macrochiropetara) originated during the early Eocene, and shared genetic material with microbats (Tsagkogeorga et al., 2013; Solari et al., 2019). The new proposed suborder is Yinpterochiroptera, including Pteropodidae (megabats) and Rhinolophoidae (horseshoe bats and megadermatidae or false vampire bats). The other suborder is Yangochiroptera (also known as Vespertilioniformes), and is composed of most of the microbat families; echolocation and eating habits evolution are one of the characteristics used in this new classification (Teeling et al., 2000; Eick et al., 2005).

The oldest fossil record of the modern bat ancestor is *Onychonycteris finneyi*, which dated back to the early Eocene (approximately 52.5 million years ago, as a part of the Tertiary Period Cenozoic Era) from the Green River Formation of Wyoming, USA. This fossil proves a piece of evidence that bats evolved the ability to fly before echolocation (Simmons et al., 2008). Currently, the order Chiroptera is composed of more than 1400 species, and various species can evolve together with different viruses. Members of both suborders act as reservoirs or susceptible hosts for several zoonotic viruses that are highly pathogenic in humans (Calisher et al., 2006; Moratelli and Calisher, 2015a; Banerjee et al., 2018). Without causing clinical disease in natural or experimental bats, these flying mammals vary widely in size, shape, and mass, with species weighting from two grams (*Craseonycteris thonglongyai*) to even one kilogram (*Acerodon jubatus*). They are distributed in the world on all continents except Antarctica (Banerjee et al., 2018; Wong et al., 2019).

Their biological diversity is as complex as their taxonomy and their feeding habits range from frugivorous (megabats) to insectivorous, carnivorous, and hematophagous in the Yangochiroptera suborder (Solari and Baker, 2007; Banerjee et al., 2018). They are nocturnal mammals, with one or two peaks of activity during the night; they inhabit a variety of ecological sites, including trees, caves, and other human-made structures, such as tunnels, deserted and occupied houses in rural areas. They spend their time in roosts, creating huge colonies like the one in Bracken cave in the United States of America (Moratelli and Calisher, 2015b), which is composed of more than 20 million bats (*Tadarida brasiliensis mexicana*) (Han et al., 2015).

Although sometimes they are considered seasonal animals. Some species have migratory behavior, especially in temperate zones. They are short-distance migrators, which raises concern about the probability of transmission of pathogens by this species (*Pipistrellus* spp, *Tadarida brasiliensis*, *Vespertilio*, and *Nyctalus* spp.). When they are active, bats provide critical services for the ecosystem by regulating crop pests, as pollinators and dispersing seeds (they are vital for the live cycle of Baobab African tree, *Adansonia digitata*), and fertilizing the soil with their excreta, which is rich in nitrogen control of vectors such as mosquitoes. Bats are used in applied immunogenetic research, as healthy aging models. They have been impacted by the White Nose Syndrome (*Pseudogymnoascus destructans)*. It has been suggested that there has been a decline in the bat population caused by this fungal infection. There are millions of pounds of insects that are not eaten and has turned into a burden for agriculture (Kasso and Balakrishnan, 2013).

Besides their intricate relationship with the environment, with repercussions on human well-being, several host pathogens cause disease in humans. Bats can store around 23 families of detected viruses; some are biological agents responsible for the zoonosis in which the coronavirus is found (Banerjee et al., 2018; Wong et al., 2019).

Knowledge about the bat coronaviruses (Bat-CoV) has increased over the past decade. It is estimated that not lees than 3,204 Bat-CoV worldwide exists. It is known now that they are the main ecological drivers of the diversity of coronaviruses and their evolutionary reservoirs (Banerjee et al., 2019). Bats possess adequate traits to host more viruses, whether these are zoonotic, providing them with a long lifespan for their body size and generating the viral persistence of chronic infections (Lacroix et al., 2017; Banerjee et al., 2019; Wong et al., 2019). The decrease in the physiological activity of the animal in a long way can reduce the immune function and viral replication (Lacroix et al., 2017; Banerjee et al., 2019; Wong et al., 2019).

Due to their role as a primary source of emerging infectious diseases worldwide, the study of the zoonosis associated with bats is vital for understanding the dynamics of transmission, prevention, and control of these pathogens (Banerjee et al., 2018). The bats' ability to be the host of hundreds of viruses without manifesting disease is an extensive area of research, and one of the peculiarities is that these mammals act as a source of infectious diseases. As bats are distributed worldwide with a wide diversity of species at multiple habitats, there is a matter of concern and a field for future related research. They are the only mammals that can fly, look for food daily, and migrate seasonally. For example, the Nathusius's bat (*Pipistrellus nathusii*) showed a mean speed of 47 km/day (Hedenström, 2009). Some species can fly over the ocean up to 14 km from the shore (Ahlén et al., 2009). They can facilitate their flight by having hollow bones to reduce body mass (Han et al., 2015; Fleming, 2019).

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Immunology of coronavirus in bats

Bats possess properties that modify their immune function and allow them to be reservoirs and vectors of diseases instead of being clinically infected (Calisher et al., 2006; Allen et al., 2009). It has been observed during the co-evolutionary history of bats and viruses that they have formed a unique host-pathogen relationship. Coronaviruses cause little or no harm to bats and can take months or years to manifest the disease (Schountz, 2014; Banerjee et al., 2018).

One of the existing hypotheses indicates that bats are not infected by the viruses due to the high body temperature during their flight. They mimic the effects of the response to fever, which results from the consumption of ATP which increases mitochondrial activities facilitating host responses. However, by itself, it is not enough to explain how bats can host viruses without signs of disease. Bats' cells are suggested to interfere with viral replication with the constitutive Interferon (IFN) activity, as well as, with active INF-stimulated Genes (ISG). It has also been suggested that bats use Pattern Recognition Receptors (PRRs) as a surveillance system for infectious threats (Schountz, 2014; Flies and Woods, 2019).

The first lines of evidence that associate a pathogen with a host are detecting combined genome fragments that may have circular DNA or linear RNA from one or two fragmented lines, A cell suitable for spreading a virus must be permissive and also be able to carry proper receptors that allow it to bind to an incoming virus. The immunological characteristics presented above are unique in bats, and allow them to be a virus carrier without getting sick (Dobson, 2005; Han et al., 2015). Some types of bats hibernate so their body temperature and metabolic rate are lowered, and immune responses are suppressed to conserve energy during the winter (George et al., 2011). It is believed that one possible mechanism of transmission of viruses from bats is through their saliva and urine, which can contaminate both the soil and fruits. So the contaminated fruits/soil can be ingested by intermediate hosts, including horses, pigs, civets, or non-human primates (Han et al., 2015). The pathogenicity of such viruses for human beings is still unknown, and possible threats need to be determined. Viruses transmitted by bats have been found in different species and geographical distribution, which indicates that they have been expanding the disease (Han et al., 2015). The related example is pandemic coronaviruses for which bats have been identified as a reservoir par excellence since they are ideal for hosting and transporting the virus to intermediate hosts through wet markets or illegal trafficking resulting in endangering human health (Huong et al., 2020).

Coronavirus

Bats have been the natural reservoir of the coronaviruses for many years (Table 1). Bats have been evolved over the years by immune and biological changes. These animals are sociable and live in colonies of at least 500 bats per square foot, facilitating the transmission of viruses from bat to bat (Han et al., 2015; Mackenzie et al., 2016).

Coronaviruses (CoV) are part of an extensive family of *Coronaviridae*, subfamily Coronavirinae and order Nidovirales. They present an envelope, and are made up of the most extended single-stranded RNA chains present in unsegmented positive sense vertebrates, and have the particularity of producing new strains and recombining; this virus has a high capacity to adapt to its host, and has been classified into four different genuses; Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus (Table 1) (Wong et al., 2019; Dos Santos Bezerra et al., 2020). Coronaviruses contain the protein S located on the surface; it will enable the start of infection by recognizing receptors and membrane fusion; it is a critical factor in the host's specificity (Banerjee et al., 2018).

This virus is highly contagious and zoonotic, which indicates that the transmission can occur from a vertebrate animal to a human, and presents great importance to global public health, being associated with multiple outbreaks that remain an unknown phenomenon. Coronaviruses circulate in nature in various animal species. Alpha-coronaviruses (alphaCoV) and beta-coronaviruses (betaCoV) can infect mammals, while gamma-coronaviruses and delta-coronaviruses mainly infect birds (Mollentze and Streicker, 2020; Rodriguez-Morales et al., 2020). These viruses have been detected globally, and alphaCoV and betaCoV have originated from bats in regions such as Asia, Africa, Europe, North and South America, as well as Australasia (Table 2).

The first case of coronavirus originated from bats and transmitted by an intermediate host was called palm civet, *Paguma larvata*, which was registered during 2002-2003 in the province of Guangdong in China. In the year 2012, the Middle East Respiratory Syndrome Coronavirus (MERS- CoV), which originated from bats, manifested itself in Saudi Arabia with dromedary camels (*Camelus dromedarius*) as an intermediate host. In December 2019, an unidentified pathogen emerged in Wuhan, China, causing severe pneumonia, and later in January 2020, a novel coronavirus (nCoV-2019, currently SARS-CoV-2) was described as the cause. Since then, a global emergency has been aroused due to the pandemic caused by SARS-CoV-2 (the current taxonomic name of the new coronavirus). It is supposed that Coronavirus disease 2019 (COVID19) is originated from bats, but the intermediate host is still undefined. Some coronavirus genuses, such as SARS-CoV and several betaCoVs of the subgenus *Merbecovirus*, are known to be closely related to MERS-CoV and have a common reservoir as horseshoe bats (Wong et al., 2019) (Table 2).

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Table 1. Reported Coronaviruses described in bats and other hosts

Coronavirus	Main affected hosts	Intermediate hosts	Natural hosts
Porcine epidemic diarrhea virus	Pigs	Unknown	Bat (*Scotophilus kuhlii*)
Swine acute diarrhea syndrome coronavirus	Pigs	Uknown	Bat (*Rhinolophus* spp.)
Middle East respiratory syndrome coronavirus	Human and other animals	Camels (*Camelus dromedarius*)	Bats (*Taphozous*)
Severe acute respiratory syndrome coronavirus	Human and other animals	Civets and Himalayan pangolin	Bats (*Rhinolophidae*)
Severe acute respiratory syndrome coronavirus 2	Human and other animals	Still unknown, suspected: pangolin	Bats?

?, Bats are still questioned and studied for SARS-CoV-2.

Table 2. Comparison of epidemic coronavirus versus other dangerous zoonotic bat viruses

	CORONAVIRUS*			FILOVIRUS	PARAMYXOVIRUS
Features	**Middle East respiratory syndrome coronavirus**	**Severe acute respiratory syndrome**	**Severe acute respiratory syndrome 2****	**Marburg virus*****	**Nipah virus**
Human functional receptor	Dipeptidyl peptidase 4 (DPP4 or D26	Angiotensin-converting enzyme 2 (ACE2) and CD209L (L-SIGN)	Angiotensin-converting enzyme 2 (ACE2) host transmembrane serine protease TMPRSS2	T cell immunoglobulin mucin domain-1 (TIM-1)	Ephrin B2 (membrane-bound ligand for the EphB class of receptor tyrosine kinases)
Clinical characteristic	Bilateral pneumonia, diarrhoea	Fever, bilateral pneumonia. Long prodrome	Peripheric bilateral pneumonia, anosmia, dysgeusia. Increasing report of neurological syndromes.	Hemorrhagic fever	Encephalitis, acute respiratory infection
Geographical Origen	Saudi Arabia	Guandong, China	Wuhan, China	Africa	Malaysia (1) Bangladesh (2)
Year of apparition	2012 (ended in sporadic cases still reported)	2002 (ended in 2004)	December 2019 (ongoing)	1967	(1) 1998 (2) 2001
Total of cases since apparition	2,494	8,096	178,879,640	587	Approximately 845
Total deaths	858	774	3,875,132	475	>800
CFR	34%	9.6%	2.17%****	24-80%	40-75%
Chiroptera associated reservoirs	*Taphozous perforatus* (Egyptian tomb bat), *Rhinopoma hardwickii* (lesser mouse-tailed bat) and *Pipistrellus kuhlii* (Kuhl's pipistrelle)	Giant horseshoe bat *(Rhinolophus ferrumequinum)*	Horseshoe bats *(Rhinolophus spp.)*	*Rousettus aegyptiacus*	*Pteropus hypomelanus*
Intermediated host	*Camelus dromedaria* (Camels)	*Paguna larvata* (Palm civet)	Pangolins?	(*Cercopithecus aethiops*) African green monkeys***, pigs?	Pigs, horses, goats, sheep, cats and dogs.
Definitive treatment	No	No	No	No	No
Vaccine	No	No	No	No	No

*Other coronaviruses that cause mild disease: HKU1, NL63, OC43, and 229E. **Up to June 22, 2021. ***Includes variants that cause the same disease: *Lake Victoria Marburgvirus* and *Ravn Marburgvirus*. ****High variation according countries.

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Porcine epidemic diarrhea virus

The swine epidemic diarrhea disease caused by the Porcine Epidemic Diarrhea Virus **(**PEDV-CoV) belonging to the *Betacoronavirus*, was identified in England in 1971. It presents digestive symptoms associated with vomiting and diarrhea manifested in adult and young animals as well as a fatal outcome in newborn animals. The contagion occurred through the oronasal and fecal routes through having contact with secretions, fecal material, food, and water contaminated with the virus. Inter-species transmission occurred in the bat-pig relationship. During 2013, the disease remained registered in Asia, Europe, and America because of high losses since its outbreaks in susceptible pigs of all ages, which defines it as contagious (Piñeros and Mogollón Galvis, 2015; Simas et al., 2015; Banerjee et al., 2019) (Table 2).

According to studies in a recombinant CoV, possibly the PEDV could have been originated from the CoV of *Scotophilus kuhlii* (Han et al., 2019). Strains related to the coronavirus were detected in two *Myotis horsfieldii* bats, in Cambodia and Lao's People Democratic Republic, where the great diversity and presence of coronavirus genetically related PEDV strains that infect pigs, and cattle may promote transmission (Lacroix et al., 2017).

In a lineage of bats of the genus *Scotophilus kuhlii* in Guangxi, China, 11 strains related to the virus were identified (Lacroix et al., 2017; Han et al., 2019). Another study found a CoV with a high relation to PEDV-CoV from Brazil in *Tadarida brasiliensis* bats, which had a zoonotic impact on the appearance of new diseases in humans and animal populations (Piñeros and Mogollón Galvis, 2015). Porcine Epidemic Diarrhea Virus (PEDV) can infect kidney cells of specific brown bats (*Eptesicus fuscus).* However, replication in bat cells needs further studies. No clinical cases of PEDV in humans have been reported. However, it has been shown that it may infect embryonic kidney cells (Zhang et al., 2017; Banerjee et al., 2019).

Swine acute diarrhea syndrome coronavirus

The Swine Acute Diarrhea Syndrome (SADS CoV) is caused by a virus classified in the Alphacoronavirus, a type of coronavirus that emerged in 2017 in the Guangdong province, China (Health, 2018; Zhou et al., 2019). This virus causes an enteric disease that led to the death of 24,693 piglets in southern China. Over time, it was believed the virus has been completely eradicated until February 2019. A new outbreak in the east caused the death of 200,000 piglets, and its transmission mechanism has not yet been clarified. The main symptoms of infected animals were severe diarrhea and weight loss. The obtained results of post-mortem analysis were indicative of intestinal lesions (Zhou et al., 2018).

The SADS-CoV can infect bats, mice, hamsters, rats, gerbils, pigs, birds such as chickens, non-human primates, and potentially humans. However, its zoonotic effect is unknown, it was transmitted through the feces of infected animals or its natural reservoirs. In China, three outbreaks of this virus have been reported so far. It was found that horseshoe bats, such as *Rhinolophus sinicus* and *Rhinolophus affinis*, were the main reservoir bats for SARS-CoV *Sarbecovirus*, MERS-CoV *Merbecovirus*, and SADS-CoV *Rhinacovirus* in South East Asia (Wong et al., 2019; Yang et al., 2019). Studies in Guangdong, China, where stool samples of bats species were collected, showed the detection of coronavirus. In a study, authors found 58 positive samples out of 591, examined by the fecal smears and processed using molecular testing, which indicated that the bats of the species *Rhinolophus sinicus*, *Rhinolophus affinis*, and *Rhinolophus rex* were potential reservoirs of this coronavirus (Zhou et al., 2018) (Table 2).

Middle East respiratory syndrome coronavirus

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a linage C, zoonotic, betacoronavirus enveloped with a positive-sense RNA genome. It was isolated from a man's sputum in Jeddah, Saudi Arabia, in 2012 (Zaki et al., 2012). Subsequent cases and clusters of patients that developed a rapidly evolving bilateral pneumonia, respiratory failure, and death have been reported in the Arabian Peninsula. Since September 2012, a total of 2494 cases have been notified with 858 deaths associated with 27 countries. Studies have indicated that this virus had a case fatality rate of 35% (Banerjee et al., 2019).

Complete genome sequence analysis and serological data provided a piece of evidence for transmission from camels to humans. MERS-CoV-specific RNAs and antibodies were also detected in camels (Reusken et al., 2013; Azhar et al., 2014), so far, there are no antiviral effective treatments for limiting any human coronavirus infection. Fortunately, since late 2020, vaccines have become available against SARS-CoV-2 (St John et al., 2015; Abuhammad et al., 2017).

It is known that bats of the genus *Taphozous perforatus* (Egyptian tomb bat), *Rhinopoma hardwickii* (lesser mouse-tailed bat), and *Pipistrellus kuhlii* (Kuhl's pipistrelle) have acted as the natural reservoir of this virus for many years. Other studies claimed that the dromedary hair participated as an intermediary host for the spread of this virus, and its contact with the man (Banerjee et al., 2019; Fan et al., 2019; WHO, 2020c).

Camels are used in the Middle East for entertainment and transportation so that people can become infected through direct contact with infected camels, and they can shed the virus in their respiratory secretions (Azhar et al., 2014). They have also been detected in camel milk, then there is a risk of infection through consumption of unpasteurized camel milk (Reusken et al., 2014; Han et al., 2015).

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The functional receptor of MERS-CoV is Dipeptidyl Peptidase 4 (DPP4 or CD26), which is present on the surface of the human non-ciliated bronchial epithelial cells (Lu et al., 2013). The receptor can also be found on bats' cells (Cui et al., 2013). Even more, there are descriptions of novel bat coronavirus in South China that attach to this receptor; the clinical implications of these results are unknown (Luo et al., 2018). A study reported that the use of the receptor by the MERS-CoV was different from that used by SARS-CoV and by SARS-CoV-2 (ACE2). The MERS-CoV receptor is conserved and can replicate in both bat cells and human cells (Müller et al., 2012; Han et al., 2015) (Table 2).

Severe acute respiratory syndrome coronavirus

The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) is a linage b *Betacoronavirus* and the MERS-CoVs (Maganga et al., 2020). It was based in Foshan, province of Guangdong, China in November 2002 (Fan et al., 2019). The index case was a medical doctor from the province of Guandong who made a trip to Hong Kong Special Administrative Region of the People's Republic of China, five days after the onset of symptoms. After that secondary cases appeared in the city and Singapore, Thailand, Vietnam, and Canada. All the index cases were from those countries where travelers returned from Hong Kong (Tsang et al., 2003). From the first cases, a novel coronavirus was isolated (Drosten et al., 2003). Following that it reached 33 countries and 5 continents, including North America and Europe, causing a global pandemic that lasted eight months, and led to 8096 cases with 774 deaths, reaching a case-fatality rate of 9.6%. The transmission occurred through respiratory drops, micro saliva, or direct contact with the mucous membranes of patients. Extraordinary efforts were made to contain these emerging conditions were deployed globally, including travel advisories by the World Health Organization (WHO) that in consequence led to the end of the epidemic in the first half of 2004 (Vaqué Rafart, 2005).

The SARS is presented as the first epidemic disease of the twenty-first century. It is known that its origin was the wet markets of Guandong, China, where the interaction with fluids and blood of different animals facilitated the propagation. Some studies indicated that the Himalayan palm civet and the palm civet o masked palm civet *(Paguna larvata)* were the intermediaries of this virus as a reservoir host as same as the giant horseshoe bat (*Rhinolophus ferrumequinum*) (Wang et al., 2006; Cleri et al., 2010).

The SARS-CoV produced unusual pneumonia because it had a prolonged prodrome with influenza-like symptoms. The patients develop the respiratory phase with progressive pulmonary infiltrates and respiratory failure. From early in the research, the virus has efficiently replicated *in vitro* (Sims et al., 2008). The functional receptors for SARS-CoV are Angiotensin-converting enzyme 2 (ACE2) and CD209L (L-SIGN) (Jeffers et al., 2004).

The SARS-CoV's genetic components circulated among the various bats' species that share the same cave and the opportunity for a new resurgence (Banerjee et al., 2019; Wong et al., 2019). The SARS virus survives for at least two to four days in feces, and two to three days on dry surfaces at room temperature (Rabenau et al., 2005). The transmission from person to person by direct or indirect contact of the mucous membranes with drops was the primary mode of spread of the pandemic produced in health centers, workplaces, homes, public transport, and air travel; the latter facilitated its rapid worldwide diffusion (Seto et al., 2003; Cheng et al., 2007). The SARS-CoV was detected in feces, urine, and respiratory secretions (Chan et al., 2004). Intra-hospital transmission of the virus was facilitated by nebulizers, intubation, or cardiopulmonary resuscitation in patients with SARS generated by many infectious droplets (Lee et al., 2003; Christian et al., 2004; Loon et al., 2004).

Regarding phylogenetic and viral diversity analyses, studies conducted in Africa, Asia, and Latin America (Peru, Bolivia, Brazil, and Mexico) indicated that intra-genus transmission of bats was higher in Africa and Asia (Health, 2018). The study was also able to confirm that the abundance of the virus was related to many bats (Health, 2018).

A study of an in-hospital outbreak at a community hospital in Toronto on February 23, 2003, indicated that cases of this virus had the age range from 21 months to 86 years, 60.2% of whom were women, and total death of 17 cases. Of the cases identified, 36.7% were hospital personnel. Other cases were locally transmitted or linked to imported SARS cases from other places (29.6%), hospitalized patients (14.1%), visitors (14.1%), or other health staff (5.5%). Of a total of 128 cases, 120 (93.8%) had contact with a SARS-positive case or a place where there was a known case of infection (Varia et al., 2003) (Table 2).

It is essential to know that bats and their products are used in food markets, and as traditional medicine, the feces of bats are used in the south from China and Asia, and consequently, it provides a constant human exposure source to bats and their tissues (Bonilla-Aldana et al., 2020e; Dhama et al., 2020a; Dhama et al., 2020b).

Severe acute respiratory syndrome coronavirus 2

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, belongs to the subgenus's genus Betacoronavirus *Sarbecovirus (*same subgenus for SARS-CoV*)*. It has affected the world since January 2020, affecting 210 countries and territories. In December 2019, a cluster of pneumonia with epidemiological nexus with an open-air-live animal market in Wuhan, China, was reported. A novel coronavirus was isolated and named SARS-CoV-2 by the International Committee on Taxonomy Viruses (ICTV). As of June 22, 2021, it has left a balance of

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178,879,640 confirmed cases and 3,875,132 deaths worldwide, but these figures continued to increase (Alanagreh et al., 2020; WHO, 2020a; Tiwari et al., 2020), fortunately 2.76 billion doses of vaccine have been administered. The outbreak was discovered in the Wuhan market, verifying that all the samples obtained from the animals were positive for SARS-CoV-2. This virus had been circulating since November, 2019. On December 1, 2019, the first registered case was found in an elderly man (Mackenzie and Smith, 2020; Yu et al., 2020). Subsequently, significant attempts have been rendered to detect the reservoirs and intermediate host of this emerging infectious disease.

Comparisons revealed that the bat CoV is approximately 79% similar to SARS-CoV at the nucleotide level. Although the MERS and SARS viruses are related to SARS-CoV-2, they have very notable biological differences. SARS-CoV 2 is much more infectious and has a remarkable capacity for local and global spread. It has been taken as a priority to determine the virological characteristics related to transmission. The respiratory pathogen has a relatively high virulence that can jump the barrier species (Chinazzi et al., 2020; Zhang and Holmes, 2020). The bats' role as a zoonotic origin is unknown. The viruses present an association with SARS-CoV-2 were sampled in Yunnan province more than 1,500 km from Wuhan (Wrapp et al., 2020). In a recent publication, a bat coronavirus, RaTG13, was found in *Rhinolophus affinis*, showed 96% genomic similarity to SARS-CoV-2. However, it does not bind efficiently to the human receptor ACE2 (Leitner and Kumar, 2020).

The bat is reported as the reservoir source, but it needs an intermediate host for SARS-CoV-2 to infect humans. A conducted study showed that SARS-CoV-2 replicates poorly in pigs, chickens, and ducks (Flores-Alanis et al., 2020). The pangolins (Pholidota) have been studied as a relevant intermediate host for SARS-CoV-2, with genetic analysis of pangolin CoV, MP789, showing similarities in receptor domains, suggesting an ancestral jump among bats (Flores-Alanis et al., 2020), humans, and pangolins (Fischer et al., 2020; Xiao et al., 2020; Zhang et al., 2020). However, if bats are susceptible to contracting the virus, ferrets, canids, and cats are still possible to directly infect humans (Damas et al., 2020; Leitner and Kumar, 2020; Tiwari et al., 2020). Researchers have proposed the potential risk of reverse zoonotic transmission to bats (anthropozoonosis), because of the magnitude of this historic pandemic and the intricate relationship of SARS-CoV-2 and Chiroptera. Researchers have proposed the potential risk of reverse zoonotic transmission from humans to bats (anthropozoonosis) of the SARS-CoV-2 (Franklin and Bevins, 2020; Olival et al., 2020).

The infection can be acquired by inhaling droplets that can spread from one to two, or by touching contaminated surfaces which can remain viable for several days with favorable atmospheric conditions. However, the viruses are destroyed with hypochlorite sodium products, hydrogen, peroxide, among other substances. The virus is also present in feces and contamination of the water supply leading to the transmission through the oral route (Chen et al., 2020; WHO, 2020b; Kampf et al., 2020).

All people are theoretically susceptible; the large droplets come from the coughs and sneezes of symptomatic or asymptomatic patients before the clinical signs appear, causing severe respiratory diseases such as pulmonary failure and pneumonia. However, the severity of COVID19 is unusually selective, rises with age and coexisting health conditions, including, chronic kidney disease, diabetes mellitus, obesity, smoking, and hypertension. There is also increasing evidence about ethnicity and income for the susceptibility of infection and poorer prognosis (de Lusignan et al., 2020). Human genetic factors, such as polymorphism of the host transmembrane serine protease (TMPRSS2) and ACE2 receptor have been proposed (Hou et al., 2020). Post-placental transmission in women has not yet been clearly described. Studies have shown that higher viral loads are found in the nasal cavity than the throat, without differentiating between symptomatic and asymptomatic people. Patients can infect other humans for as long as symptoms last and even after clinical recovery (Rothe et al., 2020; Singhal, 2020; Zou et al., 2020) (Table 2).

CONCLUSIONS

Bats are potential reservoirs of many viruses, including coronaviruses, but direct transmission to humans has not been demonstrated so far since it requires an intermediate host (Joffrin et al., 2020). Recently, in Peru, the complete genome sequence of an Alphacoronavirus isolated from vampire bats (*Desmodus rotundus*, family Phyllostomidae) from the Amazonas region was reported (Bergner et al., 2020).

Research of coronavirus in bats have become an urgent problem, so it is essential to be able to detect early warning signs, and minimize the subsequent outbreaks given by them in places like Egypt, Oman, Qatar, Saudi Arabia, the Middle East, Africa, and South Asia, have found camelids with the virus mentioned above, which their human-animal transmission can be generated by direct or indirect contact with infected animals (Banerjee et al., 2019; Fan et al., 2019; WHO, 2020c). More research is needed to clarify and understand the susceptibility of infection and variable outcomes.

However, there are still some questions that are left unanswered which necessitates the need for more deep studies addressing the effect of coronaviruses on animals and humans. In 2019, a coronavirus mutation from previous years was presented, and people would be exposed to a higher risk of new pandemics caused by CoVs. Thus, it is equally important to know which intermediate hosts are the means of transmission of bats to human beings.

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DECLARATIONS

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Authors' Contributions

DKBA and AJRM conceived the review, developed the preliminary search strategy, and drafted the manuscript. SDJD, CTO, PJS, VGM, and JLBA refined the search strategy by conducting iterative database queries and incorporating new search terms. SDJD, CTO, PJS, VGM, and JLBA searched and collected the articles. AJRM and DKBA conducted the quality assessment. All authors have critically reviewed this manuscript for relevant intellectual contributions. All the authors read and approved the final submitted version of the paper.

Conflicts of interest

All authors report no potential conflicts.

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REFERENCES

Abuhammad A, Al-Aqtash RaA, Anson BJ, Mesecar AD, and Taha MO (2017). Computational modeling of the bat hku4 coronavirus 3cl(pro) inhibitors as a tool for the development of antivirals against the emerging middle east respiratory syndrome (MERS) coronavirus. Journal of Molecular Recognition, 30(11): e2644. DOI: http://www.doi.org/10.1002/jmr.2644

Ahlén I, Baagøe HJ, and Bach L (2009). Behavior of Scandinavian bats during migration and foraging at sea. Journal of Mammalogy, 90(6): 1318-1323. DOI: http://www.doi.org/10.1644/09-MAMM-S-223R.1

Ahn DG, Shin HJ, Kim MH, Lee S, Kim HS, Myoung J, Kim BT, and Kim SJ (2020). Current status of epidemiology, diagnosis, therapeutics, and vaccines for novel coronavirus disease 2019 (covid-19). Journal of Microbiology and Biotechnology, 30(3): 313-324. DOI: http://www.doi.org/10.4014/jmb.2003.03011

Alanagreh La, Alzoughool F, and Atoum M (2020). The human coronavirus disease covid-19: Its origin, characteristics, and insights into potential drugs and its mechanisms. Pathogens, 9(5): 331. DOI: http://www.doi.org/10.3390/pathogens9050331

Allen LC, Turmelle AS, Mendonça MT, Navara KJ, Kunz TH, and McCracken GF (2009). Roosting ecology and variation in adaptive and innate immune system function in the Brazilian free-tailed bat (Tadarida brasiliensis). Journal of Comparative Physiology B, 179(3): 315-323. DOI: http://www.doi.org/10.1007/s00360-008-0315-3

Amador LI, Moyers Arévalo RL, Almeida FC, Catalano SA, and Giannini NP (2018). Bat systematics in the light of unconstrained analyses of a comprehensive molecular supermatrix. Journal of Mammalian Evolution, 25(1): 37-70. DOI: http://www.doi.org/10.1007/s10914-016-9363-8

Azhar EI, El-Kafrawy SA, Farraj SA, Hassan AM, Al-Saeed MS, Hashem AM, and Madani TA (2014). Evidence for camel-to-human transmission of MERS coronavirus. New England Journal of Medicine, 370(26): 2499-2505. DOI: http://www.doi.org/10.1056/NEJMoa1401505

Banerjee A, Kulcsar K, Misra V, Frieman M, and Mossman K (2019). Bats and coronaviruses. Viruses, 11(1): Article number 41. DOI: http://www.doi.org/10.3390/v11010041

Banerjee A, Misra V, Schountz T, and Baker ML (2018). Tools to study pathogen-host interactions in bats. Virus Research, 248: 5-12. DOI: http://www.doi.org/ 10.1016/j.virusres.2018.02.013

Basu-Ray I, Almaddah NK, Adeboye A, and Soos MP (2020). Cardiac manifestations of coronavirus (Covid-19). Statpearls, StatPearls Publishing.

Bergner LM, Orton RJ, and Streicker DG (2020). Complete genome sequence of an alphacoronavirus from common vampire bats in Peru. Microbiol Resource Announcements, 9(34): e00742-20. DOI: http://www.doi.org/10.1128/MRA.00742-20

Bonilla-Aldana DK, Dhama K, and Rodriguez-Morales AJ (2020a). Revisiting the one health approach in the context of covid-19: A look into the ecology of this emerging disease. Advances in Animal and Veterinary Sciences, 8(3): 234-237. Available at: http://nexusacademicpublishers.com/uploads/files/AAVS_8_3_234-237.pdf

Bonilla-Aldana DK, Holguin-Rivera Y, Cortes-Bonilla I, Cardona-Trujillo MC, Garcia-Barco A, Bedoya-Arias HA, Rabaan AA, Sah R, and Rodriguez-Morales AJ (2020b). Coronavirus infections reported by promed, february 2000-january 2020. Travel Medicine and Infectious Disease, 39: Article number 101575. DOI: http://www.doi.org/10.1016/j.tmaid.2020.101575

Bonilla-Aldana DK, Jimenez-Diaz SD, Arango-Duque JS, Aguirre-Florez M, Balbin-Ramon GJ, Paniz-Mondolfi A, Suarez JA, Pachar MR, Perez-Garcia LA, Delgado-Noguera LA et al. (2021). Bats in ecosystems and their wide spectrum of viral infectious potential threats: SARS-CoV-2 and other emerging viruses. International Journal of Infectious Diseases, 102: 87-96. DOI: http://www.doi.org/10.1016/j.ijid.2020.08.050

Bonilla-Aldana DK, Jimenez-Diaz SD, Patel SK, Dhama K, Rabaan AA, Sah R, Sierra M, Zambrano LI, Arteaga-Livias K, and Rodriguez-Morales AJ (2020c). Importance of bats in wildlife: Not just carriers of pandemic SARS-CoV-2 and other viruses. Journal of Pure and Applied Microbiology, 14(1): 709-712. DOI: http://www.doi.org/10.22207/jpam.14.Spl1.05

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Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Ramirez-Ocampo S, Paniz-Mondolfi A, Rabaan AA, Sah R, and Rodriguez-Morales AJ (2020d). SARS-CoV-2, MERS-CoV and now the 2019-novel CoV: Have we investigated enough about coronaviruses? - a bibliometric analysis. Travel Medicine and Infectious Disease, 33: Article number101566. DOI: http://www.doi.org/10.1016/j.tmaid.2020.101566

Bonilla-Aldana DK, Villamil-Gómez WE, Rabaan AA, and Rodriguez-Morales AJ (2020e). Una nueva zoonosis viral de preocupación global: Covid-19, enfermedad por coronavirus 2019. Iatreia, 33(2): 107-110.

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: http://www.doi.org/10.1128/CMR.00017-06

Chan WM, Yuen KSC, Fan DSP, Lam DSC, Chan PKS, and Sung JJY (2004). Tears and conjunctival scrapings for coronavirus in patients with sars. British Journal of Ophthalmology, 88(7): 968-969. DOI: http://www.doi.org/10.1136/bjo.2003.039461

Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q et al. (2020). Clinical characteristics and intrauterine vertical transmission potential of covid-19 infection in nine pregnant women: A retrospective review of medical records. The Lancet, 395: 809-815. DOI: http://www.doi.org/10.1016/S0140-6736(20)30360-3

Cheng VCC, Lau SKP, Woo PCY, and Yuen KY (2007). Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clinical Microbiology Reviews, 20(4): 660-694. DOI: http://www.doi.org/10.1128/CMR.00023-07

Chinazzi M, Davis JT, Ajelli M, Gioannini C, Litvinova M, Merler S, Piontti APY, Rossi L, Sun K, Viboud C et al. (2020). The effect of travel restrictions on the spread of the 2019 novel coronavirus (2019-Nov) outbreak. Med Rxiv, 2020.2002.2009.20021261. DOI: http://www.doi.org/10.1101/2020.02.09.20021261

Christian MD, Loutfy M, McDonald LC, Martinez KF, Ofner M, Wong T, Wallington T, Gold WL, Mederski B, Green K et al. (2004). Possible sars coronavirus transmission during cardiopulmonary resuscitation. Emerging Infectious Diseases, 10(2): 287-293. DOI: http://www.doi.org/10.3201/eid1002.030700

Cleri DJ, Ricketti AJ, and Vernaleo JR (2010). Severe acute respiratory syndrome (sars). Infectious Disease Clinics, 24(1): 175-202. DOI: http://www.doi.org/10.1016/j.idc.2009.10.005

Cui J, Eden JS, Holmes EC, and Wang LF (2013). Adaptive evolution of bat dipeptidyl peptidase 4 (dpp4): Implications for the origin and emergence of middle east respiratory syndrome coronavirus. Virology Journal, 10: Article number 304. DOI: http://www.doi.org/10.1186/1743-422X-10-304

Damas J, Hughes GM, Keough KC, Painter CA, Persky NS, Corbo M, Hiller M, Koepfli KP, Pfenning AR, Zhao H et al. (2020). Broad host range of Sars-Cov-2 predicted by comparative and structural analysis of ace2 in vertebrates. Proceedings of the National Academy of Sciences of the USA, 117(36): 22311-22322. DOI: http://www.doi.org/10.1073/pnas.2010146117

de Lusignan S, Dorward J, Correa A, Jones N, Akinyemi O, Amirthalingam G, Andrews N, Byford R, Dabrera G, Elliot A et al. (2020). Risk factors for Sars-Cov-2 among patients in the oxford royal college of general practitioners research and surveillance centre primary care network: A cross-sectional study. Lancet Infectious Diseases, 20(9): 1034-1042. DOI: http://www.doi.org/10.1016/S1473-3099(20)30371-6

Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, and Rodriguez-Morales AJ (2020a). Coronavirus disease 2019-covid-19. Clinical Microbiology Reviews, 33(4): e00028-20. DOI: http://www.doi.org/10.1128/CMR.00028-20

Dhama K, Patel SK, Sharun K, Pathak M, Tiwari R, Yatoo MI, Malik YS, Sah R, Rabaan AA, Panwar PK et al. (2020b). SARS-CoV-2 jumping the species barrier: Zoonotic lessons from sars, MERS, and recent advances to combat this pandemic virus. Travel Medicine and Infectious Diseases, 37: Article number 101830. DOI: http://www.doi.org/10.1016/j.tmaid.2020.101830

Dobson AP (2005). What links bats to emerging infectious diseases? Science, 310: 628-629. DOI: http://www.doi.org/10.1126/science.1120872

Dos Santos Bezerra R, Valenca IN, de Cassia Ruy P, Ximenez JPB, da Silva Junior WA, Covas DT, Kashima S, and Slavov SN (2020). The novel coronavirus SARS-CoV-2: From a zoonotic infection to coronavirus disease 2019. Journal of Medical Virology, 92(11): 2607-2615. DOI: http://www.doi.org/10.1002/jmv.26072

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA et al. (2003). Identification of a novel coronavirus in patients with severe acute respiratory syndrome. New England Journal of Medicine, 348(20): 1967-1976. DOI: http://www.doi.org/10.1056/NEJMoa030747

Eick GN, Jacobs DS, and Matthee CA (2005). A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). Molecular Biology and Evolution, 22(9): 1869-1886. DOI: http://www.doi.org/10.1093/molbev/msi180

Fan Y, Zhao K, Shi ZL, and Zhou P (2019). Bat coronaviruses in China. Viruses, 11(3): Article number 210. DOI: http://www.doi.org/10.3390/v11030210

Fischer H, Tschachler E, and Eckhart L (2020). Pangolins lack ifih1/mda5, a cytoplasmic RNA sensor that initiates innate immune defense upon coronavirus infection. Frontiers in Immunology, 11: Article number 939. DOI: http://www.doi.org/10.3389/fimmu.2020.00939

Fleming TH (2019). Bat migration. Encyclopedia of Animal Behavior, pp. 605-610. DOI: http://www.doi.org/10.1016/B978-0-12-809633-8.20764-4

Flies AS, and Woods GM (2019). Wild immunology-the answers are out there. Frontiers in Immunology, 10: 126-126. DOI: http://www.doi.org/10.3389/fimmu.2019.00126

Flores-Alanis A, Sandner-Miranda L, Delgado G, Cravioto A, and Morales-Espinosa R (2020). The receptor binding domain of SARS-CoV-2 spike protein is the result of an ancestral recombination between the bat-CoV ratg13 and the pangolin-CoV mp789. BMC Research Notes, 13(1): Article number 398. DOI: http://www.doi.org/10.1186/s13104-020-05242-8

Franklin AB, and Bevins SN (2020). Spillover of SARS-CoV-2 into novel wild hosts in North America: A conceptual model for perpetuation of the pathogen. Science of the Total Environ, 733: Article ID 139358. DOI: http://www.doi.org/10.1016/j.scitotenv.2020.139358

George DB, Webb CT, Farnsworth ML, O'Shea TJ, Bowen RA, Smith DL, Stanley TR, Ellison LE, and Rupprecht CE (2011). Host and viral ecology determine bat rabies seasonality and maintenance. Proceedings of the National Academy of Sciences of the USA, 108(25): 10208-10213. DOI: http://www.doi.org/10.1073/pnas.1010875108

Han HJ, Wen Hl, Zhou CM, Chen FF, Luo LM, Liu JW, and Yu XJ (2015). Bats as reservoirs of severe emerging infectious diseases. Virus Research, 205: 1-6. DOI: http://www.doi.org/10.1016/j.virusres.2015.05.006

Han Y, Du J, Su H, Zhang J, Zhu G, Zhang S, Wu Z, and Jin Q (2019). Identification of diverse bat alphacoronaviruses and betacoronaviruses in China provides new insights into the evolution and origin of coronavirus-related diseases. Frontiers in Microbiology, 10: 1900-1900. DOI: http://www.doi.org/10.3389/fmicb.2019.01900

Health NI (2018). New coronavirus emerges from bats in China, devastates young swine. Available at: https://www.nih.gov/news-events/news-releases/new-coronavirus-emerges-bats-china-devastates-young-swine

Hedenström A (2009). Optimal migration strategies in bats. Journal of Mammalogy, 90(6): 1298-1309. DOI: http://www.doi.org/10.1644/09-MAMM-S-075R2.1

Hou Y, Zhao J, Martin W, Kallianpur A, Chung MK, Jehi L, Sharifi N, Erzurum S, Eng C, and Cheng F (2020). New insights into genetic susceptibility of covid-19: An ace2 and tmprss2 polymorphism analysis. BMC Medicine, 18(1): Article number 216. DOI: http://www.doi.org/10.1186/s12916-020-01673-z

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Huong NQ, Nga NTT, Long NV, Luu BD, Latinne A, Pruvot M, Phuong NT, Quang LTV, Hung VV, Lan NT et al. (2020). Coronavirus testing indicates transmission risk increases along wildlife supply chains for human consumption in Vietnam, 2013-2014. PLoS ONE, 15(8): e0237129. DOI: http://www.doi.org/10.1371/journal.pone.0237129

Jeffers SA, Tusell SM, Gillim-Ross L, Hemmila EM, Achenbach JE, Babcock GJ, Thomas WD, Thackray LB, Young MD, Mason RJ et al. (2004). Cd209l (l-sign) is a receptor for severe acute respiratory syndrome coronavirus. Proceedings of the National Academy of Sciences of the USA, 101(44): 15748-15753. DOI: http://www.doi.org/10.1073/pnas.0403812101

Joffrin L, Goodman SM, Wilkinson DA, Ramasindrazana B, Lagadec E, Gomard Y, Le Minter G, Dos Santos A, Corrie Schoeman M, Sookhareea R et al. (2020). Bat coronavirus phylogeography in the western Indian ocean. Scientific Reports, 10(1): Article number 6873. DOI: http://www.doi.org/10.1038/s41598-020-63799-7

Kampf G, Todt D, Pfaender S, and Steinmann E (2020). Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. Journal of Hospital Infection, 104(3): 246-251. DOI: http://www.doi.org/10.1016/j.jhin.2020.01.022

Kasso M, and Balakrishnan M (2013). Ecological and economic importance of bats (order Chiroptera). ISRN Biodiversity, Article ID: 187415. DOI: http://www.doi.org/10.1155/2013/187415

King AMQ, Adams MJ, Carstens EB et al (2012). Family-coronaviridae. In: King AMQ, Adams MJ, Carstens EB et al. (eds) Virus taxonomy. San Diego: Elsevier, pp. 806-828. DOI: http://www.doi.org/10.1016/B978-0-12-384684-6.00136-1

Lacroix A, Duong V, Hul V, San S, Davun H, Omaliss K, Chea S, Hassanin A, Theppangna W, Silithammavong S et al. (2017). Genetic diversity of coronaviruses in bats in lao pdr and Cambodia. Infection, Genetics, and Evolution, 48: 10-18. DOI: http://www.doi.org/10.1016/j.meegid.2016.11.029

Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, Ahuja A, Yung MY, Leung CB, To KF et al. (2003). A major outbreak of severe acute respiratory syndrome in hong kong. New England Journal of Medicine, 348(20): 1986-1994. DOI: http://www.doi.org/10.1056/NEJMoa030685

Leitner T, and Kumar S (2020). Where did SARS-CoV-2 come from? Molecular Biology and Evolution, 37(9): 2463-2464. DOI: http://www.doi.org/10.1093/molbev/msaa162

Loon SC, Teoh SCB, Oon LLE, Se-Thoe SY, Ling AE, Leo YS, and Leong HN (2004). The severe acute respiratory syndrome coronavirus in tears. British Journal of Ophthalmology, 88(7): 861-863. DOI: http://www.doi.org/10.1136/bjo.2003.035931

Lu G, Hu Y, Wang Q, Qi J, Gao F, Li Y, Zhang Y, Zhang W, Yuan Y, Bao J et al. (2013). Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor cd26. Nature, 500: 227-231. DOI: http://www.doi.org/10.1038/nature12328

Luo CM, Wang N, Yang XL, Liu HZ, Zhang W, Li B, Hu B, Peng C, Geng QB, Zhu GJ et al. (2018). Discovery of novel bat coronaviruses in South China that use the same receptor as middle east respiratory syndrome coronavirus. Journal of Virology, 92(13): e00116-18. DOI: http://www.doi.org/10.1128/JVI.00116-18

Mackenzie JS, and Smith DW (2020). Covid-19: A novel zoonotic disease caused by a coronavirus from China: What we know and what we don't. Microbiology Austin, MA20013. DOI: http://www.doi.org/10.1071/MA20013

Mackenzie JS, Childs JE, Field HE, Wang LF, and Breed AC (2016). The role of bats as reservoir hosts of emerging neuroviruses. Neurotropic Viral Infections, pp. 403-454. DOI: http://www.doi.org/10.1007/978-3-319-33189-8_12

Maganga GD, Pinto A, Mombo IM, Madjitobaye M, Mbeang Beyeme AM, Boundenga L, Ar Gouilh M, N'Dilimabaka N, Drexler JF, Drosten C et al. (2020). Genetic diversity and ecology of coronaviruses hosted by cave-dwelling bats in Gabon. Scientific Reports, 10(1): Article number 7314. DOI: http://www.doi.org/10.1038/s41598-020-64159-1

Mollentze N, and Streicker DG (2020). Viral zoonotic risk is homogenous among taxonomic orders of mammalian and avian reservoir hosts. Proceedings of the National Academy of Sciences of the USA, 117(17): 9423-9430. DOI: http://www.doi.org/10.1073/pnas.1919176117

Moratelli R, and Calisher CH (2015a). Bats and zoonotic viruses: Can we confidently link bats with emerging deadly viruses? Memorias do Instituto Oswaldo Cruz, 110(1): 1-22. DOI: http://www.doi.org/10.1590/0074-02760150048

Moratelli R, and Calisher CH (2015b). Bats and zoonotic viruses: Can we confidently link bats with emerging deadly viruses? Mem Inst Oswaldo Cruz, 110(1): 1-22. DOI: http://www.doi.org/10.1590/0074-02760150048

Müller MA, Raj VS, Muth D, Meyer B, Kallies S, Smits SL, Wollny R, Bestebroer TM, Specht S, Suliman T et al. (2012). Human coronavirus emc does not require the sars-coronavirus receptor and maintains broad replicative capability in mammalian cell lines. mBio, 3(6): 515-512. DOI: http://www.doi.org/10.1128/mBio.00515-12

Olival KJ, Cryan PM, Amman BR, Baric RS, Blehert DS, Brook CE, Calisher CH, Castle KT, Coleman JTH, Daszak P et al. (2020). Possibility for reverse zoonotic transmission of SARS-CoV-2 to free-ranging wildlife: A case study of bats. PLoS Pathogens, 16(9): e1008758. DOI: http://www.doi.org/10.1371/journal.ppat.1008758

Piñeros R, and Mogollón Galvis JD (2015). Coronavirus en porcinos: Importancia y presentación del virus de la diarrea epidémica porcina (pedv) en Colombia. Revista de Medicina Veterinaria, pp. 73-89. Available at: http://www.scielo.org.co/scielo.php?pid=S0122-93542015000100008&script=sci_abstract&tlng=es

Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, and Doerr HW (2005). Stability and inactivation of sars coronavirus. Medical Microbiology and Immunology, 194: 1-6. DOI: http://www.doi.org/10.1007/s00430-004-0219-0

Reusken CB, Farag EA, Jonges M, Godeke GJ, El-Sayed AM, Pas SD, Raj VS, Mohran KA, Moussa HA, Ghobashy H et al. (2014). Middle east respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014. Eurosurveillance, 19(23): Article number 20829. DOI: http://www.doi.org/10.2807/1560-7917.ES2014.19.23.20829

Reusken CBEM, Haagmans BL, Müller MA, Gutierrez C, Godeke GJ, Meyer B, Muth D, Raj VS, Vries LSD, Corman VM et al. (2013). Middle east respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: A comparative serological study. The Lancet Infectious Diseases, 13(10): 859-866. DOI: http://www.doi.org/10.1016/S1473-3099(13)70164-6

Rodriguez-Morales AJ, MacGregor K, Kanagarajah S, Patel D, and Schlagenhauf P (2020). Going global - travel and the 2019 novel coronavirus. Travel Medicine and Infectious Diseases, 33: Article number 101578. DOI: http://www.doi.org/10.1016/j.tmaid.2020.101578

Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W et al. (2020). Transmission of 2019-ncov infection from an asymptomatic contact in Germany. New England Journal of Medicine, 382(10): 970-971. DOI: http://www.doi.org/10.1056/NEJMc2001468

Schountz T (2014). Immunology of bats and their viruses: Challenges and opportunities. Viruses, 6(12): 4880-4901. DOI: http://www.doi.org/10.3390/v6124880

Seto WH, Tsang D, Yung RWH, Ching TY, Ng TK, Ho M, Ho LM, Peiris JSM, and Advisors of Expert SgoHA (2003). Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (sars). Lancet, 361(9368): 1519-1520. DOI: http://www.doi.org/10.1016/s0140-6736(03)13168-6

Simas PVM, Barnabé ACdS, Durães-Carvalho R, Neto DFdL, Caserta LC, Artacho L, Jacomassa FAF, Martini MC, Bianchi Dos Santos MMA, Felippe PAN et al. (2015). Bat coronavirus in brazil related to appalachian ridge and porcine epidemic diarrhea viruses. Emerging Infectious Diseases, 21(4): 729-731. DOI: http://www.doi.org/10.3201/eid2104.141783

To cite this paper: Bonilla-Aldana DK, Toro-Ortiz C, Jimenez-Salazar P, Guevara-Manso V, Jimenez-Diaz SD, Bonilla-Aldana JL, Gutierrez-Grajales EJ, Pecho-Silva S, Paniz-Mondolfi A, Suárez JA, Pachar MR, Martinez-Pulgarin DF, Zambrano LI, Soler-Tovar D, Rodriguez-Morales AJ, and Mattar S (2021). The Broad Range of Coronaviruses Co-existing in Chiropteran: Implications for One Health. *World Vet. J.*, 11 (2): 170-180.

Simmons NB, Seymour KL, Habersetzer J, and Gunnell GF (2008). Primitive early eocene bat from wyoming and the evolution of flight and echolocation. Nature, 451(7180): 818-821. DOI: http://www.doi.org/10.1038/nature06549

Sims AC, Burkett SE, Yount B, and Pickles RJ (2008). SARS-CoV replication and pathogenesis in an in vitro model of the human conducting airway epithelium. Virus Research, 133(1): 33-44. DOI: http://www.doi.org/10.1016/j.virusres.2007.03.013

Singhal T (2020). A review of coronavirus disease-2019 (covid-19). The Indian Journal of Pediatrics, 87(4): 281-286. DOI: http://www.doi.org/10.1007/s12098-020-03263-6

Solari S, and Baker RJ (2007). Mammal species of the world: A taxonomic and geographic reference by d. E. Wilson; d. M. Reeder. Journal of Mammalogy, 88(3): 824-830. DOI: http://www.doi.org/10.1644/06-MAMM-R-422.1

Solari S, Sotero-Caio CG, and Baker RJ (2019). Advances in the systematics of bats: Towards a consensus on species delimitation and classifications through integrative taxonomy. Journal of Mammalogy, 100(3): 838-851. DOI: http://www.doi.org/10.1093/jmammal/gyy168

St John SE, Tomar S, Stauffer SR, and Mesecar AD (2015). Targeting zoonotic viruses: Structure-based inhibition of the 3c-like protease from bat coronavirus hku4--the likely reservoir host to the human coronavirus that causes middle east respiratory syndrome (MERS). Bioorganic and Medicinal Chemistry, 23(17): 6036-6048. DOI: http://www.doi.org/10.1016/j.bmc.2015.06.039

Teeling EC, Scally M, Kao DJ, Romagnoli ML, Springer MS, and Stanhope MJ (2000). Molecular evidence regarding the origin of echolocation and flight in bats. Nature, 403(6766): 188-192. DOI: http://www.doi.org/10.1038/35003188

Tiwari R, Dhama K, Sharun K, Iqbal Yatoo M, Malik YS, Singh R, Michalak I, Sah R, Bonilla-Aldana DK, and Rodriguez-Morales AJ (2020). Covid-19: Animals, veterinary and zoonotic links. Veterinary Quarterly, 40(1): 169-182. DOI: http://www.doi.org/10.1080/01652176.2020.1766725

Tsagkogeorga G, Parker J, Stupka E, Cotton JA, and Rossiter SJ (2013). Phylogenomic analyses elucidate the evolutionary relationships of bats. Current Biology, 23(22): 2262-2267. DOI: http://www.doi.org/10.1016/j.cub.2013.09.014

Tsang KW, Ho PL, Ooi GC, Yee WK, Wang T, Chan-Yeung M, Lam WK, Seto WH, Yam LY, Cheung TM et al. (2003). A cluster of cases of severe acute respiratory syndrome in hong kong. The New England Journal of Medicine, 348(20): 1977-1985. DOI: http://www.doi.org/10.1056/NEJMoa030666

Vaqué Rafart J (2005). Síndrome respiratorio agudo grave (sars). Anales de Pediatría, 62: 6-11.

Varia M, Wilson S, Sarwal S, McGeer A, Gournis E, Galanis E, Henry B, and Hospital Outbreak Investigation Team (2003). Investigation of a nosocomial outbreak of severe acute respiratory syndrome (sars) in Toronto, Canada. Canadian Medical Association Journal, 169(4): 285-292. Available at: https://pubmed.ncbi.nlm.nih.gov/12925421/

Wang LF, Shi Z, Zhang S, Field H, Daszak P, and Eaton BT (2006). Review of bats and sars. Emerging Infectious Diseases, 12(12): 1834-1840. DOI: http://www.doi.org/10.3201/eid1212.060401

Wong ACP, Li X, Lau SKP, and Woo PCY (2019). Global epidemiology of bat coronaviruses. Viruses, 11(2): Article number 174. DOI: http://www.doi.org/10.3390/v11020174

World Health Organization (WHO) (2020a). Coronavirus disease (covid-19) pandemic. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019

World Health Organization (WHO) (2020b). Coronavirus disease (covid-19) weekly epidemiological update and weekly operational update. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/

World Health Organization (WHO) (2020c). Middle east respiratory syndrome coronavirus (mers-cov) – Saudi Arabia. Available at: https://www.who.int/csr/don/02-jul-2020-mers-saudi-arabia/en/

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, and McLellan JS (2020). Cryo-em structure of the 2019-ncov spike in the prefusion conformation. Science, 367(6483): 1260-1263. DOI: http://www.doi.org/10.1126/science.abb2507

Xiao K, Zhai J, Feng Y, Zhou N, Zhang X, Zou JJ, Li N, Guo Y, Li X, Shen X et al. (2020). Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins. Nature, 583(7815): 286-289. DOI: http://www.doi.org/10.1038/s41586-020-2313-x

Yang YL, Qin P, Wang B, Liu Y, Xu GH, Peng L, Zhou J, Zhu SJ, and Huang YW (2019). Broad cross-species infection of cultured cells by bat hku2-related swine acute diarrhea syndrome coronavirus and identification of its replication in murine dendritic cells *in vivo* highlight its potential for diverse interspecies transmission. Journal of Virology, 93(24): 01448-01419. DOI: http://www.doi.org/10.1128/JVI.01448-19

Yu WB, Tang GD, Zhang L, and Corlett RT (2020). Decoding the evolution and transmissions of the novel pneumonia coronavirus (SARS-CoV-2 / hcov-19) using whole genomic data. Zoological Research, 41(3): 247-257. DOI: http://www.doi.org/10.24272/j.issn.2095-8137.2020.022

Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, and Fouchier RAM (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. New England Journal of Medicine, 367(19): 1814-1820. DOI: http://www.doi.org/10.1056/NEJMoa1211721

Zhang J, Guo L, Xu Y, Yang L, Shi H, Feng L, and Wang Y (2017). Characterization of porcine epidemic diarrhea virus infectivity in human embryonic kidney cells. Archives of Virology, 162(8): 2415-2419. DOI: http://www.doi.org/10.1007/s00705-017-3369-2

Zhang T, Wu Q, and Zhang Z (2020). Probable pangolin origin of SARS-CoV-2 associated with the covid-19 outbreak. Current Biology, 30(7): 1346-1351, e1342. DOI: http://www.doi.org/10.1016/j.cub.2020.03.022

Zhang YZ, and Holmes EC (2020). A genomic perspective on the origin and emergence of SARS-CoV-2. Cell, 181(2): 223-227. DOI: http://www.doi.org/10.1016/j.cell.2020.03.035

Zhou L, Li QN, Su JN, Chen GH, Wu ZX, Luo Y, Wu RT, Sun Y, Lan T, and Ma JY (2019). The re-emerging of sads-cov infection in pig herds in southern China. Transboundary and Emerging Diseases, 66(5): 2180-2183. DOI: http://www.doi.org/10.1111/tbed.13270

Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zhu Y, Zhang YW, Xie QM, Mani S et al. (2018). Fatal swine acute diarrhea syndrome caused by an hku2-related coronavirus of bat origin. Nature, 556(7700): 255-258. DOI: http://www.doi.org/10.1038/s41586-018-0010-9

Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J et al. (2020). SARS-CoV-2 viral load in upper respiratory specimens of infected patients. New England Journal of Medicine, 382(12): 1177-1179. DOI: http://www.doi.org/10.1056/NEJMc2001737

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A Retrospective Study on Dog Bite Associated Rabies in Human and the Use of Post-exposure Prophylaxis in Nepal during 2008 to 2017

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ABSTRACT

A 10-year (2008-2017) retrospective canine-mediated human rabies epidemiology was studied to assess the burden of rabies in Nepal. To this end, the number of dog bites, the use of post-exposure prophylaxis (PEP), and human death records from 2008 to 2017 were retrieved from Sukraraj Tropical Hospital, Kathmandu, Nepal. The findings revealed that the number of human rabies occurrences was consistent with minor fluctuations throughout the study period. There were 252,297 dog bite cases in humans recorded between 2008 and 2017. Every month, 2,102 people were bitten by mostly stray dogs. There was a gradual increase in PEP use throughout 10 years. On average, 36,995 PEP dosages were used per year for stray dog bites. The PEP consumption and the number of human deaths were negatively correlated. A total of 482 human rabies deaths were recorded in Nepal during the study period. On average, 49 people died of canine-mediated rabies each year. Although there was an increase in the use of PEP, the number of human deaths and street dog bites recorded were still high. The high mortality due to rabies could then be attributed to the flawed surveillance system and stray dog population management, and not merely the lack of PEP services. Hence, it is recommended that the government agencies and other concerned stakeholders should organize mass vaccination and population management program for stray dogs in order to reduce the country's rabies burden.

Keywords: Dog bite, Epidemiology, Prophylaxis, Rabies

INTRODUCTION

Rabies has been viewed as an extreme and dismissed general medical issue worldwide, with an estimated 55,000 human deaths each year (WHO, 2013). The majority of death occurs in developing countries like Asia and Africa ones (Knobel et al., 2005). About 21,000-24,000 people died in Southeast Asia due to rabies infection (Gongal and Wright, 2011) and this infection counts for 45% of human deaths around the world (Masiira et al., 2018). Nonetheless, due to all rabies cases not being reported, the actual case numbers are unknown. Although the disease is undoubtedly lethal once clinical signs are developed in the patient, it can be prevented with timely pre-exposure and post-exposure prophylactic vaccination (Overall and Love, 2001). The exposure regimen of human diploid vaccines is 0, 7, and 21 or 28 days and need to be renewed after a year in risky populations whereas post-exposure prophylaxis regimen is 0, 3, 5, 14 to 28 days with the primary aids, i.e. thorough washing and flushing of wounds (WHO, 1997; WHO, 2010). As per the World Health Organization (WHO), more than 15 million people annually receive PEP for rabies worldwide mostly in India and China (WHO, 2010). Children are at the greatest risk of rabies exposure and approximately 40% of PEP is given to children aged between 5 and14 years old (WHO, 2007).

Nepal is a small and landlocked country situated between India and China. There is a free and open border with India from which restriction-free trade and movement of humans and animals occur, facilitating the transmission of infectious diseases (Yadav et al., 2020). Nepal and India share similar socio-financial circumstances, given that India carries the burden of the biggest rabies cases worldwide (Knobel et al., 2005). Nepal has been considered endemic in rabies for a long time with an average mortality of 100 people every year (Annual Report of the Department of Health Services, Nepal, 2013). In order to control rabies, the government of Nepal established a national coordinating committee for dog rabies expulsion in Nepal in 1979 (Joshi, 1991). However, it took the measures in 1983 by initiating the rabies control program, supported by the Department of Livestock Development and Animal Health (DLDAH), the Department of Health, and representatives of local government (Bögel and Joshi, 1990). The program involved the organization of mass vaccination in stray dogs for the first few years, but it terminated after few years because of the political changes and other internal reformation process happened in the country during the 1990s (Joshi, 1991).

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The production of reactogenic nerve-tissue vaccines started in Nepal in 2006 as an attempt to control rabies, but it was soon replaced by tissue culture vaccines. However, the production of reactogenic nerve-tissue vaccine phased out in the middle of 2006. Thereafter, recent rabies tissue-culture vaccines, such as human diploid cell vaccine (HDCV), purified Vero cell rabies vaccine (PVRV), purified chick embryo cell vaccine (PCECV), and purified duck embryo vaccine (PDEV) have come into practice. These vaccines were mainly imported from India. The cost of the whole course of the vaccine is equivalent to 12,000 Nepalese rupees (WHO, 2005). The Department of Livestock Service, Government of Nepal had developed a 10-year rabies control program in 2010 to produce 50,000 cell culture rabies vaccine for animal use, and the production of rabies vaccine for human use is in progress (Acharya et al., 2019). According to this plan, a rabies vaccine bank was supposed to be established at least at five regional veterinary laboratories in Nepal. However, it is not yet established due to financial constraints.

Stray dog bites have been considered as the major source of the rabies in human population in Nepal, but most of the cases go unreported because of poor surveillance, lack of awareness, and improper reporting system (Devleesschauwer et al., 2016). The stray dog population in Nepal has been increasing and humane efforts to control the uncontrolled dog population are very limited (Kakati, 2010). Children aged below 15 and old aged people are at the most risk of dog bites (Pantha et al., 2020). The first humane approach to control the stray dog population was established in 2004 by the Kathmandu Animal Treatment Centre (KAT), a non-governmental organization with limited resources. The local government in the capital of Nepal joined KAT's mission to control the stray dog population in 2017.

An effective strategy for control of rabies takes into account the epidemiology of animal bites; and rabies and factors influencing post-exposure treatment which is absent in Nepal (Gongal and Wright, 2011). Rabies is certainly not a notifiable illness in Nepal, and there is no coordinated monitoring program between medical veterinary divisions (Acharya et al., 2019). The genuine number of death due to rabies might be a lot higher than the reported cases (Sharma, 2005). There are very few and incomplete epidemiological reports on rabies and the available reports have discrepancies over the rabies statistics. Therefore, the purpose of this study was to explain the rabies epidemiology in terms of PEP consumption, dog bite injuries, and human death from 2008-2017 in Nepal. As Nepal has shown solidarity with the global approach to eradicate rabies by 2030, the finding of this study would assist the government authorities and other stakeholders in improving the current rabies surveillance and monitoring system leading to building up a systematic and effective surveillance system.

MATERIALS AND METHODS

Ethical approval

The present study did not involve either humans or animals as an experimental setup. Required permission in verbal forms to conduct the research and to use the data has been received from relevant authorities involving in this study.

Data source and data management

The study was a retrospective review of epidemiological surveillance data in humans on animal bite injuries, rabies deaths, and the use of PEP reported to the national database from 2008 to 2017. The raw data were collected from the Sukraraj Tropical Hospital, Teku, Kathmandu, Nepal, which is the only Central Referral Medical Center for infectious diseases that deals with rabies issues. In addition, human rabies occurrence data were collected from other hospitals and medical centers located in different parts of the country.

Data analysis

The raw data were categorized yearly and analyzed using descriptive statistics. Likewise, the occurrence of dog bites, human deaths due to rabies, and PEP use in the last 10 years were graphically plotted to examine the respective trends. Bivariate analysis was performed and a Pearson's correlation coefficient was used to describe the strength and direction of relationship among years, number of dog bites, number of anti-rabies vaccine for PEP, number of human deaths.

RESULTS

During the past 10 years, the burden of human rabies in Nepal appeared consistently with minor variations. There were 252,297 dog bite cases recorded and 2,219,701 PEP vials were used to counter those dog bites. In the same way, 482 people died of rabies during the same period. Likewise, 25,164 bites from other species of animals, including cats, monkeys, and rats were also documented by the Department of Health Services, Nepal (Annual Report of the Department of Health Services, Nepal, 2014).

There was a trend of a gradual increase of dog bites from 2008 to 2012 with a little drop in 2009 reaching the highest bites in 2013, and an abrupt drop was recorded in 2014. For the rest of the period, it remained stable (Figure 1).

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Likewise, deaths caused by rabies in the human population showed a constant and consistent pattern from 2008 to 2012. The occurrence gradually decreased from 2013 towards 2016, though there was an abrupt increase in the number of human deaths in 2017 (Figure 2).

The descriptive analysis showed the gradual increase of PEP use from 2008 to 2011, which was slightly decreased during 2012 and 2013. However, the use of PEP increased again between 2014 and 2015. Once again, the cases were decreased in 2016 and further increased again in 2018. The PEP vaccine use ranged from 145,978 to 320,139 vials (Figure 3). On average, 221,970 vials per year were used for dog bite cases.

Table 1 presents the results of bivariate correlation analysis. The statistical results revealed that there was a very weak relationship between dog bites and deaths and a negative correlation found between dog bites and PEP consumption. The number of deaths was negatively correlated with the number of anti-rabies vaccines for PEP. In addition, the number of deaths declined from 2008 to 2017.

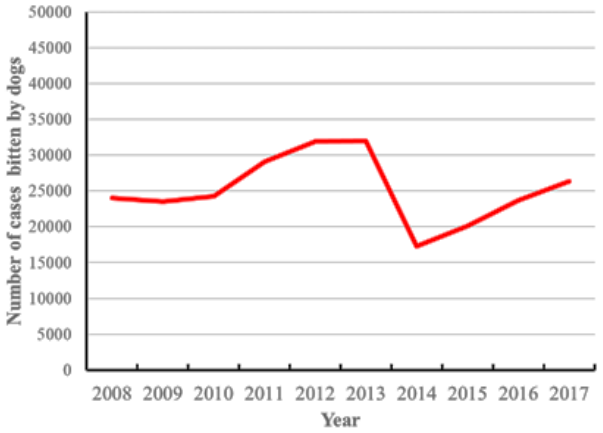

Figure 1. Dog bites cases reported to Sukraraj Tropical Hospital, Nepal from 2008 to 2017. Source: Department of Health Services, Government of Nepal.

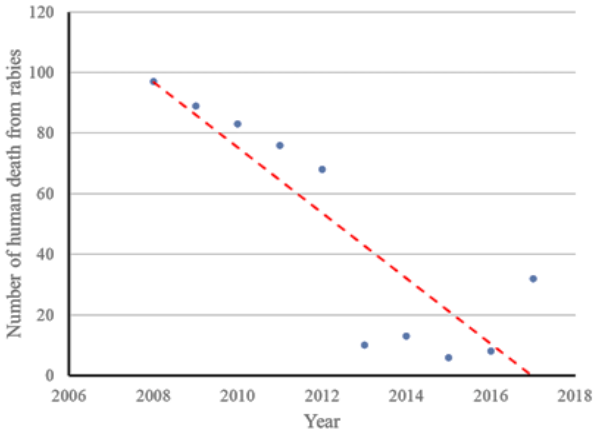

Figure 2. Human deaths due to canine-mediated rabies in Nepal from 2008 to 2017. Source: Department of Health Services, Government of Nepal.

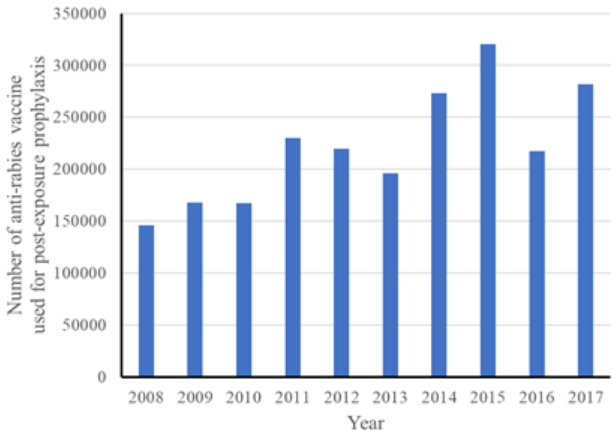

Figure 3. Number of post-exposure prophylaxis vials administered to dog bite patients in Nepal from 2008 to 2017. Source: Department of Health Services, Government of Nepal

Table 1. Correlation among year, number of cases bitten by dogs, number of anti-rabies vaccine for post-exposure prophylaxis (PEP), and number of deaths.

Variable	Year	Number of cases bitten by dogs	Number of anti-rabies vaccine for PEP	Number of Death
Year	1			
Number of cases bitten by dogs	-0.130	1		
Number of anti-rabies vaccine for PEP	0.799**	-0.316	1	
Number of deaths	-0.869**	0.221	-0.704*	1

* Correlation is significant at the $p < 0.05$. **Correlation is significant at the $p < 0.01$

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DISCUSSION

No reports of a large-scale survey have been available for appraisal of dog bites, use of PEP, and human rabies mortality. Hence, this study attempted to use available data to determine the rabies burden in Nepal. Prior to this work, small-scale-epidemiological reports based on a brief time frame had been documented (Joshi, 1991; Karki and Thakuri, 2010; Pantha et al., 2020). These previous studies are therefore used in the present study to make some relevant comparisons.

The present study showed that humans bitten by dogs occurred during 2008-2017 with an average of 25,229 dog bites per annum, and this figure accounts the 0.084% of the total population of Nepal. The dog bite injury in Nepal was 25% in 100,000 populations. On a monthly basis, 2,102 people got bitten by the dogs. Most of these bites were reported from urban areas, where the stray dog population is higher than the rural areas. Kathmandu Animal Treatment Centre reported that more than 22,500 stray dogs were roaming in Kathmandu City, the capital city, alone in 2012 (Kakati, 2010). Without precise information on patterns of human rabies and difficulties in rabies diagnosis in developing countries, the utilization of surveillance data on animal bites gives valuable data to rabies observation and improves the distribution of clinical and veterinary assets (Martin et al., 1969). It was also comparable to the dog bite occurrence rates with immediate neighboring countries, such as India and China, which recorded more than 15 million dog bites per year (WHO, 2013). It might be due to the higher population of stray dogs in those countries. At the regional level, Bhutan, another country similar in topography and cultural settings reported around 6,416 dog bites in 2018, which was significantly lower than Nepal. Another country in Asia, the Philippines reported 32,859 dog bite occurrences per annum, which were much higher than Nepal (Gongal and Wright, 2011). A study in Central India reported that 95.8% of victims were bitten by stray dogs (Marathe and Kumar, 2016), which is very much in alignment with our findings. Similar findings were also reported from Uganda, the study stated that 25,420 patients reported roaming dog bite injuries (Wangoda et al., 2019). The dog bite occurrence revealed a peak in 2103 and a sharp declined in 2014. The increasing occurrence of animal bites was suggesting that the potential exposure to rabies infection remains important to public health and so vice versa. The results were similar to the study performed in Uganda (Dodet and African Rabies Bureau, 2009).

This study also showed PEP consumption and human rabies exposure for 10 years (2008-2017) in Nepal. Results revealed that there was a gradual increase throughout the 10 years in PEP use with a small drop in 2013, which was still higher than the first three years. It was observed that the PEP use and dog bites were negatively correlated. However, dog bites and PEP consumption have both increased for the last 10 years. However, in comparison to dog bite cases, the PEP use was higher. This is in agreement with other findings (Helmick, 1983; Khkhar et al., 2003; Sriaroon et al., 2005), which might be due to the increased awareness level of people residing in urban areas. Moreover, hospital authorities informed us that they recommended patients for PEP even for minor scratching and licking because of several reasons, such as poor diagnostic facilities in the hospitals, a large number of free-roaming and stray dogs, and no quarantine facility for biting animals (WHO, 2019). This finding is also in agreement with the study performed in Illinois, USA (Helmick, 1983). Despite the increased use of PEP, there were still enormous deaths which might be due to poor access to PEP facilities for the genuine cases. The shortage and immature availability of PEP in rabies clinics had been reported from India (Hanumanthaiah and Haradanhalli, 2019). In appropriate distribution and availability of PEP was an issue in China (Qi et al., 2018).

A total of 482 human rabies deaths were recorded in Nepal from 2008 to 2017. The number of human rabies cases gradually decreased from 2013 to 2016 and a slight increase was recorded in 2017. This phenomenon may be due to rabies control being emphasized and strengthened by the central government in Nepal after 2007. However, the human rabies cases remained stable from 2007 to 2012 indicating that human rabies constitutes a real public health threat in Nepal. On average, human mortality was 49 in the last 10 years. This finding was contradictory to the study performed by Pant in 2012 (Pant, 2013). He demonstrated that rabies mortality in humans ranges between 100 and 150 deaths per year in Nepal. Similar figures were also reported by WHO in 2013 (WHO, 2013). These higher estimates might be based on projected statistics from PEP use and under-reporting scenario. Another study carried out by WHO in 2007 stated that the rabies mortality was 0.21 per 100 000 human population while our study showed 0.0048, which was lower than the studies carried out in Tanzania (Cleaveland et al., 2002). This study did not include the data before 2008, so there might be a higher number of cases during or before 2007. In the Asian regions, Nepal lies in between in terms of the human rabies burden. India has the highest human death records 3 in 100,000 and Thailand (0.012/100,000) being the lowest (Acharya et al., 2019). However, the recent study from India indicated 2 deaths per 100,000 population due to rabies (Sudarshan et al., 2007).

CONCLUSION

Based on the findings, it can be concluded that the death rate in humans inflicted by canine-mediated rabies was considered unstable during the study period. However, the dog bite cases and PEP consumption increased, which

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indicates rabies is a public health challenge affecting Nepal. Eliminating rabies by the year 2030 would be possible if the strengthening of rabies prevention and control strategies are applied in a coordinated approach at all levels of the health and veterinary areas. These sectors should adopt the "One Health" approach with a strategic focus on strengthening rabies surveillance, controlling rabies in dogs, controlling the stray dog population, and ensuring the availability of post-exposure prophylaxis at rural health offices.

DECLARATIONS

Authors' contribution

P. Pal and T. Rukkwamsuk conceive the idea, designed the project, and write the manuscript. H. Shimoda and A. Yawongsa helped with the analysis of the data. R. Bashyal helped in data collection. T. Rukkwamsuk supervises the project throughout the study and write-up process. All authors had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final draft of the manuscript and the statistical analysis of data for publication.

Competing interests

The authors certify that there is no conflict of interest.

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Ethical considerations

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

REFERENCES

Acharya KP, Adhikari N, and Tariq M (2019). Fight against rabies in Nepal: Immediate need for government intervention. One Health, 9: Article number 100114. DOI: https://www.doi.org/10.1016/j.onehlt.2019.100114

Annual Report of the Department of Health Services, Nepal (2013). Available at: http://ghdx.healthdata.org/record/nepal-department-health-services-annual-report-2012-2013.

Annual Report of the Department of Health Services, Nepal (2014). Avialable at: http://edcd.gov.np/resources/dohs-annual-report-fy-2071-72.

Bögel K, and Joshi DD (1990). Accessibility of dog populations for rabies control in Kathmandu Valley, Nepal. Bulletin of the World Health Organization, 68: 611-617. Available at: https://apps.who.int/iris/bitstream/handle/10665/53422/bulletin_1990_68%285%29_611-617.pdf?sequence=1&isAllowed=y

Cleaveland S, Fevre EM, Kaare M, and Coleman PG (2002). Estimating human rabies mortality in the United Republic of Tanzania from dog bite injuries. Bulletin of the World Health Organization, 80: 304-310. Available at: https://pubmed.ncbi.nlm.nih.gov/12075367/.

Devleesschauwer B, Aryal A, Sharma BK, Ale A, Declercq A, Depraz S, Gaire TN, Gongal G, Karki S, Pandey BD et al. (2016). Epidemiology, impact and control of rabies in Nepal: A systematic review. PloS Neglected Tropical Diseases, 10: e0004461. DOI: https://www.doi.org/10.1371/journal.pntd.0004461.

Dodet B, and African Rabies Bureau (AfroREB) (2009) The fight against rabies in Africa: From recognition to action. Vaccine, 27(37): 5027-5032. DOI: https://www.doi.org/10.1016/j.vaccine.2009.06.030.

Gongal G, and Wright AE (2011). Human rabies in the WHO Southeast Asia region: Forward steps for elimination. Advances in Preventive Medicine, Article ID: 383870. DOI: https://www.doi.org/10.4061/2011/383870

Hanumanthaiah AND, and Haradanhalli RS (2019). Assessment of procurement, distribution, availability, and utilization of rabies biologicals for postpexposure prophylaxis in seven state of India. Indian Journal of Public Health, 63: 31-36. DOI: http://www.doi.org/10.4103/ijph.IJPH_365_19.

Helmick CG (1983). The epidemiology of human rabies postexposure prophylaxis, 1980-81. Journal of the American Medical Association, 250: 1990-1996. DOI: https://www.doi.org/10.1001/jama.1983.03340150032022.

Joshi DD (1991). Organization of veterinary public health in the South Asia region. Revue Scientifique et Technique, 10: 1101-1129. DOI: https://www.doi.org/10.20506/rst.10.4.587.

Kakati K (2010). Street dog population survey, Kathmandu 2010. Final Report to the World Society for the Protection of Animals, pp. 1-20. Available at: https://www.studylib.net/doc/7636360/street-dog-population-survey-kathmandu.

Karki S, and Thakuri KC (2010). Epidemiological situation of animal rabies and its control strategy in Nepal. The proceedings of the 9th conference of Kathmandu, Nepal: Veterinary Association, pp. 105-110. Available at: http://nva.org.np/downloadsdetail.php?id=2

Khkhar A, Meena GS, and Mehara M (2003). Profile of dog bites cases attending M.C.D. dispensary at Atipur, Delhi. Indian Journal of Community Medicine, 28: 157-160. Available at:

https://www.indmedica.com/journals.php?journalid=7&issueid=37&articleid=477&action=article.

Knobel DL, Cleaveland S, Coleman PG, Fèvre EM, Meltzer MI, Miranda MEG, Shaw A, Zinsstag J, and Meslin FX (2005). Re-evaluating the burden of rabies in Africa and Asia. Bulletin of the World Health Organization, 83: 360-368. Avialable at: https://www.pubmed.ncbi.nlm.nih.gov/15976877/

Marathe N, and Kumar S (2016). Epidemiological study of animal bite victims in Central India: a cross sectional institutional study. International Journal of Community Medicine and Public Health, 3(1): 78-82. DOI: https://www.dx.doi.org/10.18203/2394-6040.ijcmph20151220.

Martin RJ, Schnurrenberger PR, and Rose NJ (1969). Epidemiology of rabies vaccinations of persons in Illinois 1967-68. Public Health Report, 84: 1069-1077.DOI: https://www.doi.org/10.2307/4593757.

Masiira B, Makumbi I, Matovu JKB, Ario AR, Nabukenya I, Kihembo C, Kaharuza F, Musenero M, and Mbonye A (2018). Long term trends and spatial distribution of animal bite injuries and deaths due to human rabies infection in Uganda, 2001-2015. PLoS One, 13: e0198568. DOI: https://www.doi.org/10.1371.journal.pone.0198568.

Overall KL, and Love M (2001). Dog bites to humans–demography, epidemiology, injury and risk. Journal of the American Veterinary Medical Association, 218: 1923-1934. DOI: https://www.doi.org/10.2460/javma.2001.218.1923.

Pant GR (2013). Rabies control strategy in SAARC member countries. In: Proceedings of the Inception Meeting of the OIE/JTF Project for Controlling Zoonoses in Asia Under One Health Concept, Japan: World Organization for Animal Health. Available at: https://pdffox.com/rabies-control-strategy-in-saarc-pdffree-html.

Pantha S, Subedi D, Poudel U, Subedi S, Kaphle K, and Dhakal S (2020). Review of rabies in Nepal. One Health, 10: 100155. DOI: https://www.doi.org/10.1016/j.onehlt.2020.100155.

Qi L, Su K, Shen T, Tang W, Xiao B, Long J, Zhao H, Chen X, Xia Y, Xiong Y et al. (2018). Epidemiological characteristics and post-exposure prophylaxis of human rabies in Chongqing, China, 2007-2016. BMC Infectious Diseases, 18: Article number 6. DOI: https://www.doi.org/10.1186/s12879-017-2830-x.

Sharma M (2005). Knowledge and attitude of dog owner's towards the dog anti-rabies vaccination. Journal of Nepal Health Research Council, 3: 11-16. DOI: https://www.doi.org/10.33314/jnhrc.v0i0.94.

Sriaroon C, Jaijaroensup W, Tantawichien T, Benjawongkunchai M, Supich C, and Wilde H (2005). Common dilemmas in managing rabies exposed subjects. Travel Medicine and Infectious Disease, 3: 1-7. DOI: https://www.doi.org/10.1016/j.tmaid.2004.05.003.

Sudarshan MK, Madhusudana SN, Mahendra BJ, Rao NSN, Narayana DHA, Rahman SA, Meslin FX, Lobo D, Ravikumar K, and Gangaboraiah (2007). Assessing the burden of human rabies in India: Results of a national multi-center epidemiological survey. International Journal of Infectious Diseases, 11: 29-35. DOI: https://www.doi.org/10.1016/j.ijid.2005.10.007.

Wangoda R, Nakibuuka J, Nyangoma E, Kizito S, and Angida T (2019). Animal bite injuries in the accident and emergency unit at Mulago Hospital in Kampala, Uganda. The Pan African Medical Journal, 33: Article number 112. DOI: https://www.doi.org/10.11604/pamj.2019.33.112.16624.

World Health Organization (WHO) (1997). WHO recommendations on rabies post-exposure treatment and the correct technique of intradermal immunization against rabies. Available at: https://www.apps.who.int/iris/handle/10665/63396

World Health Organization (WHO) (2005). World Health Organization Expert Consultation on rabies. WHO Technical Report Series 931 First Report. Available at: http://www.wpro.who.int/NR/rdonlyres/B1ED8443-0993-408C-BF09-D1D06A6E1B45/0/FINALTEXTWHOTechnicalReportSeries090605.pdf

World Health Organization (WHO) (2007). Recommendations for inactivated rabies vaccine for human use produced in cell substrates and embryonated eggs. WHO Technical Report Series No. 941, Annex 2. Available at: https://www.who.int/biologicals/publications/trs/areas/vaccines/rabies/Annex%202%20inactivated%20rabies%20vaccine.pdf?ua=1

World Health Organization(WHO) (2010). Rabies vaccines: WHO position paper. Weekly Epidemiological Record, 85: 309-320. Available at: https://www.who.int/wer/2010/wer8532.pdf?ua=1.

World Health Organization (WHO) (2013). WHO Methods and Data Sources for Global Burden of Disease Estimates, 2000–2011. Global Health Estimates Technical Paper WHO/HIS/HSI/GHE/2013.4. Department of Health Statistics and Information Systems, World Health Organization. Available at: http://www.who.int/healthinfo/statistics/GlobalDALYmethods_2000_2011.pdf.

World Health Organization (WHO) (2019). Rabies: epidemiology and burden of disease. Available at: https://www.who.int/rabies/epidemiology/en/.

Yadav MP, Singh RK, and Malik YS (2020). Emerging and transboundary animal vial diseases: perspectives and preparedness. Emerging and Transboundary Animal Viruses, pp. 1-25. DOI: https://www.doi.org/10.1007/978-981-15-0402-0_1.

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Production of Newcastle Disease Polyclonal Antibody as the Alternative of Immunohistochemistry Primary Antibody against Newcastle Disease in Poultry

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ABSTRACT

Newcastle disease (ND) is the most pathogenic viral infection in poultry. Furthermore, the availability of laboratories that support the molecular diagnosis of ND is still limited in Indonesia. The present study aimed to produce ND polyclonal antibody as the alternative of immunohistochemistry primary antibody against ND in poultry. Two adult male New Zealand White rabbits weighed 2.5 kg were vaccinated seven days after the adaptation using intraperitoneal injection of the ND live vaccine at multilevel doses weekly. The serum was collected inactivated, and purified in the sixth week. A total number of 31 chicken samples were collected and their samples of brain, lung, spleen, and intestine were tested using immunohistochemistry and Reverse Transcription Polymerase Chain reaction (RT-PCR). The result showed that 19/31 (61%) were positive against immunohistochemistry and RT-PCR and a total of 12/31 (39%) were negative. Based on the obtained results, immunohistochemistry using ND polyclonal antibody had a similar accuracy with RT-PCR. It can be concluded that ND polyclonal antibody produced by vaccination in the rabbit could be used as the alternative immunohistochemistry primary antibody for diagnosing ND in poultry.

Keywords: Immunohistochemistry, Newcastle disease, Polyclonal antibody, Poultry, RT-PCR

INTRODUCTION

Newcastle disease (ND) is the most pathogenic viral infection in poultry. From the first outbreak of it in 1926 in Java and Newcastle, ND still has high morbidity and mortality among the poultry (Absalón et al., 2019). The ND causes severe lesions, including necrosis of brain tissue, intestine, and depression of the immune system. There are several types of ND infections in poultry including viscerotropic, neurotropic, mesogenic, respiratory, and asymptomatic. On the other hand, ND can be prevented by vaccination (Sarcheshmei et al., 2016).

The success of the vaccination program is still lacking in Indonesia due to the environmental factors, weather, and maintenance program. Newcastle disease infection in poultry is mostly undetected because of its manifestation types (Ogali et al., 2020). Furthermore, the distribution of laboratories in Indonesia that support the molecular test as a gold standard is quite limited and expensive compared to histopathology. In contrast, histopathology has low sensitivity, however, the sensitivity of tissue assessment can be increased by using the detection of an immunogenic molecule, such as an immunohistochemistry test (Zhang et al., 2017).

The immunohistochemistry increases the sensitivity and specificity of the tissue assessment performed by a pathologist since it can express the immunogenic molecule within the tissue section. The immunohistochemistry can be performed by using either monoclonal and polyclonal antibodies (Ascoli and Aggeler, 2018). Each type of antibody has a different procedure to be produced. Polyclonal antibody is commonly produced by vaccination in animal models, such as rabbits. The vaccination in rabbits induces the synthesis of antibodies by plasma cells. Nasiri et al. (2017) described that the vaccination in rabbits produces greater quantities of immunoglobulin G (IgG). The IgG is applicable for immunohistochemistry. The current study aimed to produce ND polyclonal antibodies through the vaccination in rabbits as the alternative of immunohistochemistry primary antibody against Newcastle disease compared to molecular tests using Reverse Transcription Polymerase Chain reaction (RT-PCR).

MATERIALS AND METHODS

Ethical approval

The animal experimentation in the present study was approved by the local ethical clearance committee from the Faculty of Veterinary Medicine, University of GadjahMada, Yogyakarta, Indonesia with reference number 0005/EC-FKH/2020. The committee conducted the monitoring and evaluation during this study.

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Time and place of study

The study was conducted from November 2019 until April 2020 in several places. The study was separated into three steps. The first step was antibody production and it was conducted in the Department of Pathology, Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia. The second step was an antibody purification and molecular test. Both antibody purification and the molecular test were conducted in the Laboratory of Microbiology and Virology, Station of Fish Quarantine and Quality Control, Yogyakarta, Indonesia. Finally, the third step was immunohistochemistry that conducted in the Integrated Laboratory, Faculty of Health, University of MuhammadiyahSidoarjo, East Java, Indonesia.

Newcastle disease polyclonal antibody production and purification

The vaccine used for producing polyclonal antibodies of ND was a live vaccine (Medivac, ND Lasota, Medion, Indonesia). The vaccination process was conducted in two male adult New Zealand White rabbits (Integrated Research and Testing Laboratory, University of Gadjah Mada, Yogyakarta, Indonesia) weighed 2.5 kg. The vaccine was injected intraperitoneally into the rabbits in the fourth week using multilevel doses 0.5 mL in the first week, 1.00 mL in the second week, 2.00 mL in the third week, and 3.00 mL in the fourth week. The serum was collected in the sixth week. Further, the serum was inactivated and purified following the procedure of Naf'an et al. (2020). The purified ND polyclonal antibody solution was stored inside the fridge at 4°C of temperature.

Field sample collection

The samples were obtained from semi-intensive local poultry farms in Yogyakarta, Indonesia. A total of 31 samples were collected in the current study. The chickens were randomly selected regardless of sex and age. All the collected chickens should indicate the clinical signs of ND infection such as torticollis, paralysis, lethargy. The chicken samples were included 11 broiler chickens, 10 laying chickens, and 10 free-range chickens (Figure 1). The chickens were euthanized by the cervical dislocation and they were necropsied. The chicken's brain, lung, spleen, and intestine were collected and used as the tested specimens. All those organs were divided into two parts, the first part was fixed using Neutral Buffer Formalin (NBF) for immunohistochemistry and the second one was fixed in the ethanol absolute for a molecular test.

Figure 1. Several clinical manifestations of the chickens used in the present study. The broiler chicken with torticollis (**A**), laying chicken with paralysis (**B**), and free-range chicken with lethargy (**C**)

Immunohistochemistry

The immunohistochemistry staining used the purified ND polyclonal antibody solution that has been produced in the first step of the study. The purified ND polyclonal antibody was used as the primary antibody. Further, the immunohistochemistry staining was performed following the procedure of the previous study (Prakoso et al., 2020). The positive result demonstrated a brown color, and the negative result did not demonstrate a brown color within the tissue section.

Reverse transcription-polymerase chain reaction

The RT-PCR was used as the comparison against the immunohistochemistry. The RT-PCR was chosen as the comparison because the molecular test is a gold standard for the detection of ND in poultry. The following primer was used as the molecular target, F: 5'-GCTGTATCTGTCTGACAAGCTCTC-3' and R: 5'-AGGTTGAGTTCTACACCAACCTGT-3' (Angeliya and Wibowo, 2014). The primer was obtained from the Disease Investigation Center (DIC) Lampung, Indonesia. The procedure of RT-PCR was conducted following the procedure of the previous study without any modification (Dhar et al., 2018).

Data analysis

All collected data were expressed as positive and negative. The data of immunohistochemistry and RT-PCR were analyzed by using descriptive qualitative methods. The sensitivity and specificity were measured using the following formulae: Sensitivity = (number of positive samples/ number of positive samples + number of false-positive samples) × 100%; Specificity = (number of negative samples/ number of negative samples + number of false-negative samples) ×

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100%.

RESULTS AND DISCUSSION

The results indicated that immunohistochemistry staining using ND polyclonal antibody stained brown color in the tissue that belonging to ND infection. The brown color appeared because of the utilization of diethylaminobenzidine (DAB) that was used as a chromogen during immunohistochemistry. The brown color was expressed as the reflection of antigen-antibody binding within the tissue (Figure 2A). In contrast, the negative control specimen did not present any reactivity (Figure 2B).

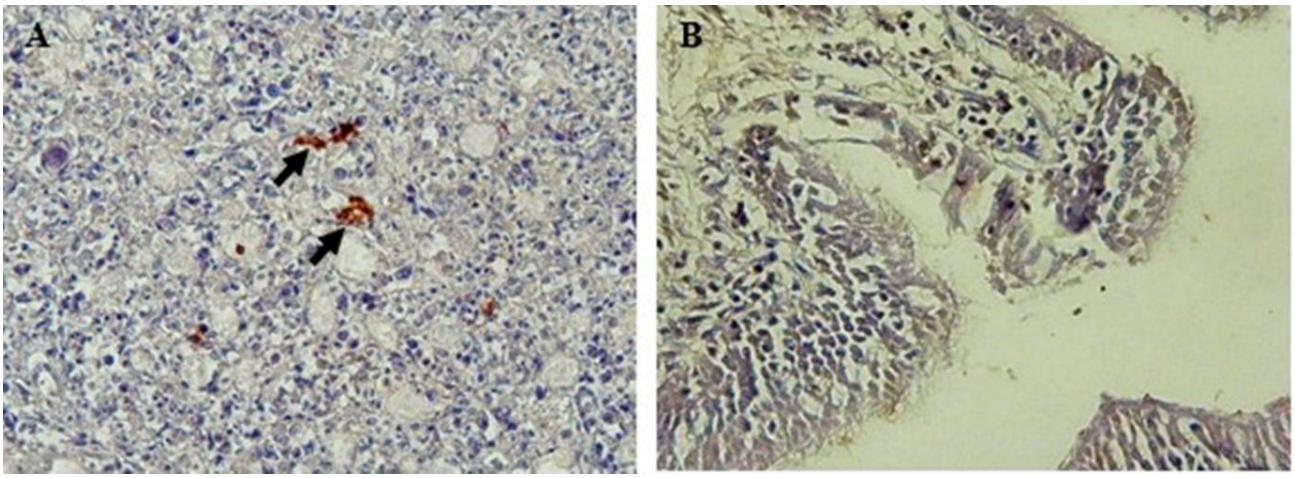

Figure 2. Positive and negative immunoreactivity of Newcastle Disease polyclonal antibodies within the spleen and intestine tissues from broiler chickens. The brown color expressed a positive immunoreactivity within a spleen tissue (**A**), there is no brown color within the intestine tissue that indicated negative immunoreactivity (**B**). Immunohistochemistry, ND polyclonal antibody, 200 × (A, B).

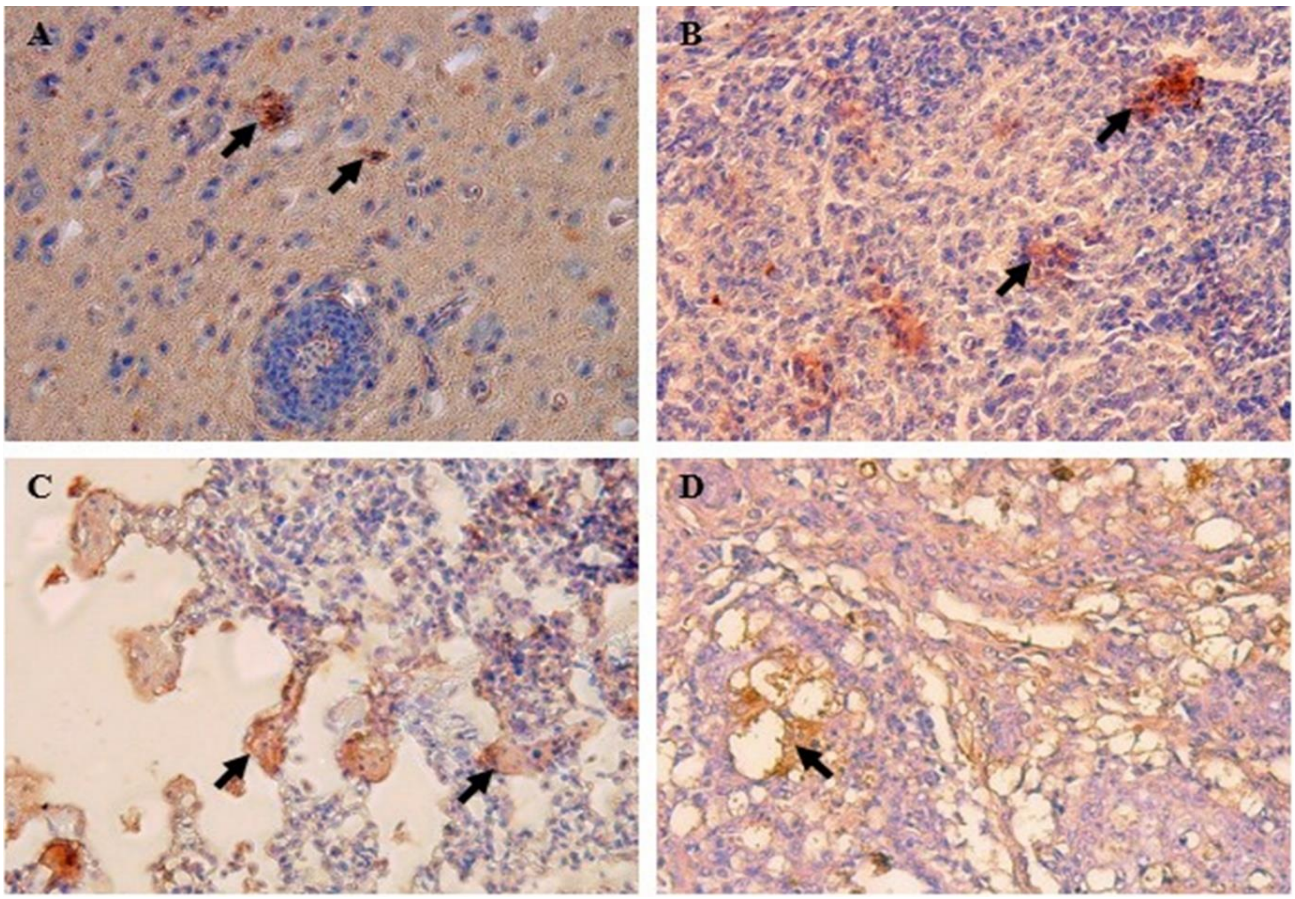

Figure 3. Immunoreactivity of Newcastle disease polyclonal antibodies in several organs of infected chickens. Positive immunoreactivity in the brain (**A**), spleen (**B**), lung's parabronchus (**C**), intestine mucosa (**D**). Immunohistochemistry, ND polyclonal antibody, 200 × (A, B).

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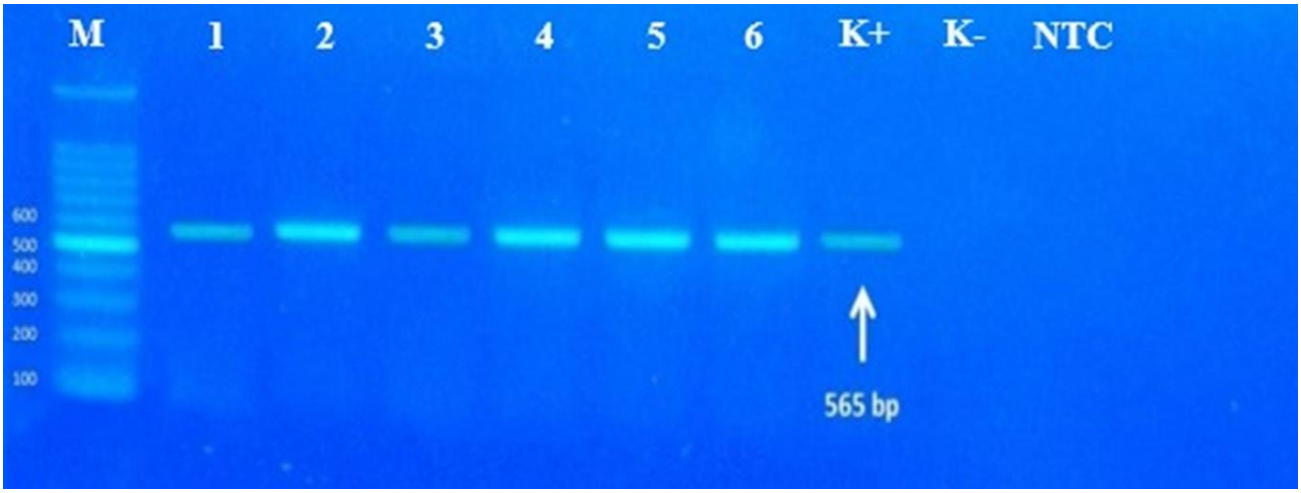

Figure 4. The RT-PCR results from the samples that have been stained with immunohistochemistry using Newcastle disease polyclonal antibody. M = marker, 1-6 = samples; K+ = positive control; K- = negative control; NTC = non template control.

Table 1. The comparison of immunohistochemistry staining using Newcastle disease polyclonal antibody and RT-PCR results against Newcastle disease infection from the collected specimens of infected chickens

Status of the sample		RT-PCR		Total
		Positive	Negative	
Immunohistochemistry using ND polyclonal antibody	Positive	19 (61%)	0 (0%)	19 (61%)
	Negative	0 (0%)	12 (39%)	12 (39%)
Total		19 (61%)	12 (39%)	31 (100%)

The immunohistochemistry using ND polyclonal antibody indicated that 19/31 (61%) of the collected specimens were positive against ND infection and the others were negative. The antigen-antibody binding within the tissue was detected on several organs including the brain (Figure 3A), spleen (Figure 3B), lung (Figure 3C), and intestine (Figure 3D). As the confirmation test, all the specimens that have been running with ND polyclonal antibodies were tested using RT-PCR. The RT-PCR demonstrated that 19/31 (61%) were positive and 12/31 (39%) were negative. That is similar to the result of immunohistochemistry using the ND polyclonal antibody (Table 1). Representation of RT-PCR from the collected specimens could be identified in Figure 4.

ND infection causes several clinical manifestations with high economic losses among the poultry industry. The disease is marked by its ability to spread and cause death. The primary gold standard test for the detection of ND infection is based on a molecular test, such as RT-PCR, However, RT-PCR is one of the expensive laboratory procedures for Indonesian farmers. Besides, the availability of RT-PCR in Indonesia is still limited, and not all veterinary laboratories can perform this test (Adi et al., 2010). Those limitations aggravate the incidence and prevalence of ND infection in a local poultry farm in Indonesia. The diagnosis of ND in the local poultry farm is commonly conducted based on its clinical signs, macroscopic, and microscopical lesion. The microscopic lesion is majority tested using histopathology (Cardiff et al., 2006).

The local farmer has been familiar to send the chickens to the laboratory for histopathology. Histopathology becomes the recommended laboratory test, because it is cheaper than the molecular test, even though histopathology is time-consuming, and has low sensitivity. The histopathology has low sensitivity because the pathognomonic lesion is not always representing within the tissue section (Knoblaugh et al., 2018). On the other hand, the sensitivity of tissue assessment can be increased by using immunohistochemistry. Immunohistochemistry has more accurate results because it detects the immunogenic molecule either in cells and tissue (Kim et al., 2016). It takes a long time to obtain the primary antibody for immunohistochemistry in Indonesia since limited companies are producing the primary antibody for immunohistochemistry in Indonesia. These factors potentially impact the availability of primary antibodies in the Indonesian market, and it makes both the researcher and technician waiting more than two months for the chemicals. One of the alternatives to conduct immunohistochemistry is by producing the polyclonal antibody through the vaccination of rabbits. The vaccination of New Zealand White rabbit produces a specific polyclonal antibody (Wicaksono et al., 2015).

The vaccination of the rabbit generates serum contained immunoglobulins which are specific for the detection of antigen within the samples. The polyclonal antibody is produced by the presentation of antigen with numerous epitopes during the vaccination. The numerous epitope antigen activates a large number of lymphocytes (Lipman et al., 2005). Further, the lymphocytes proliferate and differentiate into plasma cells that synthesize antibodies (Pioli, 2019). In the

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current study, the produced antibodies were determined as the polyclonal antibody. These polyclonal antibodies have high specificity because of their ability to recognize a specific epitope rather than forming cross-reaction.

The previous study described that utilization of polyclonal antibody produced by rabbit vaccination could be used as coagglutination kit with high sensitivity for diagnosing ND in poultry (Naf'an et al., 2020) and diagnosing viral nervous necrosis in fish (Sulistiyono et al., 2020). The findings of the present study indicated that ND polyclonal antibodies produced by vaccination can be used as the primary polyclonal antibody in chickens' tissue staining. The present study proved that ND polyclonal antibody has similar sensitivity and specificity compared to RT-PCR. It is also representing that produced ND polyclonal antibody in the current study had high affinity rather than avidity because it was able to bind the specific location and forming specific antigen-antibody binding. The present study provided an alternative primary antibody for immunohistochemistry against the ND virus that could be replaced the RT-PCR dependency to diagnosing ND infection. Like many other breakthroughs in veterinary medicine, the production of a polyclonal antibody for immunohistochemistry by using rabbit vaccination is a brilliantly simple idea.

CONCLUSION

The vaccination of ND live vaccine in New Zealand White rabbit generates serum consisted of polyclonal antibody. The ND polyclonal antibody produced by vaccination in the current study could be applied as an alternative polyclonal antibody for immunohistochemistry in diagnosing ND infection with high sensitivity and specificity compared to RT-PCR.

DECLARATIONS

Authors' contribution

All the authors contributed equally to the present study.

Competing interests

The authors have not declared any conflict of interests.

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All the staff from the Department of Pathology, Faculty of Veterinary Medicine, University of Gadjah Mada; staff from Laboratory of Microbiology and Virology, Station of Fish Quarantine and Quality Control, Indonesia, and staff from Integrated Laboratory, Faculty of Health, University of Muhammadiyah Sidoarjo, Indonesia were acknowledged for their assistance in the present study.

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

Absalón AE, Cortés-Espinosa DV, Lucio E, Miller PJ, and Afonso CL (2019). Epidemiology, control, and prevention of Newcastle disease in endemic regions. Latin America. Tropical Animal Health and Production, 51(5): 1033-1048. DOI: https://www.doi.org/10.1007/s11250-019-01843-z.

Adi AA, Astawa NM, Putra KS, Hayashi Y, and Matsumoto Y (2010). Isolation and characterization of a pathogenic Newcastle disease virus from a natural case in indonesia. The Journal of Veterinary Medical Science, 72(3): 313-319. https://www.doi.org/10.1292/jvms.09-0303.

Angeliya L, and Wibowo MH (2014). Molecular characterization of fusion gene fragment of Newcastle disease virus isolates in Disease Investigation Center Lampung working area from 2010-2013. Theses. Universitas Gadjah Mada, Indonesia. Available at: https://repository.ugm.ac.id/130667/.

Ascoli CA, and Aggeler B (2018). Overlooked benefits of using polyclonal antibodies. Biotechniques, 65(3): 127-136. DOI: https://www.doi.org/10.2144/btn-2018-0065.

Cardiff RD, Gregg JP, Miller JW, Axelrod DE, and Borowsky AD (2006). Histopathology as a predictive biomarker: Strengths and limitations. The Journal of Nutrition, 136(10): 2673-2675. DOI: https://www.doi.org/10.1093/jn/136.10.2673S

Dhar PK, Dutta A, Das A, Jalal MS, Barua H, and Biswas PK (2018). Validation of real-time reverse transcription-polymerase chain reaction to detect virus titer and thermostability of Newcastle disease live virus vaccine. Veterinary World, 11(11): 1597-1603. DOI: https://www.dx.doi.org/10.14202%2Fvetworld.2018.1597-1603.

Kim SW, Roh J, and Park CS (2016). Immunohistochemistry for pathologist: Protocols, pitfalls, and tips. Journal of Pathology and Translational Medicine, 50(6): 411-418. DOI: https://www.doi.org/10.4132/jptm.2016.08.08.

Knoblaugh SE, Hohl TM, and La Perle K (2018). Pathology principles and practices for analysis of animal models. ILAR Journal, 59(1): 40-50. https://www.doi.org/10.1093/ilar/ilz001.

Lipman NS, Jackson LR, Trudel LJ, and Weis-Garcia F (2005). Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. ILAR Journal, 46(3): 258-268. DOI: https://www.doi.org/10.1093/ilar.46.3.258.

Naf'an MK, Kurniasih K, Untari T, and Prakoso YA (2020). Development of coagglutination kit as rapid test for diagnosing

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Newcastle disease in poultry. Veterinary World, 13(8): 1719-1724. DOI: https://www.doi.org/10.14202/vetworld.2020.1719-1724.

Nasiri H, Valedkarimi Z, Aghebati-Maleki L, Abdolalizadeh J, Kazemi T, Esparvarinha M, and Majidi J (2017). Production and purification of polyclonal antibody against F(ab')2 fragment of human immunoglobulin G. Veterinary Research Forum : An International Quarterly Journal, 8(4): 307-312. Available at: https://www.ncbi.nlm.nih.gov/pubmed/29326789.

Ogali IN, Okumu PO, Mungube OO, Lichoti JK, Ogada S, Moraa GK, Agwanda BR, and Ommeh SC (2020). Genomic and pathogenic characteristic of virulent Newcastle disease virus isolated from chicken in live bird markets and backyard flocks in Kenya. International Journal of Microbiology, pp. 1-11. DOI: https://www.doi.org/10.1155/2020/4705768.

Pioli PD (2019). Plasma cells, the next generation: beyond antibody secretion. Frontiers in Immunology, 10(2768): 1-7. DOI: https://www.doi.org/10.3389/fimmu.2019.02768.

Prakoso YA, Rini CS, Rahayu A, Sigit M, and Widhowati D (2020). Celery (Apiumgraveolens) as a potential antibacterial agent and its effect on cytokeratin-17 and other healing promoters in skin wounds infected with methicillin-resistant *Staphylococcus* aureus. Veterinary World, 13(5): 865-871. DOI: https://www.doi.org/10.14202/vetworld.2020.865-871.

Sarcheshmei M, Dadras H, Mosleh N, and Mehrabanpour MJ (2016). Comparative evaluation of the protective efficacy of different vaccination programs against a virulent field strain of the Newcastle disease virus in broilers. Brazilian Journal of Poultry Science, 18(3): 363-370. DOI: http://www.dx.doi.org/10.1590/1806-9061-2015-0128.

Sulistiyono A, Kurniasih K, and Untari T (2020). Detection of nervous necrosis virus of grouper fish in Lombok Island, Indonesia based on serological and molecular studies. Egyptian Journal of Aquatic Biology and Fisheries, 24(1): 329-335. DOI: https://www.dx.doi.org/10.21608/ejabf.2020.70593.

Wicaksono WP, Permana DS, Maryam R, and Einaga Y (2015). Synthesis of polyclonal antibody against aflatoxin B1. Makara Journal of Science, 19(3): 91-95. DOI: https://www.doi.org/10.7454/mss.v19i3.4847.

Zhang H, Wen W, and Yan J (2017). Application of immunohistochemistry in hydrobiological studies. Aquaculture and Fisheries, 2(3): 140-144. DOI: https://www.doi.org/10.1016/j.aaf.2017.04.004.

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Transcriptional, Mitochondrial Activity, and Viability of Egyptian Buffalo's Granulosa Cells *In Vitro* Cultured under Heat Elevation

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ABSTRACT

It is documented that heat stress caused impairment on the reproductive performance of dairy animals. However, there are few reports that have focused on the molecular and intracellular responses of *in vitro* cultured buffalo granulosa cells during heat elevation. The present study was conducted to investigate the effect of heat elevation during *in vitro* culture of buffalo granulosa cells on their viability, quality, mitochondrial activity, and transcriptional activity. Granulosa cells were harvested after aspiration of cumulus-oocytes complexes that were collected from abattoir ovaries. The granulosa cells were cultured *in vitro* either at a normal physiological temperature suitable for oocyte maturation and embryo development (38.5°C) or exposed to the elevated temperature of 40.5°C on day 3 of culture (the first two days were for confluence) for two hours of culture then continued at 38.5°C up to day 7 of culture. The viability of granulosa cells was measured using trypan blue and quality was estimated by measuring the level of intracellular reactive oxygen species (ROS) on day 7. Moreover, metabolic activity was performed by measuring the fluorescent intensity of mitochondria. Moreover, transcriptional activity was done by profiling four selected candidate genes using quantitative real-time PCR. The results indicated that the granulosa cells viability rate significantly decreased in the heat stress group (25.1 ± 3.7), compared to the control group (36.6 ± 5.3) on confluence day (day 3). In addition, the viability rate on the last day of culture (day 7) decreased in heat stress, compared to control (83.7 ± 4.5 and 97.4 ± 0.4, respectively). On the other hand, there was a nonsignificant difference in ROS profile between the control ($21.7*10^4$ ± 1.3) and the heat-stressed group (15.7 ± 0.7) on day 7 of culture. However, the mitochondrial fluorescent intensity was higher in the control (21.9 ± 1.9) than in the heat-stressed group (15.4 ± 0.8) on day 7 of culture. The expression of cellular defense (HSF1) and apoptosis-inducing gene (P53) were significantly up-regulated in granulosa cells exposed to heat elevation, compared to the control group. On the other hand, the steroidogenesis-regulating gene (StAR) was down-regulated in granulosa cells cultured under heat shock, compared to the control group. In conclusion, heat stress reduced the viability of granulosa cells by inducing the expression of an apoptosis-related gene (P53) and compromised expression of genes regulating the steroid biosynthesis, which resulted in up-regulation of cell defense gene (HSF1) in an attempt to ameliorate the deleterious effect of heat stress on the biological activity of the granulosa cells.

Keywords: Apoptosis, Granulosa, Heat stress, Gene expression

INTRODUCTION

There are many different challenges that the livestock sectors face in developing countries, including nutrition deficiency, poor management, and heat stress. Among the environmental stressors, heat stress (HS) has a negative impact on animal reproductive performance causing great economic losses (Sammad et al., 2020). The HS impairs both ovarian functions and the developmental competence of oocytes (Sammad et al., 2020). The mammalian ovarian follicle consists of an oocyte that is surrounded by granulosa (GCs) and theca cells producing molecules, hormones, and nutrients to maintain the oocyte development potential, ovulation, and preimplantation embryo development (Albertini et al., 2001).

Granulosa cells are ovarian cells that enclose the follicle cavity and have a cross talk with oocytes through physical contact with zona pellucida and gap junctions, which facilitate the exchange of biological factors (Jancar et al., 2007). This crosstalk allows GCs to control oocyte maturation and its transcription activity (Carabatsos et al., 2000). Indeed, granulosa cells play a critical role in oocyte maturation and subsequent embryonic development (Gilchrist et al., 2004) by providing growth factors, amino acids, ions, and hormones (estrogen and progesterone). In addition, the ruptured follicle forms the corpus luteum after ovulation, and luteinized GCs become the main source for progesterone synthesis, which is the key to placenta development and pregnancy maintenance (Denkova et al., 2004; Matsuda et al., 2012). The steroidogenic activity of granulosa cells is controlled by many genes such as Steroidogenic Acute Regulatory Protein (StAR), Cytochrome P450 17A1 (CYP17A1), and 3-beta-Hydroxysteroid dehydrogenases (HSD3B2). In bovine, HS compromises follicular development, *in vitro* maturation, and fertilization of oocytes by impairing steroidogenic activity and viability of granulosa cells (Roth et al., 2001a; Roth et al., 2001b).

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Therefore, the current study focused on the evaluation of granulosa cells viability and transcriptional activity when the cells were cultured *in vitro* under HS, compared to normal conditions.

MATERIAL AND METHODS

Experimental design

A constant concentration of granulosa cells was cultured in six-well cell culture plates. Cells were divided into two groups. The first group was the control that cultured under *in vitro* normal temperature 38.5°C while the second treated group was exposed to heat stress at 40.5°C for 2 hours on day 3 of culture (granulosa cells were confluent), followed by normal temperature until day 7 of culture. Cells viability was measured using trypan blue and quality was estimated using intracellular reactive oxygen species successively on day 3 after heat treatment and day 7. Moreover, metabolic activity was performed by measuring the mitochondrial activity and transcriptional activity was done by profiling four selected candidate genes (HSF1, StAR, and P53, BCL2) using quantitative real-time PCR (Ghanem et al., 2020b).

Chemicals and reagents

Collection of ovaries and granulosa cells preparation

The collection of GCs was done according to Ghanem et al. (2020a). Ovaries were collected from local slaughtered houses in physiological saline supplemented with gentamycin and kept at approximately 37°C. Granulosa cells were aspirated from buffalo's follicles (2-8 mm). After oocyte selection, granulosa cells were centrifuged at 1500 rpm for 5 minutes. The pellet of granulosa cells then was re-suspended in the washing medium [TCM-199 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal calf serum (Gibco, Thermo Fisher Scientific, USA) and 1% antibiotic (streptomycin and penicillin)]. An 18-gauge needle mechanically broke the clumps of the cell. Finally, a total of 500000 cells were cultured per well under 38.5°C 5% CO2 and humidified air until the treatment.

Granulosa cells trypsinization

The trypsinization of GCs was performed according to Ghanem et al. (2020a). The medium was aspirated from each well slowly and the cell layer was washed twice with sterilized phosphate buffer saline (PBS). A total volume of 100 µl of trypsin EDTA (Sigma-Aldrich, St. Louis, MO, USA) solution (10%) was added to every well slowly then the plate was incubated at 38.5°C for 30 seconds. In the next step, 1 ml of washing medium was added and the suspension was centrifuged at 1500 rpm for 5 minutes. The pellets of GCs were mixed with 1 ml of washing medium.

Granulosa cells viability

Cells viability was determined using trypan blue (0.4%) according to Ghanem et al. (2020a). A total volume of 10 µl of cell suspension was mixed with 10 µl of trypan blue and incubated at room temperature for 1-2 minutes. Total cell count and viable cell count were calculated by hemocytometer using a magnification of 10 X (Inverted Microscope, Leica DMI 3000B, Wentzler, Germany).

Intracellular reactive oxygen species detection

Intracellular reactive oxygen species (ROS) were detected by 6-carboxy-2′, 7′-dichlorodihydro fluorescein diacetate (H2DCFDA; life technologies, California, USA) according to the protocol described by the manufacturer with some modifications according to Ghanem et al. (2020a). Granulosa cells from each group were incubated with 985 µl of 15 µl MH2DCFDA mixed with 970 µl PBS at 38.5°C for 45 minutes. The cells were washed with PBS and images were captured with a Nikon Eclipse Ti-S microscope (Nikon Instruments Inc., Tokyo, Japan) using a blue-fluorescence filter, and images were acquired by LAS Core software.

Mitochondrial activity

Mitochondrial activity of buffalo GCs was determined using MitoTracker® Green FM (M7514, life technologies) according to the manufacturer's instructions with some modifications according to Ghanem et al. (2020a). The GCs from each group were incubated with 200 µl MitoTracker green dye to 800 µl PBS for 45 minutes, followed by washing with PBS. The images were captured with a Nikon Eclipse Ti-S microscope (Nikon Instruments Inc., Tokyo, Japan) using a blue-fluorescence filter, and images were acquired by LAS Core software.

Image analysis after fluorescent staining

Captured images (13 images) per stain were processed using Image J software. The data of fluorescence intensity were presented as mean ± SD.

RNA isolation

Total RNA was extracted using GeneJet RNA Purification Kit (ThermoFisher Scientific, USA) from three different biological replicates of granulosa cells of each experimental group according to Faheem et al. (2021). First, a volume of

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600 µL of Lysis buffer was supplemented with 12 µL of β-mercaptoethanol added to each sample tube, and mixed with vortex until homogenization was reached. The sample tubes were centrifuged at 16000 × g for 5 minutes. The mix was transferred into a new RNase-free microcentrifuge tube. After that 600 µL of ethanol (96-100%) were added and the solution was mixed by pipetting. Up to 700 µL of lysate were transferred to the GeneJET RNA Purification Column inserted in a collection tube. The columns were centrifuged at 12000 × g for 1 minute. The flow-through was discarded and the purification column was placed back into the collection tube. This step was repeated until all of the lysates were transferred into the column and centrifuged. The collection tube containing the flow-through solution was discarded and the GeneJET RNA Purification Column was placed into a new 2 mL collection tube. Afterwards, 700 µL of wash buffer 1 (supplemented with ethanol) was added to the GeneJET RNA purification column and centrifuged at 12000 × g for a minute. The flow-through was discarded and the purification column was placed back into the collection tube. Moreover, 600 µL of Wash Buffer 2 (supplemented with ethanol) was added to the GeneJET RNA purification column and centrifuged at 12000 × g for a minute. The flow-through was discarded and the purification column was placed back into the collection tube. The previous step was repeated using 250 µL of wash buffer 2 that was added to the GeneJET RNA purification column and centrifuged at 12000 × g for 2 minutes. The flow-through solution was removed and the purification column was moved to a new tube. Finally, RNA was eluted by adding 20 µL of nuclease-free to the center of the GeneJET RNA purification column membrane and centrifuged at 12000 × g for 1 minute. The DNA residue was removed by adding 1 µl of DNases and 1 µl of MgCl2 buffer (ThermoFisher Scientific, USA) to each RNA sample and incubated at 37°C for 30 minutes in a PCR instrument (ThermoFisher Scientific, USA) then 1µl of EDTA was added and incubated at 65°C for 10 minutes. The purification column was discarded and eluted total RNA was measured using a nanodrop spectrophotometer (ThermoFisher Scientific, USA) and purity was estimated using measurement at 260/280 ratio. The extracted total RNA was stored at -70°C in an ultra-cool freezer (ThermoFisher Scientific, USA) until further use.

The synthesis of cDNA

The reverse transcription of RNA samples to cDNA was done using RevertAid first-strand cDNA synthesis kit (ThermoFisher Scientific, USA) according to Ghanem et al. (2020b). The following chemicals were added to each of RNA samples, 1 µl of oligo dt18 primer, 4 µl of PCR buffer, 2 µl of dNTPs, 1 µl of RNase inhibitor, 1 µl RNase inhibitor enzyme, 1 µl of reverse transcriptase enzyme were gently mixed by pipetting. The PCR mix was incubated in PCR thermocycler (Thermo Fisher Scientific, USA) at 42°C for 60 minutes, then at 70°C for 5 minutes and at 4°C overnight.

Quantitative real-time PCR analysis

Three replicates from each treatment were used for profiling selected candidate genes using quantitative real-time PCR analysis. Each pair of primers (Table 1) of selected candidate genes (HSF1 and StAR) and housekeeping gene (GAPDH) were designed using primers3 software (https://primer3.ut.ee/). The real-time PCR reaction mix was prepared by adding 12 ul of Maxima Sybr green PCR master mix (ThermoFisher Scientific, USA), 5.4 ul nuclease-free water, and 0.3 ul of forward and reverse primers which were incubated in StepOnePlus™ instrument (ThermoFisher Scientific, USA). The PCR mix was incubated at 50°C for 2 minutes, initial denaturation was done at 95°C for 10 minutes followed by 40 cycles of 95°C (Denaturation) for 15 minutes than at 60°C for 1 minute (annealing), and finally, melt curve at 95°C for 15 seconds and then 60°C for 1 minute. The expression data were analyzed using the delta-delta Ct method after normalization of the target genes with the housekeeping gene.

Table 1. List of primers used for quantitative real-time PCR analysis

Gene Name	Gene bank accession number	Primer sequence	Fragment size (bp)
StAR	XM_006183353.3 DQ062682.1	F:5′-CCATGGAGAGGCTTTATGAA-3′ R:5′-TCTTTCCCAATCTTCTGCAG-3′	103
HSF1	KC568561	F:5’-CGACCACCCTCATTGACTCC-3’ R:5’-CATCTTTGGAGTGCGAGCCA-3’	170
P53	XM_006175816.3	F:5′ - CCACCTGAAGTCTAAGAAGG-3′ R:5′ - AGTGCAGGTCAACTTCTTTA-3′	250
BCL2	XM_010979993.	F:5′ACATCCACTATAAGCTGTCG3′ R:5′ -TAGCGCCGAGAGAAGTCAT3′	241
GAPDH	NM_001034034.2	F:5′-TGCCCAGAATATCATCCCT-3’ R:5′- CTCATCATACTTGGCAGGTT-3’	166

Statistical analysis of data

The viability of granulosa cells, metabolic activity, and ROS level data were analyzed by applying a One-way Analysis of Variance (ANOVA). The analyzed data were expressed as mean ± standard error (SE) of means (SEM). Comparisons were significantly different if $p < 0.05$. Statistical analysis of data was performed using the IBM SPSS

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Statistics 22 program (SPSS Inc., Chicago, Illinois, USA). The expression profiles of selected target genes were analyzed using the SAS (SAS, 2004) using the general linear model (GLM) procedure. In addition, Duncan's test was used to detect differences among means of the two studied groups. Values of means were considered significant at $p < 0.05$.

RESULTS

Granulosa cells viability rate

At the beginning of the experiment, the viability rate was 88.3. The viability rate of granulosa cells showed a significant increase in control (36.6 ± 5.3), compared to the heat-treated group (25.1 ± 3.7) on day 3 of *in vitro* culture (Table 2). Moreover, there was a significant ($p < 0.05$) increase in granulosa cells viability rate in control (97.4 ± 0.4), compared to the HS group (83.7 ± 4.5) at the end of the culture period (day 7) as shown in Table 2.

Table 2. Viability rate of buffalo granulosa cells after heat shock at 40.5°C for 2 hours on days 3 and 7 of *in vitro* culture

Items	Initiate	At confluence (day 3)	End of culture (day 7)
Control	88.3 ± 0.0	36.6 ± 5.3	97.4 ± 0.4[a]
Heat-treated GCs	88.3 ± 0.0	25.1 ± 3.7	83.7 ± 4.5[b]

GCs: Granulosa cells

Mitochondrial activity

Mitochondrial activity was detected at the end of the culture period (day 7). Moreover, the mitochondrial fluorescent intensity was higher ($p < 0.05$) in the control (21.9 ± 1.9) than in the heat-stressed group (15.4 ± 0.8) as shown in figures 1 and 2 (a and b).

Reactive oxygen species level

There was an insignificant difference between the control (21.7 ± 1.3) and the heat-stressed group (15.7 ± 0.7) on day 7 of culture figures 3 and 4 (a and b).

Gene transcriptional profile

Heat shock factor 1 expression

The transcriptional profile of HSF1 gene was significantly up-regulated in granulosa cells exposed to HS compared to that cultured under normal temperature (Figure 5).

Steroidogenic acute regulatory gene (star) expression

The expression of StAR gene was up-regulated significantly ($p < 0.05$) in the granulosa cells of the control, compared to the HS group (Figure 6).

Antiapoptosis-related gene

The expression of BCL2 was similar in the granulosa cells of the control group and HS group (Figure 7).

Apoptosis-related gene

The transcript abundance of P53 gene was increased significantly in the granulosa cells of control compared with that of the HS group (Figure 8).

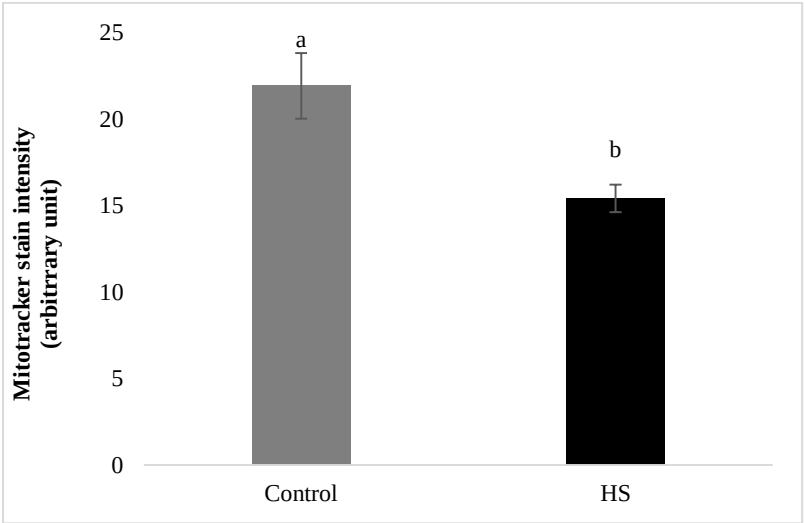

Figure 1. Mitochondrial fluorescent intensity of buffalo granulosa cells after heat shock at 40.5°C for 2 hours on day 7 of *in vitro* culture

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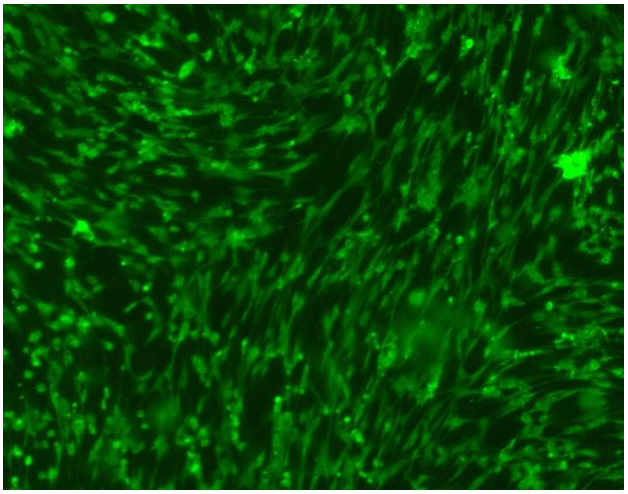

Figure 2a. Mitochondria of granulosa cells cultured *in vitro* under normal temperature and stained with Mitotracker

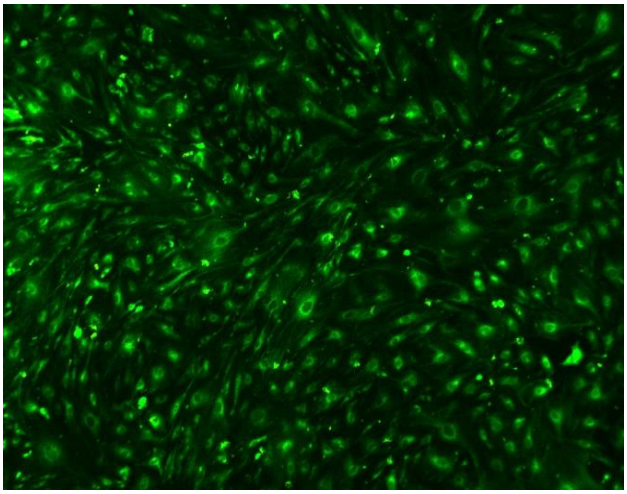

Figure 2b. Mitochondria of granulosa cells cultured *in vitro* under heat stress conditions and stained with Mitotracker

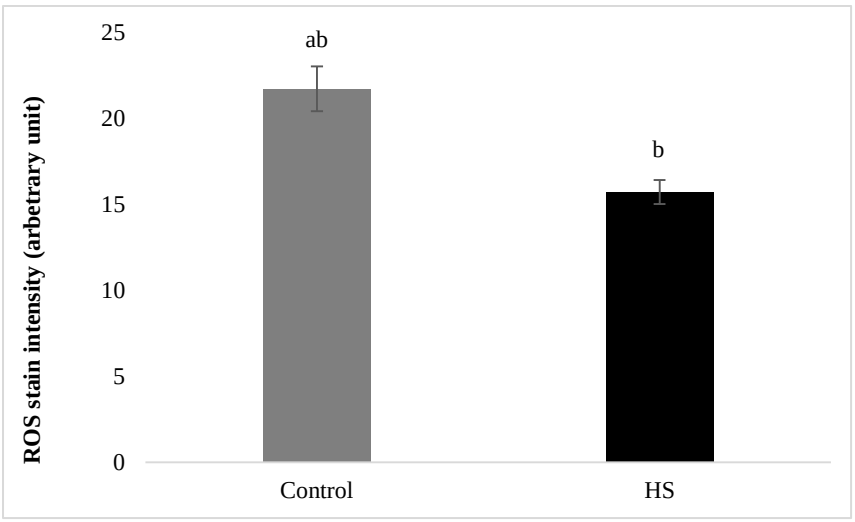

Figure 3. The level of reactive oxygen species of buffalo granulosa cells after heat shock at 40.5°C for 2 hours on day 7 of *in vitro* culture

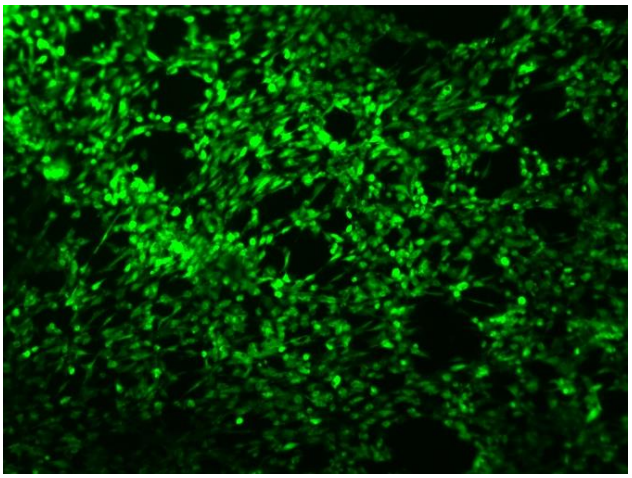

Figure 4a. Reactive oxygen species of granulosa cells cultured under normal condition and stained with MH2DCFDA

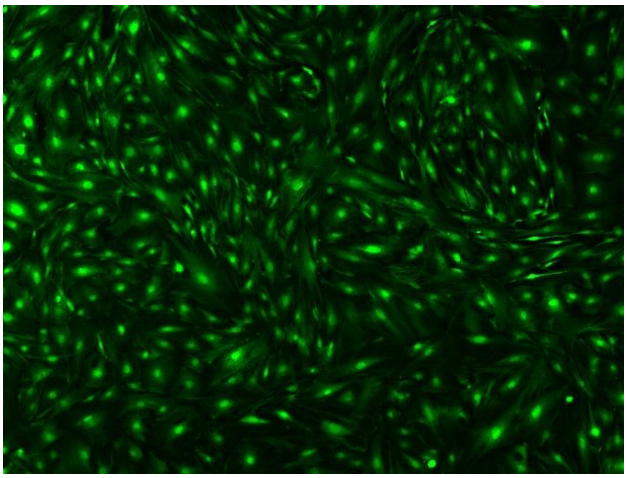

Figure 4b. Reactive oxygen species of granulosa cells cultured under heat stress and stained with MH2DCFDA

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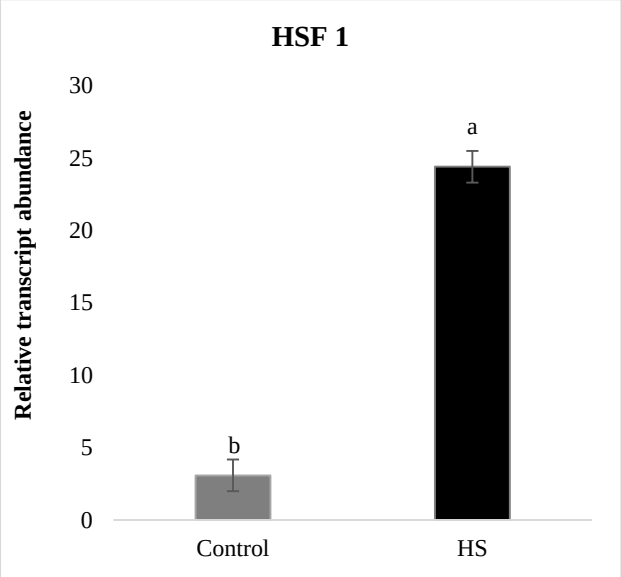

Figure 5. The expression profile of heat shock factor 1 of buffalo granulosa cells after heat shock at 40.5°C for 2 hours on day 7 of *in vitro* culture

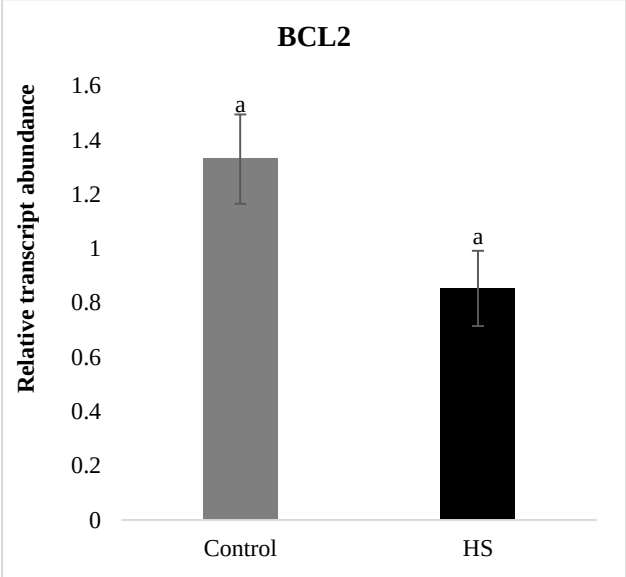

Figure 7. The expression profile of anti-apoptotic related transcript (BCL2) of buffalo granulosa cells following heat shock at 40.5°C for 2 hours on day 7 of *in vitro* culture

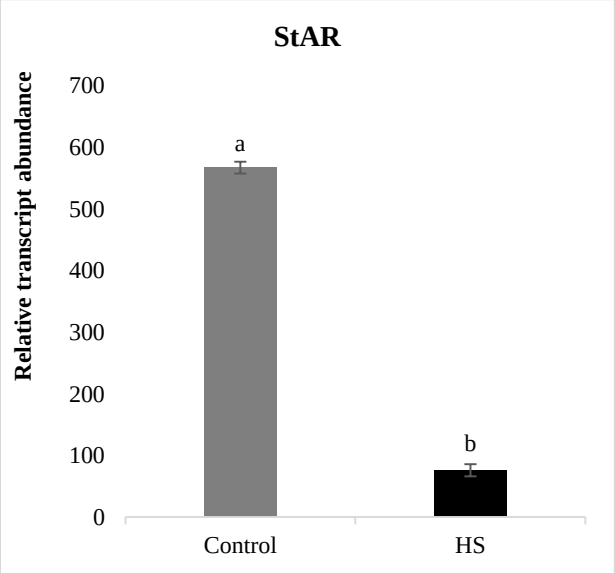

Figure 6. The expression profile of steroidogenic acute regulatory protein of buffalo granulosa cells after heat shock at 40.5°C for 2 hours on day 7 of *in vitro* culture. StAR: Steroidogenic Acute Regulatory

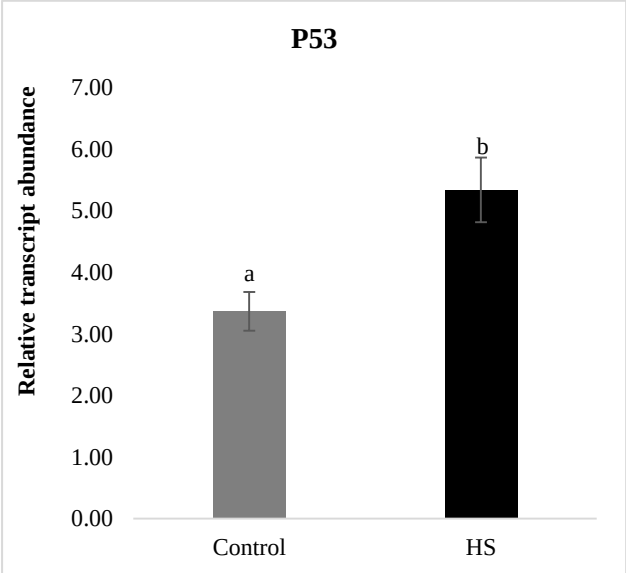

Figure 8. The expression profile of apoptosis-related transcript (P53) of buffalo granulosa cells after heat shock at 40.5°C for 2 hours on day 7 of *in vitro* culture

DISCUSSION

Elevation of ambient temperature caused HS on dairy animals and resulted in a reduction in fertility that manifested in impairment of follicle development, estradiol biosynthesis, ovulation, oocyte quality, and early embryonic development (Badinga et al., 1993; Wakayo et al., 2015; Li et al., 2016a). In the present study, the increased temperature on day 3 during *in vitro* culture of granulosa cells reduced the viability of granulosa cells, compared to the control group. However, Faheem et al. (2021) have observed that cultured GCs exposed to 40.5°C for different time durations (24, 48, and 72 hours) showed no significant differences in the GCs viability of the post-heat-treatment group, compared to the control group that was exposed to 37°C. In support to the current findings, it was demonstrated that exposure of bovine granulosa cells to heat shock at 39°C, 40°C, and 41°C significantly for 2 hours reduced the cell viability, increased incidence of apoptosis, and finally impaired steroidogenesis by reducing estradiol and progesterone levels (Khan et al., 2020). The results of the current study (Table 2) revealed that a reduction in cellular viability was linked with decreased metabolic activity after exposure to heat elevation at the end of the culture period (day 7). This could be a sign of an

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intracellular demise due to thermal stress through the incidence of apoptosis that compromises all biological activity of granulosa cells.

Indeed, granulosa cells proliferate and synthesize hormones required for follicular growth and development (Petro et al., 2012). This ability depends on the antioxidant capacity of granulosa cells to sustain the optimum microenvironment inside the follicle. The results of the present study (Figures 3 and 4a, b) indicated a high capacity of granulosa cells to scavenge the ROS, which either produced by reducing endogenous metabolic activity or induced by heat stress as there was no significant difference between the control and heat-stressed group on day 7 of culture. These results were in accordance with those obtained by Faheem et al. (2021) who detected stability on the steroidogenic activity of GCs under heat elevation by stable expression of SOD2 and sustaining intracellular antioxidant capacity under heat elevation. However, the steroidogenic activity that was indicated in the present study by StAR expression (Figure 6) might be compromised due to the differences in the culture condition and low GCs concentration. In addition, heat elevation increased intracellular ROS level (Paul et al., 2009), subsequently induced apoptosis (Liu et al., 2015), and finally impaired the development competence of oocyte (Blondin et al., 1997). Similarly, the results of the current study indicated higher expression of apoptosis, inducing genes, namely P53 (Figure 8) in heat-stressed buffalo granulosa cells although there was no difference in the expression of anti-apoptotic related transcript (BCL2). Similarly, earlier investigations revealed apoptosis incidence in bovine granulosa cells coupled with increased expression of HO-1 (play role in the protective response to stress), however, the precise molecular mechanism is still unknown (Li et al., 2016b; Luo et al., 2016). Recently, heat stress caused apoptosis induction and incidence of oxidative stress and by increasing expression of NRF2 and HO-1 genes in *in vitro* cultured granulosa cells (Wang et al., 2019). Indeed, the maintenance of the cellular antioxidant system of granulosa cells under heat stress is regulated by the suppression of apoptosis and increased proliferative activity (Regan et al., 2018) however, when the cells cannot tolerate intense heat stress subsequently the viability of cells is compromised.

The HS impairs the development of the ovarian follicle and the cells increase the biosynthesis of heat shock proteins to repair cellular damaged proteins (Li et al., 2016b). In a study done in bovine granulosa cells, heat shock genes, such as HSP32, HSP60, HSP70, HSP90, and HSP105, were upregulated in response to heat shock (Li et al., 2016a). In agreement with this observation, the results of the current study showed increased expression of HSF1 (Figure 5) in buffalo granulosa cells exposed to heat elevation for 2 hours on day 3. Moreover, the GCs reduced the expression profile of gene-regulating steroidogenic activity (StAR). It was demonstrated a lower expression of the StAR gene in heat-stressed bovine granulosa cells (Khan et al., 2020). Additionally, the transcript abundance of HSF1 was upregulated in *in vitro* matured buffalo cumulus-oocyte complexes that developed under heat stress (El-Sayed et al., 2018). The elevation of heat during *in vitro* culture of granulosa cells, reduced viability that might attenuate steroidogenic activity by reducing expression of StAR. Moreover, the stability of ROS level and upregulation of HSF1 is the key cellular response of defense mechanism that might protect GCs functionality under suboptimal heat elevation conditions.

CONCLUSION

Based on the findings of the present study, heat stress reduced the viability of granulosa cells by inducing the expression of an apoptosis-related gene (P53) and compromised the expression of genes regulating the steroid biosynthesis. In response to this suboptimal intracellular condition, GCs up-regulated cell defense gene (HSF1) in an attempt to ameliorate the deleterious effect of heat stress on their biological activity.

DECLARATIONS

Competing interests

All authors declare that there is no conflict of interest.

Authors' contributions

All authors are contributed equally to the current manuscript by designing the experiment, writing, and revising it. All authors confirmed the final draft of this manuscript and data analysis.

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Ethical considerations

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

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REFERENCES

Albertini DF, Combelles CM, and Benecchi E (2001). Cellular basis for paracrine regulation of ovarian follicle development. Reproduction, 121: 647-653. DOI: https://www.dx.doi.org/10.1530/rep.0.1210647.

Badinga L, Thatcher WW, Diaz T, Drost M, and Wolfenson D (1993). Effect of environmental heat stress on follicular development and steroidgenesis in lactating Holstein cows. Theriogenology, 39: 797-810. DOI: https://www.dx.doi.org/10.1016/0093-691x(93)90419-6.

Blondin P, Coenen K, and Sirard MA (1997). The impact of reactive oxygen species on bovine sperm fertilizing ability and oocyte maturation. Journal of Andrology, 18(4): 454-460. DOI: https://www.dx.doi.org/10.1095/JAndrol.104.029264.

Carabatsos MJ, Sellitto C, and Goodenough DA (2000). Oocyte-granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. Developmental Biolology, 226: 167-179. DOI: https://www.dx.doi.org/10.1006/dbio.2000.9863.

Denkova R, Bourneva V, and Staneva-Dobrovski L (2004). *In vitro* effects of inhibin on apoptosis and apoptosis related proteins in human ovarian granulosa cells. Endocrine Regulation, 38: 51-55. DOI: https://www.dx.doi.org/10.2478/enr-2004-0013.

El-Sayed A, Nagy R, El-Asheeri A, and Eid L (2018). Developmental and molecular responses of buffalo (Bubalus bubalis) cumulus-oocyte complex matured *in vitro* under heat shock conditions. Zygote, 26: 177-190. DOI: https://www.dx.doi.org/10.1017/S0967199418000072.

Faheem MS, Dessouki SM, Abdel-Rahman FES, and Ghanem N (2021). Physiological and molecular aspects of heat-treated cultured granulosa cells of Egyptian buffalo (Bubalus bubalis). Animal Reproduction Science, 224: Article ID 106665. DOI: https://www.dx.doi.org/10.1016/j.anireprosci.2020.106665.

Ghanem N, Amin A, Moustafa AS, Abdelhamid SM, El-Sayed A, Farid OA, Dessouki SM, and Faheem MS (2020a). Effects of curcumin supplementation on viability and antioxidant capacity of buffalo granulosa cells under *in vitro* culture conditions. World Veterinary Journal, 10: 146-159. DOI: https://www.dx.doi.org/10.36380/scil.2020.wvj19.

Ghanem N, Salilew-Wondim D, Hoelker M, Schellander K, and Tesfaye D (2020b). Transcriptome profile and association study revealed STAT3 gene as a potential quality marker of bovine gametes. Zygote, 13: 1-15. DOI: https://www.dx.doi.org/10.1017/S0967199419000765

Gilchrist RB, Ritter LJ, and Armstrong DT (2004). Oocyte-somatic cell interactions during follicle development in mammals. Animal Reproduction Science, 83: 431-446. DOI: https://dx.doi.org/10.1016/j.anireprosci.2004.05.017

Jancar N, Kopitar AN, and Ihan A (2007). Effect of apoptosis and reactive oxygen species production in human granulosa cells on oocyte fertilization and blastocyst development. Journal of Assisted Reproductive Genetics, 24: 91-97. DOI: https://www.dx.doi.org/10.1007/s10815-006-9103-8.

Khan A, Dou J, Wang Y, Jiang X, Khan MZ, Luo H, Usman T, and Zhu H (2020). Evaluation of heat stress effects on cellular and transcriptional adaptation of bovine granulosa cells. Journal of Animal Science and Biotechnology, 11: Article number 25. DOI: https://www.dx.doi.org/10.1186/s40104-019-0408-8.

Li J, Gao H, Tian Z, Wu Y, Wang Y, and Fang Y (2016a). Effects of chronic heat stress on granulosa cell apoptosis and follicular atresia in mouse ovary. Journal of Animal Science and Biotechnology, 7: Article number 57. DOI: https://www.dx.doi.org/10.1186/s40104-016-0116-6.

Li L, Wu J, Luo M, Sun Y, and Wang G (2016b). The effect of heat stress on gene expression, synthesis of steroids, and apoptosis in bovine granulosa cells. Cell Stress Chaperones, 21(3): 467-475. DOI: https://www.dx.doi.org/10.1007/s12192-016-0673-9.

Liu ZQ, Shen M, Wu WJ, Li BJ, Weng QN, and Li M (2015). Expression of PUMA in follicular granulosa cells regulated by FoxO1 activation during oxidative stress. Reproductive Science, 22(6): 696-705. DOI: https://www.dx.doi.org/10.1177/1933719114556483.

Luo M, Li L, Xiao C, Sun Y, and Wang GL (2016). Heat stress impairs mice granulosa cell function by diminishing steroids production and inducing apoptosis. Molecular Cell Biochemistry, 412: 81-90. https://www.dx.doi.org/10.1007/s11010-015-2610-0

Matsuda F, Inoue N, and Manabe N (2012). Follicular growth and atresia in mammalian ovaries: regulation by survival and death of granulosa cells. Journal of Reproduction and Development, 58: 44-50. DOI: https://www.dx.doi.org/10.1262/jrd.2011-012.

Paul C, Teng S, and Saunders PT (2009). A single, mild, transient scrotal heat stress causes hypoxia and oxidative stress in mouse testes, which induces germ cell death. Biology of Reproduction, 80: 913-919. DOI: https://www.dx.doi.org/10.1095/biolreprod.108.071779.

Petro EM, Leroy JL, Van Cruchten SJ, Covaci A, Jorssen EP, and Bols PE (2012). Endocrine disruptors and female fertility: focus on (bovine) ovarian follicular physiology. Theriogenology, 78: 1887-1900. DOI: https://www.dx.doi.org/10.1016/j.theriogenology.2012.06.011.

Regan SLP, Knight PG, Yovich JL, Leung Y, Arfuso F, and Dharmarajan A (2018). Granulosa cell apoptosis in the ovarian follicle-a changing view. Frontiers Endocrinology, 9: 1-10. DOI: https://www.dx.doi.org/10.3389/fendo.2018.00061.

Roth Z, Arav A, Bor A, Zeron Y, Braw-Tal R, and Wolfenson D (2001a). Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. Reproduction, 122(5): 737-44. DOI: https://www.dx.doi.org/10.3168/jds.s0022-0302(02)74207-0.

Roth Z, Meidan R, Shaham-Albalancy A, Braw-Tal R, and Wolfenson D (2001b). Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles. Reproduction, 121: 745-751. Available at: https://www.pubmed.ncbi.nlm.nih.gov/11427162/

Sammad A, Umer S, Shi R, Zhu H, Zhao X, and Wang Y (2020). Dairy cow reproduction under the influence of heat stress. Journal of Animal Physiology and Animal Nutrition, 104: 978-986. DOI: https://www.dx.doi.org/10.1111/jpn.13257.

Wakayo BU, Brar PS, and Prabhakar S (2015). Review on mechanisms of dairy summer infertility and implications for hormonal intervention. Open Veterinary Journal, 5: 6-10. DOI: https://www.dx.doi.org/10.1016/s0378-4320(00)00102-0.

Wang Y, Yang C, Elsheikh NAH, Li C, Yang F, Wang G, and Li L (2019). HO-1 reduces heat stress-induced apoptosis in bovine granulosa cells by suppressing oxidative stress. Aging (Albany NY), 11: 5535-5547. DOI: https://www.doi.org/10.18632/aging.102136.

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Supplementation of *Moringa oleifera* Leaf Meal in Layer Chickens' Feed: A Review

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ABSTRACT

As a dietary supplement for animals, *Moringa oleifera* is very useful because its leaves are very nutritious. *Moringa oleifera* leaves are rich in fats, proteins, vitamins, and minerals with antimicrobial effects. Leaf tea is used to treat ulcers in the stomach and diarrhea. Moringa leaves are considered healthy food sources and are recommended for anemia from malnutrition due to the high protein, fiber, and iron content of the leaves. *Moringa oleifera* leaves are primarily used for medicinal and human consumption purposes since they are abundant in antioxidants and other nutrients. Due to the low energy and digestibility of proteins, *Moringa oleifera* leaf meal supplementation increases feed intake and feed conversion ratio, as well as decreasing egg mass yield, percentage of egg production, and egg weight. More research in these areas is required to make full use of the potential advantages of the *Moringa oleifera* plant as layer feed.

Keywords: Layer chicken, Laying performance, Leaf meal, Moringa oleifera

INTRODUCTION

Chickens are a major and inexpensive source of animal protein, so poultry production plays a vital role in social and economic securities in developing countries (Olwande et al., 2010; Melesse et al., 2013). Nutrition and environmental factors affect the production output of poultry (Gakuya et al., 2014). Protein supplementation is very important for egg production, with the key sources being soya bean meal and fish meal (Gakuya et al., 2014).

In most developed countries, poultry production industries face the challenge of raising feed costs by 60-70% (Tesfaye et al., 2013). Modern poultry husbandry systems strive to achieve full benefit at the lowest cost of production. This situation has led to the need to look for cheap, locally available, and less competitive alternatives for some poultry feed ingredients, and in particular, protein sources (Gadzirayi et al., 2012). In this context, the best alternative protein source may be *Moringa oleifera* leaves.

Many parts of *Moringa oleifera* (leaves, fruits, immature pods, and flowers) are incorporated into typical human foods in many tropical and subtropical countries (Siddhuraju and Becker, 2003). *Moringa oleifera* is the supplement chosen as a leaf meal in animal diets (Siddhuraju and Becker, 2003). India, the Philippines, Ethiopia, and Sudan are widely known for the *Moringa oleifera* cultivation. The *Moringa oleifera* tree is cultivated in tropical Asia, Latin America, the Caribbean, the Pacific Islands, South Africa as well as West and East Florida (Fahey, 2005). In Ethiopia, the Moringa tree is called Shiferaw or Cabbage Tree and is usually found in the southern region.

As a feed for livestock, supplementation of *Moringa oleifera* is potentially beneficial, as its leaves are highly nutritious. The most nutritious components are Vitamin B complexes, Vitamin C, beta-carotene, Vitamin K, manganese, and protein, among other essential nutrients, present in *Moringa oleifera* leaves (Leone et al., 2015). *Moringa oleifera* leaves have high levels of fats, proteins, minerals, and vitamins and have antimicrobial properties (Onunkwo and George, 2015). Supplementing the diets of farm animals with *Moringa oleifera* was inadequate to increase production quality and health. Although *Moringa oleifera* has medicinal importance for the health of chickens, the supplementation of *Moringa oleifera* levels in the poultry diet is low due to the farmers' and producers' lack of knowledge (Mahfuz and Piao, 2019). *Moringa oleifera* has a high nutritional content, but there is little information on its use as a source of protein in the layer ration in poultry feeding. Consequently, data on the effects of feeding *Moringa oleifera* leaf meal (MOLM) is scarce when it comes to laying efficiency or egg production, egg quality and egg shelf life, chicken fertility, and hatchability (Alebachew et al., 2016). This review, therefore, intended to evaluate the role of *Moringa oleifera* leaf meal in layer chicken growth results.

Nutritional value of *Moringa oleifera*

Moringa oleifera is rich in protein, amino acids, carbohydrates, minerals, vitamins, and organic acids, and is known for its nutritional value (Raja et al., 2013). *Moringa* leaves have anti-bacterial, anti-inflammatory effects (Abbas et al.,

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2018). Gastric ulcers and diarrhea are treated with *Moringa oleifera* leaf tea. Moringa leaves are safe food sources for those suffering from anemia due to malnutrition because of the high protein and fiber content and iron content (Abbas et al., 2018). The most nutritious component is the leaves of *Moringa oleifera*, which are an important source of Vitamin B complex, Vitamin C, beta-carotene pro-vitamin A, Vitamin K, manganese, and protein, among other essential nutrients (Mahfuz and Piao, 2019).

Compared to seeds, a greater quantity of minerals is found in *Moringa oleifera* leaves (Annongu et al., 2014). In reality, moringa is said to supply 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than drain, 9 times more protein than yoghurt, 15 times more potassium than bananas, and 25 times more press than spinach (Gopalakrishnan et al., 2016).

Moringa oleifera's fresh leaves contain seven times the Vitamin C of oranges and four times the Vitamin A of carrots. However, *Moringa oleifera's* dried leaves contain 10 times the Vitamin A and 1/2 times the Vitamin C of oranges (Mahmood et al., 2010). As an animal feed supplement, the leaves of *Moringa oleifera* are very useful as their leaves are highly nutritious. *Moringa oleifera* is an excellent source of vitamins and amino acids that are alleged to improve the immune system (Olugbemi et al., 2010). *Moringa oleifera* seeds and leaves contain all essential amino acids and, when compared to the prescribed amino acid pattern requirements for most farm animals, these are higher than sufficient amounts (Alikwe and Omotosho, 2013). The leaves of *Moringa oleifera* have between 16 and 19 amino acids, out of which 10 are known as essential amino acids (Sonkar et al., 2019).

Table 1. Mineral compositions of *Moringa oleifera* leaves (state whether these values are on a fresh or dry matter basis)

Minerals	Leaves (mg)
Calcium (Ca)	99.1
Phosphorous (P)	70.8
Magnesium (Mg)	35.1
Iron (Fe)	1.3
Zinc (Zn)	0.85
Sodium (Na)	70
Manganese (Mn)	0.119
Potassium (K)	471

Reference: Abbas et al. (2018)

Table 2. Vitamin compositions of *Moringa oleifera* leaves and seeds

Vitamins	Leaf	Seed
Vitamin A (mg)	6.3-6.8	0.3-0.8
Vitamin B1 (mg)	2.59-2.64	0.05-0.06
Vitamin B2 (mg)	20.5-21	0.06-0.08
Vitamin B3 (mg)	8.2-9.6	1.2-1.9
Vitamin B5 (mg)	0.13-1.6	0.7-0.9
Vitamin B9 (mg)	39.5-40	46-48
Vitamin C (mg)	17.3-19.4	124-130
Vitamin E (mg)	113-121	-

Reference: Chelliah et al. (2017)

Phytochemical composition of *Moringa oleifera* leaf meal

A high concentration of phytochemicals accumulates in plants, which can defend against free radical damage (Alagbe, 2019). Vitamins (A, C, and E), flavonoids, and tannins, have been found to have high levels of antioxidants, which have a variety of health benefits for humans and animals (Alagbe, 2019). The synergistic combination of these phytochemicals in *Moringa oleifera* causes an increase in feed intake, palatability, and disease prevention (Alagbe, 2019). A massive wide variety of bioactive compounds have been identified in *Moringa oleifera* (Martin et al., 2013). Vitamin-rich leaves, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins are the most commonly used parts of the plant (Leone et al., 2015). *Moringa oleifera* leaves are often used for medicinal and human nutrition purposes since they have high levels of antioxidants and other nutrients usually lacking in people living in undeveloped nations (Popoola and Obembe, 2013). *Moringa oleifera* leaves have been used to treat various diseases, ranging from malaria and typhoid fever to high blood pressure and diabetes (Sivasankari et al., 2014).

Effect of *Moringa oleifera* leaf meal on the performance of layers

Melesse et al. (2011) found that using *Moringa* stenopetala leaf meal in the diet of Rhode Island Red chicks resulted in a significant improvement ($p < 0.05$) in feed and crude protein consumption, average weight gain, feed efficiency ratios, and protein efficiency ratios as compared to the control diet. As a supplement for sunflower seed meal, the addition of 10 percent and 20 percent *Moringa oleifera* leaf meal to the laying hen diet significantly ($p < 0.05$) increased feed intake and dry matter feed intake and decreased the development of egg mass (Kakengi et al., 2007). As the amount of *Moringa oleifera* leaf meal increased, the percentage of egg production decreased. The feed conversion ratio (kg feed/kg egg) is improved when 20% *Moringa oleifera* leaf meal was added to the laying hen diet. At a level of 20%, the addition of 5% *Moringa oleifera* leaf meal significantly increased egg weight ($p < 0.05$) but leading to lower egg weight (Abbas, 2013)

The increase in feed intake and feed conversion ratio and the decrease in the production of egg mass, percentage of egg production, and egg weight at higher levels of *Moringa oleifera* leaf meal was mainly due to low energy and protein digestibility (Abbas, 2013). Olugbemi et al. (2010) noted that *Moringa oleifera* leaf meal supplementation had no

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significant impact on feed consumption, feed conversion ratio, and laying percentage at levels of up to 10% in a cassava chip-based diet provided to laying hens. In contrast to the control diet (free of *Moringa oleifera* leaf meal and cassava chip), egg weight substantially increased as a result of the supplementation of *Moringa oleifera* leaf meal with cassava chip (Abbas, 2013).

Abou-Elezz et al. (2011) agreed that up to 10% of the use of *Moringa oleifera* leaf meal had no adverse effect on laying hens' efficient output, but levels above that (15% and 20%) are expected to have adverse effects. *Moringa oleifera* leaf powder increases the number of eggs per week, egg weight, egg width, egg surface, yolk height, yolk weight, albumen weight, and yolk ratio in the layer diet of chickens by 2.5% and 5%, compared to the control diet (Ebenebe et al., 2013).

Effect of *Moringa oleifera* leaf meal on the egg quality

There was no difference between the control groups and dietary inclusion of *Moringa oleifera* leaves ($p > 0.05$) in terms of the Haugh units, eggshell intensity, or egg shape index (Durmus et al., 2004). The addition of *Moringa oleifera* leaf had no impact on the egg shape index, which is linked to eggshell intensity and egg grade (Ebenebe et al., 2013). Mabusels et al. (2018) discovered that adding 10% *Moringa oleifera* seed meal to layer diets increased shell thickness, compared to the control diet. However, both 5% and 7.5% *Moringa oleifera* seed meal supplementation had the same effect on eggshell thickness. With regard to the effect of different *Moringa oleifera* leaf levels on layer diet, lower levels of inclusion increased egg production and egg quality, but higher levels of inclusion resulted in lower productivity and lower quality indices in the Isa Brown Breed layer chickens (Ebenebe et al., 2013). The R2 value for the relationship between egg weight and MOL inclusion amount in the diet was 0.99. Increased MOL levels in the diet resulted in a decrease in egg weight, egg width, yolk weight, and yolk ($p < 0.05$), but MOL had no effect on egg volume, yolk duration, yolk index, or shell weight and thickness (Raphael et al., 2015).

Increased levels of MOLM did not affect feed consumption, weight gain, customer acceptance of boiled eggs, and egg weight ($p \geq 0.05$, Gakuya et al., 2014). In terms of the impact of different MOL levels on layer diet, MOL inclusion at lower levels improved egg production and egg quality, but higher levels of inclusion resulted in lower productivity and lower quality indices of Isa Brown Breed layers (Ebenebe et al., 2013). There was, however, no substantial difference between the eggs from MOLM diets and those from supermarkets in the egg yolk color ratings (Gakuya et al., 2014). There are no major variations in egg weights from layers of *Moringa oleifera* leaf and twig meals at levels ranging from 0.2% to 0.8% (Paguia et al., 2014).

Table 3. Effects of feeding various levels of *Moringa oleifera* leaf meal as a replacement for soya bean meal on dual-purpose of egg quality in Koekoek hens

***Moringa oleifera* leaf meal incorporation** / **Parameters**	**T1 (0)**	**T2 (5%)**	**T3 (10%)**	**T4 (15%)**
Egg weight (g)	48.66 ± 0.36^{b}	54.51 ± 0.47^{a}	49.94 ± 0.91^{b}	50.31 ± 0.33^{b}
Egg shape index	80.92 ± 0.56^{a}	75.80 ± 0.10^{c}	77.23 ± 0.76^{bc}	78.79 ± 0.25^{ab}
Shell weight (g)	5.67 ± 0.33^{b}	8.66 ± 0.33^{a}	6.33 ± 0.66^{b}	6.00 ± 0.57^{b}
Albumen weight (g)	21.67 ± 0.33^{c}	29.66 ± 1.20^{a}	25.33 ± 0.33^{b}	23.33 ± 0.88^{cb}
Haugh unit	75.33 ± 0.33^{b}	87.33 ± 0.33^{a}	79.00 ± 0.57^{b}	77.67 ± 1.45^{b}
Yolk weight (g)	15.33 ± 0.33^{b}	20.66 ± 0.33^{a}	17.00 ± 1.00^{b}	17.33 ± 0.33^{b}
Yolk index	0.27 ± 0.57^{b}	0.34 ± 0.33^{a}	0.33 ± 0.57^{ab}	0.32 ± 0.33^{ab}

Reference: Tesfaye et al. (2018). Different superscript letters in a row mean statistical differences ($p \leq 0.05$). MOLM: *Moringa oleifera* leaf meal, SBM: Soybean meal, T: Ration containing 0% MOLM, T2: Ration containing 5% MOLM, T3: Ration containing 10% MOLM, T4: Ration containing 15% MOLM.

Effect of *Moringa oleifera* leaf meal on fertility and hatchability

Moringa oleifera used as an alternative feed ingredient in the layer ration showed a non-significant effect on fertility, hatchability, and embryonic mortality (Etalem et al., 2014). Moyo et al. (2011) mentioned higher levels of zinc and Vitamin E in *Moringa oleifera* leaf played a beneficial role in egg hatchability. Likewise, Durmus et al. (2004) noted increased hatchability with increasing zinc concentration in the diets of Brown parent stock layers. Moringa contains large amounts of iron, calcium, and phosphorus, and Vitamin C is moderately high (Agbaje et al., 2007). Adesola et al. (2012) reported improved hatchability in the diets of indigenous Venda hens as a result of ascorbic acid supplementation. However, the relatively poor hatchability and higher embryonic mortality observed in the control group may be due to a lack of critical nutrients, such as zinc and Vitamin E, which are necessary for better hatchability (Mahmood and Al-Daraji, 2011).

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Effects of *Moringa oleifera* on health status in laying hens

In determining the health status of chickens the analysis of blood parameters is incredibly important (Mahfuz and Piao, 2019). In line with Aye and Adegun (2013), higher levels of albumin levels were observed in laying hens fed with 3 % *Moringa* leaf meal, compared to the control group, but the number of white blood cells (WBCs), red blood cells, lymphocytes, and packed cell volume were lower than the control diets in Moringa-fed groups. The antimicrobial activity of the phytochemicals in *Moringa* leaves may be attributed to the fact that *Moringa*-fed chickens have fewer WBCs and lymphocytes. A high WBC count is commonly linked to an infection caused by bacteria in the host (Mahfuz and Piao, 2019). The findings of this study indicated that *Moringa* pod meal supplementation was found to have lower serum cholesterol levels (Mahfuz and Piao, 2019). Lower plasma levels of malondialdehyde (MDA) and higher glutathione peroxidase have been linked to higher antioxidant activity in laying hens fed *Moringa* leaf meal (Lu et al., 2016). Plasma total protein levels were dietary 5% higher after *Moringa* leaf meal supplementation, which is a good predictor of liver synthetic activity. Furthermore, lower plasma uric acid indicated that supplemented laying hens retained more protein (Lu et al., 2016). Increased antioxidant enzyme activity and lower MDA levels in plasma and egg yolks indicated that Moringa supplementation could improve antioxidant activity and *Moringa oleifera* has strong phytobiotic properties.

CONCLUSION

In developing countries, poultry production plays a significant socio-economic role since chickens are an important and inexpensive source of animal protein. As a feed for livestock, supplementation of *Moringa oleifera* is potentially beneficial and its leaves are highly nutritious. The most nutritious components are Vitamin B complexes, Vitamin C, beta-carotene, Vitamin K, manganese, and protein, among other essential nutrients that are present in *Moringa oleifera* leaves. The increase in feed intake and feed conversion ratio and the decrease in the production of egg mass, percentage of egg production, and egg weight at higher levels of *Moringa oleifera* leaf meal were mainly due to low energy and protein digestibility. The effect of different *Moringa oleifera* leaf levels on layer diet, lower levels of inclusion increased egg production and egg quality, but higher levels of inclusion resulted in lower productivity and lower quality indices in the Isa Brown Breed layer chickens. *Moringa oleifera* leaves are mostly used both for medicinal and human nutrition purposes since they are rich in antioxidants and other nutrients, which are usually deficient in people living in undeveloped countries. More research in these areas is required in order to make full use of the potential advantages of the *Moringa oleifera* plant as layer feed.

DECLARATIONS

Competing interests

None.

Ethical considerations

All ethical issues (including plagiarism, double publication and/or submission, and redundancy) have been checked and approved by the author.

REFERENCES

Abbas RK, Elsharbasy FS, and Fadlelmula AA (2018). Nutritional values of *Moringa oleifera*, total protein, amino acid, vitamins, minerals, carbohydrates, total fat and crude fiber, under the semi-arid conditions of Sudan. Journal of Microbial Biochemical Technology, 10: 56-58. DOI: https://www.doi.org/10.4172/1948- 5948.1000396

Abbas TE (2013). The use of *Moringa oleifera* in poultry diets. Turkish Journal of Veterinary and Animal Sciences, 37: 492-496. DOI: https://www.doi.org/10.3906/vet-1211-40

Abou-Elezz FMK, Sarmiento-Franco L, Santos-Ricalde R, and Solorio-Sanchez F (2011). Nutritional effects of dietary inclusion of Leucaena leucocephala and *Moringa oleifera* leaf meal on Rhode Island Red hens' performance. Cuban Journal of Agricultural Science, 45: 163-169. Available at: http://cjascience.com/index.php/CJAS/article/view/137

Adesola A, Ng'ambi J, and Norris D (2012). Effect of ascorbic acid supplementation level to diets of indigenous Venda hens on egg production, hatchability and subsequent productivity of chicks. African Journal of Biotechnology, 11: 1260-1261. Available at: https://www.ajol.info/index.php/ajb/article/view/129059

Agbaje G, Tayo O, Chioma G, and Ajomale K (2007). Evaluation of yellow-rooted cassava varieties for differences in β-carotene and gross energy. Journal of Applied Sciences Research, 3: 946-948. Available at: https://publication.babcock.edu.ng/asset/docs/publications/AGRI/9511/2301.pdf

Alagbe J (2019). Role of *Moringa olifera* leaf meal on the growth performance of poultry/African catfish–a review. International Journal of Advanced Biological and Biomedical Research, 7(3): 237-245. Available at: http://www.ijabbr.com/index.php/journal/article_34924.html

Alebachew W, Tesfaye E, and Tamir B (2016). Effects of different dietary levels of *Moringa oleifera* Leaf meal on egg production, quality, shelf life, fertility and hatchability of dual purpose koekoek hens. Addis Ababa University, Ethiopia, MSc, thesis. Middle-East Journal of Scientific Research, 24(9): 2909-2920. DOI: http://www.doi.org/10.5829/idosi.mejsr.2016.24.09.23907

Alikwe PCN, and Omotosho MS (2013). An evaluation of the proximate and phytochemical composition of *Moringa oleifera* leaf meal as potential feedstuff for non-ruminant livestock. Agrosearch, 13(1): 17-27. DOI: http://www.doi.org/1010.4314/agrosh.v13i1.2

Annongu A, Karim OR, Toye AA, Sola-Ojo FE, Kayode RMO, Badmos AHA, Alli OI, and Adeyemi KD (2014). Geo-assessment of chemical composition and nutritional evaluation of *Moringa oleifera* seeds in nutrition of broilers. Journal of Agricultural Science, 6(4): 119-124. DOI: http://www.dx.doi.org/10.5539/jas.v6n4p119

Aye PA, and Adegun MK (2013). Chemical composition and some functional properties of Moringa, Leucaena and Gliricidia leaf meals. Agriculture and Biology Journal of North America, 4(1): 71-77. DOI: http://www.doi.org/10.5251/abjna.2013.4.1.71.77

Chelliah R, Ramakrishnan S, and Antony U (2017). Nutritional quality of *Moringa oleifera* for its bioactivity and antibacterial properties. International Food Research Journal, 24: 825-833. Available at: http://www.ifrj.upm.edu.my/24%20(02)%202017/(50).

Durmus I, Atasoglu C, Mizrak C, Ertas S, and Kaya M (2004). Effect of increasing zinc concentration in the diets of Brown parent stock layers on various production and hatchability traits. Archives Animal Breeding, 5: 483-489. DOI: https://www.doi.org/10.5194/aab-47-483-2004

Ebenebe CI, Anigbogu CC, Anizoba MA, and Ufele AN (2013). Effect of various levels of *Moringa leaf* meal on egg quality of Isa Brown Breed of layers. Advances in Life Science and Technology, 14: 45-49. Available at: https://iiste.org/Journals/index.php/ALST/article/viewFile/8612/8809

Etalem T, Getachew A, Mengistu U, and Tadelle D (2014). Cassava root chips and *Moringa oleifera* leaf meal as alternative feed ingredients in the layer ration. Journal of Applied. Poultry Research, 23(4): 614-624. DOI: http://www.doi.org/10.3382/japr.2013-00920

Fahey JW (2005). *Moringa oleifera*. A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Trees for Life Journal, 1(5): 1-15. Available at: http://www.TFLJournal.org/article.php/20051201124931586

Gadzirayi CT, Masamha B, Mupangwa JF, and Washaya S (2012). Performance of broiler chickens fed on mature *Moringa oleifera* leaf meal as a protein supplement to soybean meal. International Journal of Poultry Science, 11(1): 5-10. DOI: http://www.doi.org/10.3923/ijps.2012.5.10

Gakuya DW , Mbugua PN , Mwaniki SM , Kiama SG, Muchemi GM, and Njuguna A (2014). Effect of supplementation of *Moringa oleifera* (LAM) leaf meal in layer chicken feed. International Journal of Poultry Science, 13(7): 379-384. DOI: http://www.doi.org/10.3923/ijps.2014.379.384

Gopalakrishnan L, Kruthi D, and Devarai K (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food Science and Human Wellness, 5(2):49-56. DOI: https://www.doi.org/10.1016/j.fshw.2016.04.001

Kakengi AMV, Kaijage JT, Sarwatt SV, Mutayoba SK, Shem MN, and Fujihara T (2007). Effect of *Moringa oleifera* leaf meal as a substitute for sunflower seed meal on performance of laying hens in Tanzania. Livestock Research for Rural Development, 19(8): Article #120. Available at: http://www.lrrd.org/lrrd19/8/kake19120.htm

Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, and Bertoli S (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. International Journal of Molecular Sciences, 16: 12791-12835. DOI: http://www.doi.org/10.3390/ijms160612791

Lu W, Wang J, Zhang HJ, Wu SG, and Qi GH (2016) Evaluation of *Moringa oleifera* leaf in laying hens: Effects on laying performance, egg quality, plasma biochemistry and organ histopathological indices. Italian Journal of Animal Science, 15(4): 658-665. DOI: https://www.doi.org/10.1080/1828051X.2016.1249967

Mabusels SP, Nkukwana TT, Mokoma M, and Muchenje V (2018). Layer performance, fatty acid profile and the quality of eggs from hens supplemented with *Moringa oleifera* whole seed meal. South African Journal of Animal Science, 48(2): 235-243. DOI: http://www.dx.doi.org/10.4314/sajas.v48i2.4.

Mahfuz S, and Piao XS (2019). Review on application of moringa (*Moringa oleifera*) as natural feed supplement in poultry diets. A review. Animals, 9: Article number 431. DOI: http://www.doi.org/10.3390/ani9070431

Mahmood H, and Al-Daraji H (2011). Effect of dietary supplementation with different levels of zinc on sperm egg penetration and fertility traits of broiler breeder chicken. Pakistan Journal of Nutrition, 10: 1083-1088. DOI: http://www.doi.org/10.3923/pjn.2011.1083.1088

Mahmood KT, Mugal T, and Ul Haq I (2010). *Moringa oleifera*: A natural gift-A review. Journal of Pharmaceutical Sciences and Research, 2(11): 775-781. Available at: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.193.7882&rep=rep1&type=pdf

Martin C, Martin G, Garcia A, Fernández T, Hernández E, and Puls L (2013). Potential applications of *Moringa oleifera*. A critical review. Pastosy Forrajes, 36: 150-158. Available at: http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S0864-03942013000200001&lng=es&nrm=iso&tlng=en

Melesse A, Getye Y, Berihun K, and Banarjee S (2013). Effect of feeding graded levels of *Moringa stenopetala* leaf meal on growth performance, carcass straits and some serum biochemical parameters of Koekoek chickens. Livestock Science, 157: 498-505. http://www.dx.doi.org/10.1016/j.livsci.2013.08.012

Melesse A, Tiruneh W, and Negesse T (2011). Effects of feeding *Moringa stenopetala* leaf meal on nutrient intake and growth performance of Rhode Island Red chicks under tropical climate. Tropical and Subtropical Agro ecosystems, 14: 485-492. Available at: https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/876

Moyo B, Masika PJ, Hugo A, and Muchenje V (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African Journal of Biotechlogy, 10(60): 12925-12933. DOI: http://www.doi.org/10.5897/AJB10.1599

Olugbemi TS, Mutayoba SK, and Lekule FP (2010). Effect of *Moringa* (*Moringa oleifera*) inclusion in cassava based diets fed to broilers chickens. International Journal of Poultry Science, 9: 363-367. DOI: http://www.doi.org/10.3923/ijps.2010.363.367

Olwande PO, Ogara WO, Okuthe SO, Muchemi G, Okoth E, Odindo MO, and Adhiambo RF (2010). Assessing the productivity of indigenous chickens in an extensive management system in southern Nyanza, Kenya. Tropical Animal Health and Production, 42: 283-288. DOI: http://www.doi.org/10.1007/s11250-009-9418-4

Onunkwo DN, and George OS (2015). Effects of *Moringa oleifera* leaf meal on the growth performance and carcass characteristics of broiler birds. Journal of Agriculture and Veterinary Sciences, 8: 63-66. DOI: http://www.doi.org/10.9790/2380-08326366

Paguia M, Rina Q, Paguiab, Balbac C, and Floresd RC (2014). Utilization and evaluation of *Moringa oleifera* L. as poultry feeds. APCBEE Procedia, 8: 343-347. DOI: http://www.doi.org/10.1016/j.apcbee.2014.03.051

Popoola JO, and Obembe OO (2013). Local knowledge, use pattern and geographical distribution of *Moringa oleifera Lam.* (Moringaceae) in Nigeria. Journal of Ethnopharmacology, 150: 682-691. DOI: http://www.doi.org/10.1016/j.jep.2013.09.043

Raja S, Bagle BG, and More TA (2013). Drumstick (*Moringa oleifera* Lamk.) improvement for semiarid and arid ecosystem: Analysis of environmental stability. Journal of Plant Breeding and Crop Science, 5(8): 164-170. DOI: http://www.doi.org/10.5897/JPBCS12.029

Raphael KJ, Christian KT, Juliano RS, Lisita F, Soultan MY, Hervé MK, and Alexis T (2015). Effects of substituting soybean with *Moringa oleifera* meal in diets on laying and eggs quality characteristics of KABIR chickens. Journal of Animal Research and Nutrition, 1: 4. DOI: https://www.doi.org/10.21767/2572-5459.100004

Siddhuraju P, and Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.). Journal of Agricultural and Food Chemistry, 15: 2144-2155. DOI: http://www.doi.org/10.1021/jf020444

Sivasankari B, Anandharaj M, and Gunasekaran P (2014). An ethnobotanical study of indigenous knowledge on medicinal plants used by the village peoples of Thoppampatti, Dindigul district, Tamilnadu, Indian Journal Of Ethnopharmacology, 153: 408-423.DOI: http://www.doi.org/10.1016/j.jep.2014.02.040

Sonkar N, Singh N, Santra AK, Mishra S, Verma LP, and Soni A (2019). Application of munga (*Moringa oleifera*) in livestock feed: A review. International Journal of Chemical Studies, 8(1): 1729-1735. DOI: https://www.doi.org/10.22271/chemi.2020.v8.i1y.8513.

Tesfaye E, Alebachew W, and Tamir B (2018). Performance of koekoek chicken fed with different levels of *Moringa oleifera* leaf meal. Journal of Animal Science and Research 2(3): 1-9. DOI: http://www.dx.doi.org/10.16966/2576-6457.115

Tesfaye E, Animut G, Urge M, and Dessie T (2013). *Moringa oleifera* leaf meal as an alternative protein feed ingredient in broiler ration. International Journal of Poultry Sciences, 12(5): 289-297. DOI: http://www.doi.org/10.3923/ijps.2013.289.297

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Assessing the Chronic Poisoning of White Mice Affected by Mospilan RP and Actara 25 WG

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ABSTRACT

Neonicotinoids are a relatively small group of organic compounds that are widely used in crop production as insecticides. They are highly toxic to insects, and much less toxic to mammals, including humans. Accordingly, the present study aimed to investigate the effects of chronic toxicity of insecticides from the group of neonicotinoids Mospilan RP (active substance acetamiprid) and Actara 25 WG (active substance thiamethoxam) on white mice. The chronic toxicity was induced by daily internal introduction of Mospilan RP and Actara 25 WG to mice for 30 days at the doses of 1/10 of Median Lethal Dose reported as 65 and 363 mg/kg of body weight, respectively. The affected mice showed thrombocytosis, neutrophilic leukocytosis, and lymphocytopenia. Blood plasma hyperproteinemia in mice treated with Mospilan RP and Actara 25 WG was characterized by an increase in globulins content by almost 30.0% in both groups. In Mospilan RP and Actara 25 WG treated groups, there was a reduction in urea content by 43.6% and 31.5%, respectively, an increase in aspartate aminotransferase activity by 80% and 60.0%, and γ-glutamyltranspeptidase by 80% and almost 400%, respectively. Compared to the control group, the activity of alanine aminotransferase increased to 23.0% only in mice that were given Mospilan RP but not in mice that were given Actara 25 WG.

Keywords: Actara 25 WG, Chronic Toxicity, Insecticides Toxicity, Mospilan PP, Neonicotinoids, White Mice

INTRODUCTION

The latest agricultural technologies require the extensive use of pesticides and agrochemicals. There is an annual growth of pesticide usage in Ukraine and the world. For instance, 15 years ago, the prevalence of pesticide usage in Ukraine mounted to more than 300 active substances with about 400 preparative forms (Kovalenko et al., 2010).

The use of pesticides and agrochemicals in agriculture is a necessary condition for obtaining a high yield. Scientific research and practical experience have proven that one of the most effective methods of protecting plants from diseases and pests is the use of chemicals. There has been a growing concern regarding the negative effects of pesticides on animal and human health (Christen et al., 2017; Wood et al., 2018; Wood et al., 2019). Concurrently, the agricultural pests develop a certain level of insensitivity to the chemicals. The adaptation of pests to pesticides develops over 10-30 generations and it supports the validity of evolution theory, meaning that in the process of microevolution, a new property is produced (Lavryshyn et al., 2016). For instance, the Colorado potato beetle has developed immunity to insecticides. Being aware of this, the farmers may increase the concentration of insecticides used on fields up to 10 times over recommended dosages which is harmful to many other organisms and poses the threat to food chain contamination (Smith et al., 2016).

Over the past decades, the volume and geography of pesticide usage in the world have significantly changed. With the introduction of modern agricultural technologies, there is a tendency to increase the use of highly effective pesticides with low consumption rates, which can reduce their negative impact on the environment. For example, a new generation of neonicotinoids synthetic analogs of natural nicotine is quickly developed to protect plants from harmful insect species. Unlike nicotine, they have almost no skin-resorptive effects, which enhance their use in fighting against ectoparasites in animals (Bal et al., 2012).

Accordingly, a large number of preparations have been made due to the high insecticidal activity of neonicotinoids. For example, in the United States in 2008, there were about 190 commercial preparations and 600 ones in 2011. Moreover, 45 preparations were registered in Russia in 2012, 35 in Australia in 2011, 30 in the UK in 2010, and 27 in New Zealand in 2010 (Bazaka et al., 2017).

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In 2010, the list of permitted insecticides in Ukraine included 44 preparations based on five active substances (thiamethoxam, imidacloprid, thiacloprid, clothianidin, acetamiprid) containing almost 11% of the total number of registered insecticides in the world (Bazaka et al., 2018).

A large number of neonicotinoid preparations are available on the market. The spread of these pesticides requires a deep study of their toxic effect on the organism of animals, including poultry. Neonicotinoids have a number of advantages, compared to insecticides of other groups. However, the stability of active substances, acetamiprid, and thiamethoxam, contributes to the long-term migration of residual amounts of the preparations in the environment and the food chain (soil-plant-animal-human) contamination (Bal et al., 2013; Bazaka et al., 2018).

Due to the widespread use of neonicotinoids in agricultural production, veterinary medicine, and general sanitary practice, their negative impact on the animals cannot be ignored. In case of violations of the Standard operative procedures for the use of these substances, the poisoning of animals and people is quite possible (Felsot, 2001; Yermalova et al., 2004).

There are few scientific publications on the toxicity of neonicotinoid preparations for laboratory and productive animals. Only a few articles showed concerns about the effects that occur in acute poisoning (Boyko and Gonohova, 2012). Therefore, it is of utmost importance to study the chronic poisoning of animals with acetamiprid (active substance of Mospilan RP) and thiamethoxam (active substance of Actara 25 WG).

MATERIALS AND METHODS

Ethical approval

Experimental studies were performed on white non-linear mice in accordance with the guidelines of "Toxicological control of new animal protection products" (Lavryshyn et al., 2016), as well as existing documents regulating the organization of work using experimental animals and in compliance with the principles of the European Convention (Strasbourg, 1986) and Article 26 of Law on Ukraine No. 5456-VI of 16.10.2012 "On the protection of animals from cruelty". Provision of the commission on bioethics of the National University of Life and Environmental Sciences of Ukraine (the order №544) was also obtained.

Studies to determine the chronic toxicity of Mospilan RP and Actara 25 WG were performed on non-linear white male mice, aged 2.5 to three months old, weighing 18–20 g. The animals were kept in the vivarium of the faculty of veterinary medicine of the *National University* of Life and Environmental Sciences of *Ukraine*. Feeding was carried out according to the diets and norms that are recommended for laboratory animals of this species (Zapadnyuk et al., 1983). While determining the chronic toxicity of Mospilan RP and Actara 25 WG, the mice became adapted to the laboratory conditions for seven days before the initiation of the experiment. During this period, the mice were closely monitored for their clinical condition. Mice were active and mobile, and they consumed food and water well. They had a neat appearance, hair was smooth, clean, well-fitting to the skin. Visible mucous membranes were pink. There were no pathological changes on the skin or injuries. No signs of dysfunction of the respiratory, cardiovascular, and digestive systems were observed. The behavioral responses were characteristic of animals of this species.

Experimental mice were divided into three groups based on the principle of analogs, with seven animals in each group. The dose of the drugs was calculated in mg of the Active Substance (AS) per one kg of body weight. In animals of the experimental group M, chronic poisoning with Mospilan RP was reproduced by daily drinking of an aqueous solution of the drug at a dose of 1/10 Median Lethal Dose (LD_{50}) - 65 mg/kg of body weight. In animals of the experimental group A, chronic poisoning with Actara 25 WG was reproduced by drinking a watering suspension of the drug at a dose of 1/10 LD_{50} (360 mg/kg of body weight).

The volume of liquid forms for both drugs was 0.4 ml. The animals of group III (n=7) served as the control group, and received the same amount of distilled water. The duration of the experiment was 30 days. During the experiment, the animals were monitored, and changes in their clinical condition were constantly noted. The appearance, their reaction to stimuli, changes in body position, behavior, food and water intake, intensity and nature of the motor activity, condition of the skin and mucous membranes were taken into account, and the time of the intoxication development was recorded. At the end of the experiment, when the chronic toxicity was recognized, the mice were euthanized by bloodletting after light chloroform anesthesia. The blood from the heart was taken from all three groups of animals for morphological and biochemical studies of the toxic effects of the Mospilan RP and Actara 25 RG.

Laboratory studies

The blood for morphological studies was previously stabilized with heparin. The investigated morphological factors were hemoglobin content (HGB), hematocrit (HCT), number of erythrocytes (RBC), platelets (PLT), and leukocytes (WBC) and their forms. Hematological parameters were measured using a specialized veterinary automatic hematology analyzer Micros ABX Vet (Horiba Diagnostics, France). In the plasma, the total protein, albumin, globulin, urea, and creatinine contents, activity of alanineaminotransferase (AlAT), aspartateaminotransferase (AsAT), and

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gamma-glutamyl transpeptidase (GGTP) were studied. Biochemical parameters were examined with the use of a semi-automatic photo electro colorimetric biochemical analyzer Stat-Fax (Awareness Technology, USA) with reagents kits from the HUMAN Company (Germany). The changes in the mass coefficients of the liver, spleen, lungs, kidneys, and heart were determined at the end of the experiment (Gerunova et al., 2012). The results of clinical and biochemical studies were presented in accordance with the International System of Units (SI) recommended for use in clinical laboratory practice (Giknis and Clifford, 2008).

Statistical analysis

Statistical analyses were carried out using the Microsoft Office Excel program (Shelamova et al., 2010). The reliability of differences among indicators was evaluated using the Student's t-test.

RESULTS AND DISCUSSION

Considering the available literature, it can be inferred that acetamiprid and thiamethoxam are low-toxic substances (Bal et al., 2012). Half of the LD_{50} of acetamiprid for white rats is 213 mg/kg of body weight, and it is 98 mg/kg of body weight for poultry. Half of the LD_{50} of thiamethoxam for white rats is 1563 mg/kg of body weight, and it is 576 mg/kg of body weight for poultry (Gerunova et al., 2003).

During the entire observation period, there was no significant difference between the experimental groups and the control group of animals in terms of behavioral responses as well as feed and water consumption. The animals were active and mobile, and their coordination was not impaired. Clinical signs of poisoning and death of animals were not observed. This was indicative of the fact that the used dose of studied preparations was not sufficient to cause clinical changes. However, it should be noted that the animals of both experimental groups were more aggressive, and often attacked each other, compared to the control group. The HCT, RBC, and HGB in the blood of animals of experimental groups did not undergo significant changes when comparing with the indicators of the control group (Table1). At the same time, the number of WBC and platelets in the blood reliably increased in white mice of both treated groups.

It should be noted that by microscopic evaluation of the blood smears of the animals in both experimental groups, anisocytosis and microcytosis of WBC were noted. The found leukocytosis we are inclined to explain as a consequence of the development of inflammatory phenomena in the digestive canal after the enteral introduction of drugs (Table 1). More pronounced changes were noted in mice undergoing Mospilan RP treatment, and this indicated that its degree of toxicity was more pronounced than in Actara 25 WG.

Table 1. Blood counts in white mice treated with Mospilan RP and Actara25 WG, M ± m* (n=7)

Indicator	Group of animals		
	Control	Group M	Group A
Hematocrit (%)	0.34 ± 0.03	0.33 ± 0.01	0.37 ± 0.02
Erythrocytes (T/L)	4.43 ± 0.33	4.37 ± 0.15	4.68 ± 0.24
Hemoglobin (g/L)	112.70 ± 9.44	110.97 ± 4.60	122.43 ± 7.41
Leukocytes (G/L)	7.44 ± 0.38	8.70 ± 0.27**	8.49 ± 0.28**
Platelets(G/L)	274.60 ± 7.31	306.04 ± 4.90**	308.34 ± 8.05**

*Data are presented as Mean ± Standard Error of Mean,**p≤0.05; Group M: Mice treated with Mospilan RP, Group A: Mice treated with Actara 25 WG.

Due to the impacts of Mospilan RP and Actara 25 WGT, thrombocytosis in the blood of the experimental mice was considered to be the result of the isolation of immunomodulatory cytokines, which secondarily stimulated the production of platelets. For example, interleukin-6 is known as an anti-inflammatory cytokine, and also stimulates polyploidization of megakaryocytes and platelet formation (Heinrich et al., 1990; van Gool et al., 1990). An analysis of a blood leukogram of the mice suffering from chronic poisoning caused by Mospilan RP showed that leukocytosis had a neutrophilic character with a shift of the neutrophil nucleus to the right leading to an increased number of segment-nuclear neutrophils to 1.5 times (Figure 1).

The data indicated the depletion of hematopoietic organs after 30 daily administration of Mospilan RP to white mice at a dose of 1/10 of LD_{50}. Along with neutrophilia, a decrease in lymphocyte count showed that their number in the blood was almost 10% lower than in the control group ($p \leq 0.05$). No significant deviations were observed in the leukogram of white mice undergoing Actara 25 WG treatment at a dose of 1/10 of LD_{50}, but there was a tendency to neutrophilia with lymphocytopenia, as with Mospilan RP. The difference in the degree of manifestation of the influence of Mospilan RP and Actara 25 WG on the blood system and leukogram indicators was probably related to the degree of toxicity of each drug. Taking into account these parameters, the Mospilan RP indicated higher toxicity, compared to Actara 25 WG. To establish the functional state of organs and systems under toxic load, the determination of

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biochemical parameters in the animals' blood plasma played an important role (Table 2). Chronic poisoning of white mice with Mospilan RP was accompanied by an increase in the total protein content of blood plasma by 18.9%, comparing to the control group, while in mice poisoned with Actara 25 WG only a tendency to total protein increase was observed.

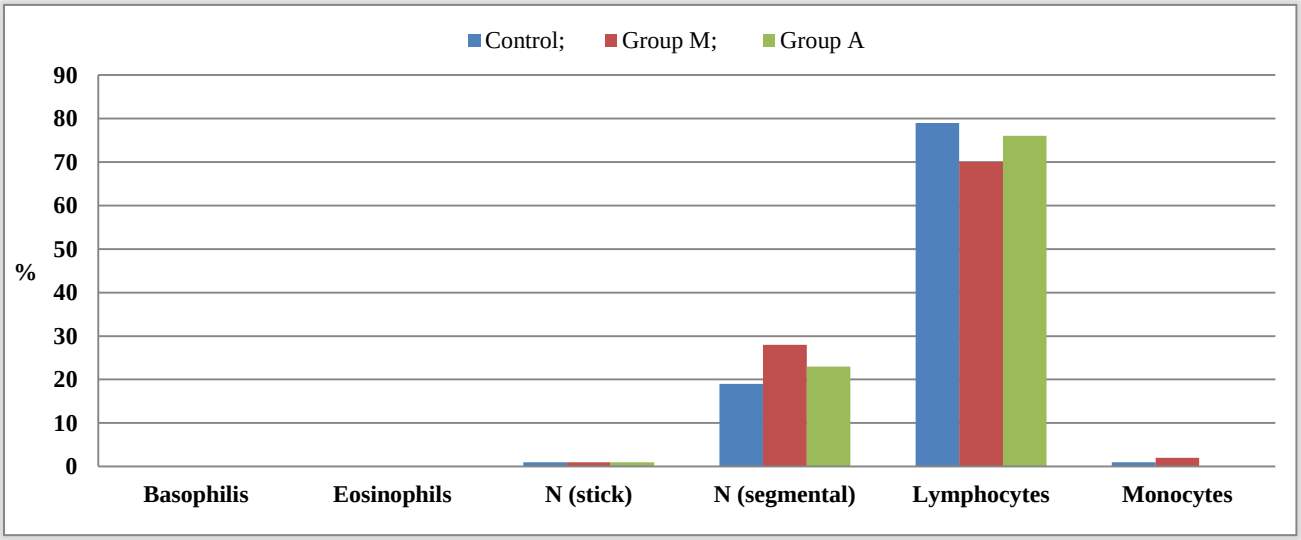

Figure 1. Leukogram of white mice treated with Mospilan RP and Actara 25 WG (n=7). Group M: Mice treated with Mospilan RP (65 mg/kg of body weight), Group A: Mice treated with Actara 25 WG (360 mg/kg of body weight)

Table 2. Biochemical parameters of blood plasma in white mice undergoing Mospilan RP and Actara 25 WG chronic poisoning, M ± m* (n= 7)

Indicators	Group of animals		
	Control	Group M	Group A
Total protein (g/l)	50.61 ± 1.72	60.19 ± 2.26**	55.16 ± 1.75
Albumins (g/l)	24.36 ± 0.73	26.97 ± 1.27	21.64 ± 1.43
Globulins (g/l)	26.25 ± 1.95	33.22 ± 2.69**	33.52 ± 1.71**
Urea (mmol/l)	6.03 ± 0.22	3.41 ± 0.16**	4.13 ± 0.18**
Creatinine (mcmol/l)	59.20 ± 2.13	55.91 ± 1.41	54.29 ± 1.53**

Notes: *data are presented as Mean ± Standard Error of Mean, **p≤0.05; Group M: Mice treated with Mospilan RP, Group A: Mice treated with Actara 25 WG.

The content of albumins in blood plasma remained unchanged although it was slightly higher in animals that were given Mospilan RP. Thus, the increase in the total protein content was associated with an increase in the content of globulins by almost 30.0% in the blood plasma of animals of both experimental groups. This may due to "irritation" of the phagocytic mononuclear system that originated from the toxic liver damage.

Synthesizing liver function is evaluated by the content of urea in the blood plasma because the urea is formed in the liver. There was a 43.6% decrease in the urea content in the blood plasma of white mice undergoing chronic Mospilan RP poisoning, and 31.5 % for Actara 25 WG poisoning (p≤0.05). This result indicated an infringement of the urea-forming function of the liver.

Urea is the final product of protein metabolism, the synthesis of which neutralizes ammonia. It is formed mainly in the ornithine cycle in the liver and partially in the kidneys. The energy required for urea synthesis is provided by the Krebs cycle. The concentration of urea in the blood plasma depends on the intensity of its synthesis and excretion. Therefore, the determination of its content is an important diagnostic test of both, the liver function, where it is synthesized, and the kidneys, through which it is excreted (Heinrich et al., 1990). A decrease in urea content in the blood plasma of both experimental groups of mice indicated a severe course of liver pathology. There was also an 8.3% decrease in creatinine content in the blood plasma of white mice that were given Actara 25 WG, comparing to the control animals (p≤0.05). As soon as Actara 25 WG showed less toxic impacts compared to Mospilan RP, the changes in the total protein and urea content in the blood plasma of white mice from chronic poisoning were less pronounced in group A than in animals that were given Mospilan RP.

Along with a decrease in urea content in the blood plasma, there was also a tendency for the decreased liver mass coefficient in mice of the experimental group M, compared with the indicator in the control group. At the same time, a significant decrease in the spleen mass coefficient in this group indicated the hematopoietic function suppression and the

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immunotropic effect of Mospilan RP. These data supported the results of morphological and biochemical studies of mice in experimental group M. The mice that were chronically poisoned with Actara 25 WG revealed no changes in the mass coefficients of internal organs (Table 3).

Table 3. The mass coefficients of internal organs of white mice undergoing Mospilan RP and Actara 25 WG chronic poisoning, M ± m* (n= 7)

Indicators	Group of animals		
	Control	Group M	Group A
Liver	6.19 ± 0.48	5.60 ± 0.22	6.28 ± 0.35
Spleen	0.80 ± 0.08	0.52 ± 0.08**	0.82 ± 0.12
Lungs	0.69 ± 0.03	0.63 ± 0.02	0.65 ± 0.03
Kidneys	1.66 ± 0.13	1.60 ± 0.11	1.69 ± 0.10
Heart	0.53 ± 0.03	0.55 ± 0.04	0.56 ± 0.02

Notes:*data are presented as Mean ± Standard Error of Mean, **p≤0.05; Group M: Mice treated with Mospilan RP; Group A: Mice treated with Actara 25 WG.

Aminotransferases (AlAT, AsAT) are localized in most organs and systems. They transfer amino groups from aspartic acid and alanine in alpha-ketoglutarate acid. Both enzymes are allocated in the cytoplasm of cells (AsAT also in mitochondria), and their activity in blood plasma increase with minor tissue damage (van Gool et al., 1990). The study of AsAT and AlAT activity in blood plasma is mostly used for the diagnosis of liver diseases. Their activity increases in several conditions, including acute hepatitis and other inflammatory processes. A moderate increase is observed in mechanical jaundice and cirrhosis of the liver (van Gool et al., 1990; Moser et al., 2015). The presence of hepatotoxic action of Mospilan RP is confirmed by changes in the activity of aminotransferases. In the present study, the activity of AlAT increased by 23.0% (p≤0.05). At the same time, there were no changes in the activity of AlAT in the blood plasma of mice undergoing chronic poisoning with Actara 25 WG (Figure 2).

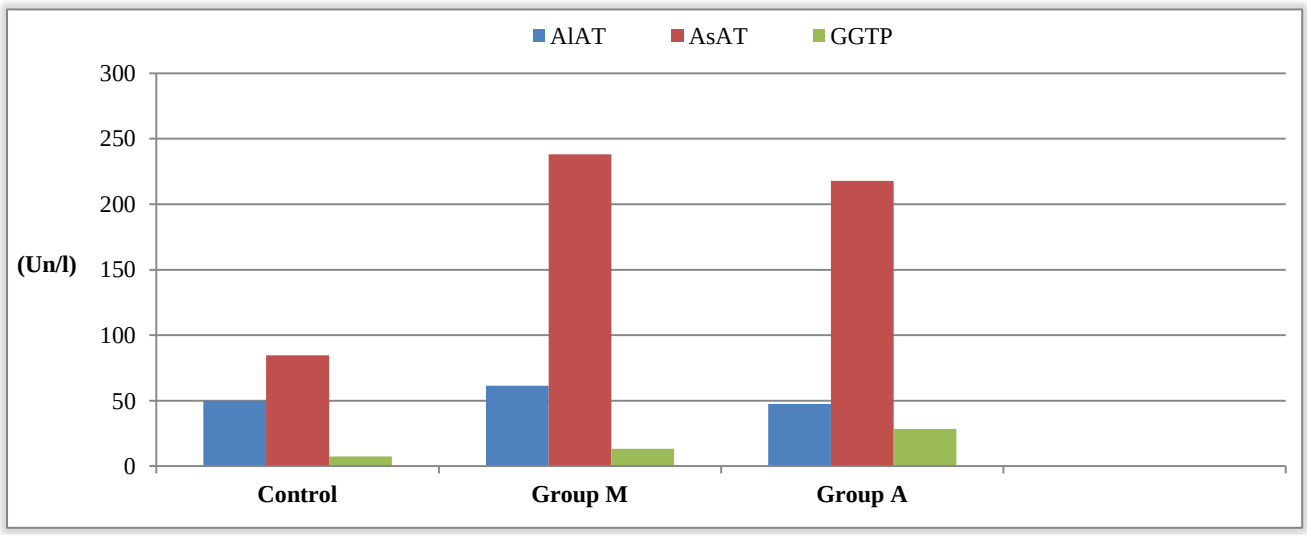

Figure 2. Activity of plasma enzymes in mice undergoing Mospilan RP (group M) and Actara 25 WG (group A) chronic poisoning. Group M: Mice treated with Mospilan RP (65 mg/kg of body weight), Group A: Mice treated with Actara 25 WG (360 mg/kg of body weight)

The affinity of neonicotinoids (acetamiprid and thiamethoxam) with nicotine plus their ability to interact with nicotine-sensitive muscle receptors are the reasons for the pronounced effect of both substances on the activity of AsAT in chronic poisoning of white mice (Bal et al., 2013). In the mice treated with Mospilan RP and Actara 25 WG, the activity of this enzyme increased by 2.8 and 2.6 times, respectively ($p \leq 0.05$).

Gammaglutamyltranspeptidase catalyzes the transfer of a glutamyl residue and a gamma-glutamylpeptide to an acceptor peptide or an alpha-amino acid. The enzyme is highly active in the kidneys, liver, especially in the cells that form the renal tubules and bile ducts, as well as in the pancreas. An increase in GGTP level is an early and reliable test of inter hepatic bile stasis, damage to hepatocyte membranes near the biliary pole, and to epithelial cells that line the lumen of the bile ducts (Chakroun et al., 2017). In mice undergoing Mospilan RP chronic poisoning, GGTP activity increased by 1.8 times, and a simultaneous increase in AlAT and AsAT activity indicated the manifestation of hepatocytolysis. At the same time, the increase in GGTP activity in mice undergoing Actara 25 WG chronic poisoning by almost four times

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without changes in AlAT activity and with less prominent changes of AsAT activity indicated the development of lesions mainly in the bile ducts.

Other studies claim that neonicotinoids are capable to accumulate in the components of treated plants (Buszewski et al., 2019), are found in human serum and urine (Yamamuro et al., 2014), and has the potential to act as neurotoxic compounds (Christen et al., 2017). We consider that these findings further support the importance of our results and sustain the necessity to continue the research in this avenue.

CONCLUSION

Daily oral administration of Mospilan RP and Actara 25 WG at 1/10 of the median lethal dose in the experimental mice for 30 days caused the development of thrombocytosis, neutrophilic leukocytosis, lymphocytopenia, hyperproteinemia due to an increase in the content of globulins and liver dysfunction. The liver dysfunction was more expressed in mice undergoing a chronic poisoning by Mospilan RP that was indicated by a more pronounced decrease of urea content and an increase in AsAT and AlAT activity comparing with animals that underwent a chronic poisoning by Actara 25 WG. Consequently, the above results indicated the feasibility of further studies addressing the effects of Mospilan RP and Actara 25 WG on others species of animals while paying attention to the changes of potentially more susceptible indexes, such as genetic apparatus or reproductive function.

DECLARATIONS

Acknowledgments

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Competing interests

The authors have declared that no competing interest exists.

Authors' contribution

VD, VS, and NK planned and conducted the research and collected data. IL, VH, and VK elaborated the study design, performed the statistical analysis, and wrote the manuscript. All authors have read and approved the final manuscript.

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.

REFERENCES

Bal R, Türk G, Tuzcu M, and Yilmaz O (2012). Assessment of imidacloprid toxicity on reproductive organ system of adult male rats. Journal of Environmental Science and Health, Part B Pesticides, Food Contaminants, and Agricultural Wastes, 47(5): 434-444. DOI: https://www.doi.org/10.1080/03601234.2012.663311

Bal R, Türk G, Tuzcu M, Yilmaz O, Kuloglu T, Baydas C, Naziroglu M, Vener Z, Ttem E, and Tuxcu Z (2013). Effects of the neonicotinoid insecticide, clothianidin, on the reproductive organ system in adult male rats. Drug and Chemical Toxicology, 36(4): 421-429. DOI: https://www.doi.org/10.3109/01480545.2013.776575

Bazaka GY, Dukhnitskyi VB, and Ishchenko VD (2017). Comparative study of mospilana and akhtar chronic toxicity for white mice. Ukrainian Journal of Veterinary Sciences, 265: 8-16. Available at: http://journals.nubip.edu.ua/index.php/Veterenarna/article/view/10606

Bazaka G, Borysevich B, and Dukhnitskiy V (2018). Pathoanatomical changes in the laying hens' organism at mospilan RP and aktara 25 WG chronic poisoning. Ukrainian Journal of Veterinary Sciences, 285: 21-26. Available at: http://journals.nubip.edu.ua/index.php/Veterenarna/article/view/12497

Boyko TV, and Gonohova MN (2012). Kliniko-morfologicheskie proyavleniya ostrogo otravleniya ptits neonikotinoidami. Vesnik veterinarii, 63(4): 104-106. Available at: http://www.stgau.ru/science/conference/conference_21.11.12/doklad/10.pdf

Buszewski B, Bukowska M, Ligor M, and Staneczko-Baranowska I (2019). A holistic study of neonicotinoids neuroactive insecticides-properties, applications, occurrence, and analysis. Environmental Science and Pollution Research, 26(34): 34723-34740. DOI: https://www.doi.org/10.1007/s11356-019-06114-w

Chakroun S, Grissa I, Ezzi L, Ammar O, Neffati F, Kerkeni E, Najjar M, Haouas Z, and Cheikh H (2017). Imidacloprid enhances liver damage in Wistar rats: Biochemical, oxidative damage and histological assessment. Journal of Coastal Life Medicine, 5(12): 540-546. DOI: https://www.doi.org/10.12980/jclm.5.2017J7-149

Christen V, Rusconi M, Crettaz P, and Fent K (2017). Developmental neurotoxicity of different pesticides in PC-12 cells *in vitro*. Toxicology and Applied Pharmacology, 15(325): 25-36. DOI: https://www.doi.org/10.1016/j.taap.2017.03.027

Felsot A (2001). Admiring risk reduction. Does imidacloprid have what it takes? Agrichemical and Environmental News, 186: 1-12. Available at: http://www.aenews.wsu.edu/Oct01AENews/Oct01AENews.htm#Admiring

Gerunova L, Gerunov V, Boyko T, and Gonokhova M (2012). Patomorfologicheskie izmeneniya i dinamika nakopleniya ostatkov imidakloprida v pecheni kryis pri eksperimentalnom otravlenii. Agrarnyiy vesnik Urala, 5(97): 32-34. Available at: http://www.m-avu.narod.ru/PDFkee/AVU_05_2012.pdf

Gerunova LK, Okolelov VI, and Boiko TV (2003). Hematotoxicological action of pesticides on hematothermal animals. Agricultural Biology, 4: 75-78. Available at http://www.agrobiology.ru/4-2003.html

Giknis MLA, and Clifford CB (2008). Clinical laboratory parameters for Crl: WI (Han) rats. Charles River Laboratories, pp. 1-14. Available at: https://www.criver.com/sites/default/files/resources/rm_rm_r_Wistar_Han_clin_lab_parameters_08.pdf

Heinrich PC, Castell JV, and Andus T (1990). Interleukin-6 and the acute phase response. Biochemical Journal, 265(3): 621-636. DOI: https://www.doi.org/10.1042/bj2650621

Kovalenko VG, Tyurina NM, and Kazadaeva SV (2010). Pesticides in the system of integrated control over the development and spread of pests and diseases of crops. Agricultural Chemistry, 4: 43-52. Available at: https://naukabooks.ru/zhurnali/katalog/agrohimija/

Lavryshyn YY, Varkholyak IS, Martyschuk TV, Guta ZA, and Ivankiv LB (2016). The biological significance of the antioxidant defense system of animals animals' body. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Veterinary Sciences, 2(66): 100-111. DOI: https://www.doi.org/10.15421/nvlvet6622

Moser VC, Stewart N, Freeborn DL, Crooks J, MacMillan DK, Hedge JM, and Herr DW (2015). Assessment of serum biomarkers in rats after exposure to pesticides of different chemical classes. Toxicology and Applied Pharmacology, 282(2): 161-174. DOI: https://www.doi.org/10.1016/j.taap.2014.11.01620.

Shelamova MA, Insarova NI, and Leshchenko VG (2010). Statistical analysis of biomedical data using the Excel program. BSMU Repository, p. 96. Available at: http://rep.bsmu.by/handle/BSMU/3865

Smith HA, Nagle CA, MacVean CA, and McKenzie CL (2016). Susceptibility of bemisia tabaci MEAM1 (*Hemiptera: Aleyrodidae*) to imidacloprid, thiamethoxam, dinotefuran and flupyradifurone in South Florida. Insects, 7(4): Article number 57. DOI: https://www.doi.org/10.3390/insects7040057

Strasbourg (1986). European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Council of Europe. ETS - No. 123. Available at: https://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/123

van Gool J, van Vugt H, Helle M, and Aarden LA (1990). The relation among stress, adrenalin, interleukin-6 and acute phase proteins in the rat. Clinical Immunology and Immunopathology, 57(2): 200-210. DOI: https://www.doi.org/10.1016/0090-1229(90)90034-N

Wood S, Kozii I, Medici de Mattos I, de Carvalho Macedo Silva R, Klein C, Dvylyuk I, Moshynskyy I, Tasha E, and Simko E (2019). Chronic high-dose neonicotinoid exposure decreases overwinter survival of *Apis mellifera L.* Insects, 11(1): Article number 30. Available at: https://www.mdpi.com/2075-4450/11/1/30

Wood S, Kozii I, Koziy R, Epp T, and Simko E (2018). Comparative chronic toxicity of three neonicotinoids on New Zealand packaged honey bees. PLoS One, 13(1): e0190517. DOI: https://www.doi.org/10.1371/journal.pone.0190517

Yamamuro T, Ohta H, Aoyama M, and Watanabe D (2014). Simultaneous determination of neonicotinoid insecticides in human serum and urine using diatomaceous earth-assisted extraction and liquid chromatography-tandem mass spectrometry. Journal of Chromatography B Analytical Technologies in the Biomedical and Life Sciences, 15(969): 85-94. DOI: https://www.doi.org/10.1016/j.jchromb.2014.06.008

Yermalova L, Prodanchuk N, Zhminko P, and Lepeshkin I (2004). Comparative toxicological assessment of new neonicotinoid insecticides. Modern problems of toxicology, 42: 4-7. Available at: http://medved.kiev.ua/arhiv_mg/st_2004/042_1en.htm

Zapadnyuk IP, Zapadnyuk VI, Zechariah EA, and Zapadnyuk BV (1983). Laboratory animals: Breeding, maintenance, use in the experiment. Kiev, Vischa shkola, p. 383. Available at: https://www.twirpx.com/file/453758/

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Analysis of Notifications of Rapid Alert System concerning Parasites in Fishery Products

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ABSTRACT

Fish and fishery products are one of Morocco's most important export products. Fish parasitism is a natural worldwide phenomenon. Fish parasites have a very wide distribution and are found in both the northern and the southern hemispheres of the globe. The present study aimed to assess parasitic infestation in fishery products by analyzing notifications available in the European rapid alert system for food and feed. The analysis involved 663 notifications registered from 2001 to 2019 on the grounds of parasitic infestation. For Morocco, 651 notifications concerning the different exported food products were analyzed. Among the 663 notifications for the presence of parasites, 161 (24.3%) were border rejections. A total number of 20 countries have been detected with the presence of parasites in their exported fish and fish products. The main fish species concerned with this hazard were Hake (26%), Silver Scabbardfish (10.5%), and Angler (9.3%). In Morocco, among the 651 notifications, 373 concerned with seafood (57.2%). The number of border rejections of fishery products was 220 that is 33.8% of overall notifications. Fish and fish products category are the most concerned with 170 rejections (26.1%), with 64 notifications due to the presence of parasites (37.6%). The Silver Scabbardfish was the species most affected by parasite infestations (23.5%), followed by European Anchovy (12.5%) and Swordfish (10.9%). In conclusion, the nematode *Anisakis* is the most common parasite in fish infestation while the plerocercoïd larvae of the Cestoda *Gymnorhynchus gigas* seems to have a predilection to infest the Atlantic Pomfret (*Brama brama*).

Keywords: Fish, Morocco, Notification, Parasite, Rapid alert system for food and feed

INTRODUCTION

The fishing sector plays a major socio-economic role in Morocco and it is one of the pillars of the national economy. Morocco is one of the major fish producers worldwide, ranked 15 after Malaysia (FAO, 2020). In 2018, national fishery production reached a volume of more than 1.37 million metric tons, which represents 2% of world production. More than half of this production (52.7%) was exported to the European Union (EU) and Japan (DPM, 2018).

Fishery products represent a valuable source of nutrients and micronutrients that are crucial for healthy and diverse diets (FAO, 2018). The role of these products in the transmission of parasitic diseases to humans has been recognized (Koepper et al., 2020; Teixeira et al., 2020). The range of parasites involved is very wide, including nematodes, trematodes, and cestodes (Huss, 1998; Chai et al., 2005). Among nematodes, the *Anisakidae* family is the most overwhelming in human infestations with essentially two genera of *Anisakis* and *Pseudoterranova* (Smith and Wootten, 1978; Oshima, 1987).

Anisakiasis is a disease caused by ingestion of the L3 larva of parasites belonging to the anisakidae family, in raw fish, undercooked, or having undergone little or no sanitizing treatment (marinating, cold smoking, etc; Oshima, 1987). In addition, it is well established that the anisakid infestation has a significant economic impact, especially during export and discarding infested fishery products from the market at the national level since it can pose risks to customers.

The main objective of this study was to assess the extent of the parasitism phenomenon in Moroccan fishery products through the analysis of data from the European Rapid Alert System for Food and Feed (RASFF) portal between 1979 and 2019 and the development of a map of RASFF notifications concerning parasites in fishery products covering all countries, including Morocco.

MATERIALS AND METHODS

Description of the rapid alert system for food and feed system

The European RASFF was introduced in 1979. It is a notification system which mentions the hazards related to agri-food products exported to EU countries. This system is governed by Regulation (European Commission) n° 178/2002 (European Commission, 2002) and Regulation (European Union) n° 16/2011 which relates to the modalities of

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its application (European Commission, 2011). It has been repealed by Regulation (European Union) 2019/1715 laying down rules for the functioning of the system.

Search procedure in the rapid alert system for food and feed portal

The scope of this study was at first the notifications received from the RASFF system concerning the hazard "parasitic infestation" on a global scale, in particular, all countries exporting their fishery products to the EU market, then, as a practical case study, notifications from Morocco.

World database

Data were retrieved from the RASFF portal (Accessed April 23, 2020). The search criteria for RASFF notifications of parasites in fishery products are as follows: date of notification (From 01/01/1979 to 31/12/2019), type of product (Food and product category, entailing Fish and fish products), and Risk category, including parasitic infestation. This research on the RASFF portal provided access to 663 entries.

Morocco database

The RASFF notifications from Morocco are as follows: date of notification: From 01/01/1979 to 31/12/2019, type of product: Food, Country (flagged as origin: Morocco). Searching on "Morocco database" yielded 651 entries representing all the notifications involving Moroccan food exported to the EU. The obtained findings from RASFF notifications are summarized in Table 1.

Table 1. Results for notifications on the rapid alert system for the food and feed portal

Database	Search time interval	Effective time interval (given by the portal RASFF)	Reason for notifications	Number of data
World database	From 01/01/1979 to 31/12/2019	01/01/2001-31/12/2019	Parasitic infestation	663
Morocco database	From 01/01/1979 to 31/12/2019	01/10/1981 -31/12/2019	All hazards motives	651

Data processing

For the World database, the data was processed according to the type of notifications. Subsequently, a detailed analysis of backflow notifications was carried out according to the country of origin, species of fish, and parasites. Regarding the Morocco database, an overall analysis of notifications by product was carried out to show the importance of notifications of fishery products. Afterwards, rejections were analyzed according to the category and the nature of the fishery products, and the evolution of the rejections between 2008 and 2019. Furthermore, an analysis of the causes for rejections of fish exports was carried out to highlight the importance of parasitic infestation in fish and fish products. Finally, an analysis of parasite rejections, depending on the species of fish and the species of parasite, was conducted.

RESULTS AND DISCUSSION

Global analysis of the rapid alert system for food and feed portal notifications concerning parasites in fishery products

Global analysis of notifications

Over the past 40 years (1979 to 2019), the RASSF portal has reported 663 notifications of parasites for fishery products exported from countries. Those notifications (Table 2) can be divided into alerts (21%), information (22.2%) and border rejections accounting for 161 notifications (24.3%). The average annual number of parasite notifications is 35 (one notification/10 days). The maximum number of notifications was recorded in 2009, 2010, and 2011 with a total of 250 notifications. Since 2011, the number of notifications has gradually decreased to stabilize at 39 during 2017-2019 (Figure 1). Official control on the European market was the main source of these notifications (50.4%), followed by border control (32%). Notifications following consumer complaints and the establishment of self-monitoring accounted for 8.1% and 5.1%, respectively (Table 3). The reporting basis was not defined in 2001 and 2002 (3.2%).

Table 2. Distribution of notifications concerning parasites in fishery products

Type of notifications	Number	Percentage
Rejection	161	24.3
Information	147	22.2
Alert	139	21.0
information for follow-up	136	20.5
Information for attention	80	12.0
Total	663	100

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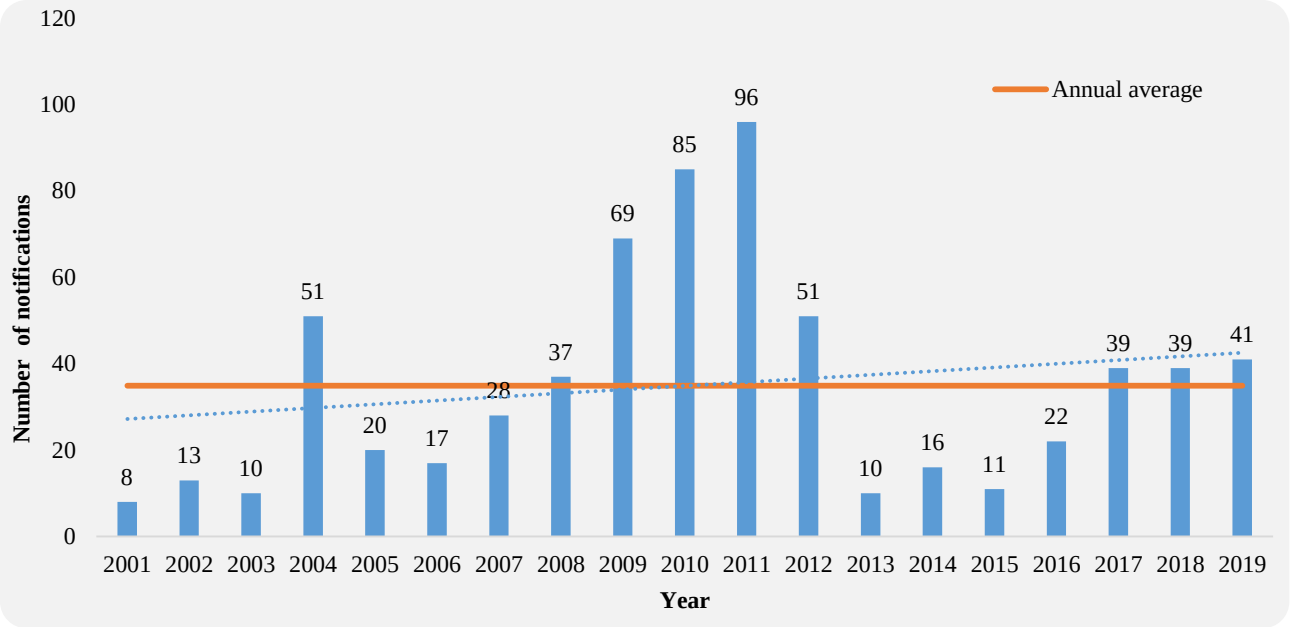

Figure 1. Evolution of rapid alert system for food and feed notifications of fishery products for parasitic infestation

Table 3. Main sources of notifications for the parasitic infestation in fishery products

Notification base	Number	Percentage
Border control	212	32
Establishment self-monitoring	34	5.1
Consumer complaints	54	8.1
Official control on the market	334	50.4
Official control after of notifications RASFF	8	1.2
Without notification base	21	3.2
Total	663	100

Analysis of rejections

Evolution of rejections between 2008 and 2019

The number of rejections is a valuable indicator of the effectiveness of control and self-monitoring systems implemented by third-world countries exporting their products to the EU. Their analysis allows establishing a health profile by the country. The first rejections of fishery products due to parasitic infestation were recorded in the portal in 2008. Over 12 years, the total number of rejections concerning parasites in fishery products was 161, which represents an average of 13 to 14 rejections per year. The most critical period was between 2009 and 2011, during which 105 rejections (65%) were notified. Subsequently, the number gradually decreased to reach only 8 rejections in 2019 (Figure 2).

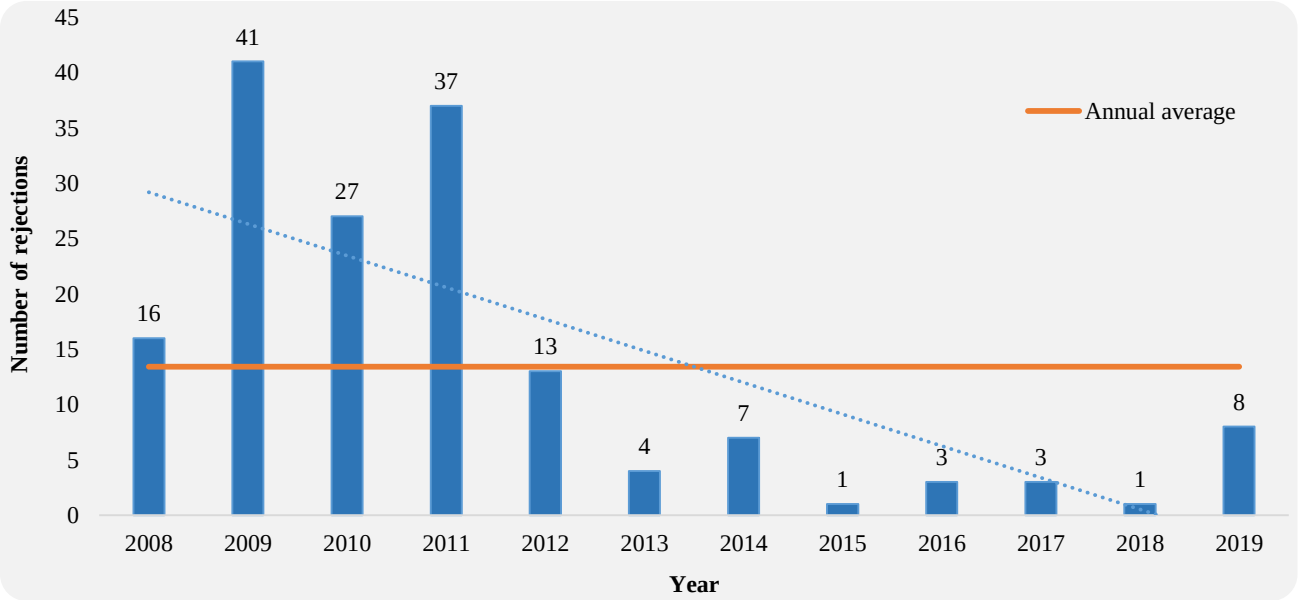

Figure 2. Evolution of rejections of fishery products for parasitic infestation

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Main causes for fish products rejections

Notifications by countries

About 20 countries have been notified on grounds of parasitic infestation. Table 4 presents the concerned countries as well as their corresponding number of seafood rejections. As shown in Table 4, Morocco was mostly notified for rejections with 64 notifications, followed by Croatia (21 rejections), China (15 rejections), and Argentina (12 rejections).

These numbers should be treated with caution as they must be related to the number and/or the quantities of exported seafood. Furthermore, it should be noted that fishery products most affected by parasites were fresh products. Given Morocco's geographic proximity to EU countries, it is common to export large quantities of fresh fishery products, which explains Morocco's ranking as the first notified country, which is much more related to the nature of the exported product. Still, these data showed with supporting evidence that the parasite infestation of fish products is a worldwide concern.

Regarding worldwide repartitions of rejections by continent, the African countries record the maximum rejections number with 49% due to parasite infestation, followed by European (22%) and American countries (15%). Asian countries and Australia account for only 10% and 4%, respectively. Table 5 shows the rejections of fishery products according to the countries of origin and the reporting countries. Spain is the gateway for fishery products to the EU. As a result, it a notified products just from 9 countries. Italy comes second by 6 countries, followed by Lithuania (n = 4). For countries of origin, China has been notified by 5 member states, followed by Argentina (n = 4).

Table 4. List of countries notified of parasitic infestation in fishery products

Country of origin	Number of rejections	Country of origin	Number of rejections
Morocco	64	Spain	3
Croatia	21	Namibia	3
China	15	South Africa	2
Argentina	12	Albania	2
Tunisia	7	Chile	2
USA	6	Ghana	1
New Zealand	6	Mauritania	1
Russia	5	Senegal	1
Canada	4	Sri Lanka	1
Falkland Islands	4	Ukraine	1

Table 5. Notifications of rapid alert system for parasitic contamination of food and feed in different countries and origins

	Notifying countries							
Notified countries	Germany	Bulgaria	Spain	Estonia	Greece	Italy	Lithuania	Poland
South Africa			2					
Albania						2		
Argentina	2		3		3	3		
Canada					1		2	1
Chile	2							
China		4	5	3	1		2	
Croatia						21		
Spain								3
USA						5		
Falkland Islands			4					
Morocco			64					
Mauritania			1					
Namibia			3					
New Zealand			6					
Russia							5	
Senegal			1					
Sri Lanka						1		
Tunisia						7		
Ukraine							1	

Notifications by category of commodity

Fresh, frozen, chilled, and canned fishery products have been subjected to border rejection. The maximum number of rejections was for fish in the chilled state (n = 76), followed by fish in the frozen state (n = 43). Notifications for

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which the nature of the product was not specified constitute a proportion of 25%. The occurrence of parasites was hence more prevalent in chilled and frozen fish. The parasitic infestation was a minor problem with canned goods.

Notifications by fish species

Regarding families and fish species, Figure 5 illustrates the main fish species as well as their corresponding number of rejections. The number of notified species is 23, but this number could be higher since several notifications do not mention the species name. During the last 12 years, hake (*Merluccius merluccius*) was the most rejected species for the presence of parasites (n = 42, 26.4%), followed by Silver Scabbardfish (*Lepidopus caudatus*, n = 17, 10.7%), and John Dory (*Zeus faber*) recorded 12 rejections (7.5%). Swordfish (*Xiphias gladius*) and European anchovy (*Engraulus encrasicolus*) were reported 10 and 8 times, respectively, for parasitic infestation (Figure 3). As can be seen in Figure 3, the two species of freshwater fish were subjected to rejections (Northern pike, *Esox lucius*) and pike-perch (*Stizostedion lucioperca*). The Hake family was the most rejected family for parasitic infestation (26.7%), followed by the Trichiuridae (10.6%), and Lophiidae (9.3%) families. The families of Gadidae and Zeidae constitute 7.5% of rejections each. Xiphiids represents 6.2% of the rejections.

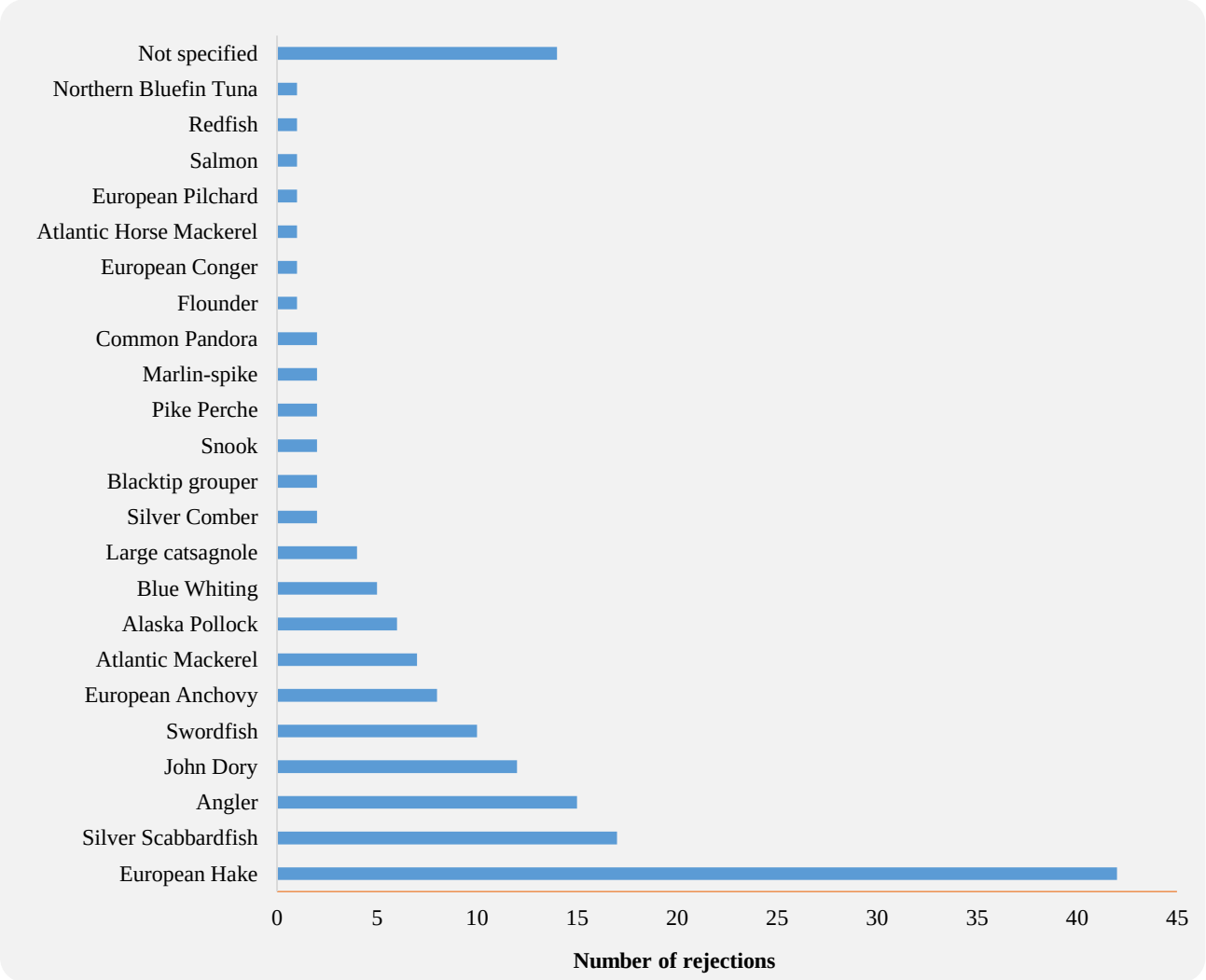

Figure 3. Number of rejections depending on the species of fish between 2008 and 2019

Parasite species

Concerning parasite species, out of 161 border rejections for parasitic infestation, *Anisakis* represents the most frequently implicated parasite responsible for 72.7% of rejections, followed by the plerocercoïd larvae of the Cestoda *Gymnorhynchus gigas*, *Microsporidia*, and *Philometra* spp. with the same proportion, 2.5% each (Table 6). Nematodes of the genus *Anisakis* are, therefore, a problem of a global dimension. Table 7 shows a list of fish species rejected for *Anisakis* infestation as well as their countries of origin. Using the data in tables 4 and 7, a map was established to show the origin of the fishery products rejected for parasite reasons (Figure 4). This map shows that fish parasites have a very wide distribution and are found in both the northern and the southern hemispheres.

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Table 6. Number of rejections depending on the species of parasite

Group	Type	Species	Number of rejections	Percentage
Helminths	Nematodes	*Anisakis*	117	72.7
		Philometra spp.	4	2.5
		Other	2	1.2
	Cestodes	*Gymnorhynchus gigas*	4	2.5
		Nybelinia spp.	1	0.6
		Trypanorhyncha	1	0.6
Total			129	80.2
Microsporidia		*Microsporidia*	4	2.5
		Spraguealophii	1	0.6
Myxosporidia		*Kudoa*	1	0.6
Total			6	3.7
Not-specified			26	16.1

Table 7. Rejections of fish species infested by *Anisakis* and their countries of origin

Species	Number of rejections	Countries of origin (rejections)
European Hake	32	Croatia (18) Argentina (4) China (3) Morocco (3) New Zealand (1) Canada (1) Spain (1) Albania (1)
Silver Scabbardfish	13	Morocco
John Dory	10	Morocco (6) Tunisia (3) Mauritania (1)
EuropeanAnchovy	8	Morocco
Swordfish	7	Morocco
Angler	5	United States
Chub Mackerel	4	Croatia (2) Morocco (2)
Atlantic mackerel	3	Croatie Morocco Tunisia
Hake	3	Albania Canada China
Silver Hake	2	Argentina Spain
Largehead Hairtail	2	Morocco
Silver Comber	2	Argentina
Blacktip Grouper	1	New Zealand
Southernhake	1	
European Conger	1	Morocco
European Pilchard	1	
Northern Bluefin Tuna	1	
Flounder	1	China
Alaska Pollock	1	
North Pacific Hake	1	Canada
Redfish	1	
Atlantic Horse Mackerel	1	Croatia
Blue Whiting	1	Falkland Islands
Common Pandora	1	Argentina
Pike-Perch	1	Ukraine
Pink Salmon (*Oncorhynchus gorbuscha*)	1	Russia
Not specified	13	Morocco

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Figure 4. Distribution of the fishery products rejected for the presence of parasites

Important actions taken by the notifying countries

The major source of rejection notifications is border control. Fishery products turned away due to parasites are either disposed of at border posts (37.3%) or returned to the countries of origin (35.4%). These two actions are not well specified in some cases (13.7%). The rest of the products are owned by member states (6.2%) or banned from entering European territory (5%, Figure 5). The majority of notifications were reported by Spain (55.3%), followed by Italy (24.2%), Lithuania (6.2%), and Greece (3.1%, Table 8).

Table 8. List of notifying countries with the number of notifications

Notifying countries	Number of notifications (%)
Spain	89 (55.3)
Italy	39 (24.2)
Lithuania	10 (6.2)
Greece	5 (3.1)
Bulgaria	4 (2.5)
Germany	4 (2.5)
Poland	4 (2.5)
Estonia	3 (1.9)
France	1 (0.6)
Romania	1 (0.6)
United Kingdom	1 (0.6)

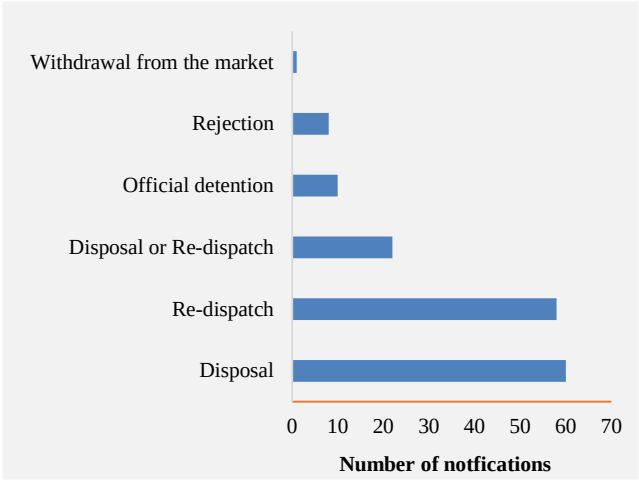

Figure 5. Importance of actions taken by notifying countries

Analysis of rapid alert system for food and feed notifications of Moroccan fishery products exported to the European Union

Analysis of notifications relating to fishery products number and evolution of notifications

Between 1981 and 2019, the RASFF portal reported 373 notifications for fishery products exported from Morocco to the EU. The "Fish and fish products" category is the one most affected with 295 notifications (80%). The two categories "Cephalopods and derived products" and "Crustaceans and derived products" constitute 9.4% and 9% of the notifications, respectively (Table 9).

Global analysis of border rejections of Moroccan fishery products

Over 12 years, the total number of rejections of fishery products was 220, which corresponds to an average rejections number of 18 per year. Fishery products account for more than two-thirds of rejected products (70%). Table 10 indicates the number and percentage of rejections for each category of fishery products. The "Fish and derived products"

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category was the most affected with 170 rejections (77.3%), followed by cephalopods and derived products with 33 rejections (15%, Table 10).

Table 9. Notification numbers of fishery products between 1981 and 2019

Product category	Number de notifications	Percentage
Fish and fish products	295	79.1
Cephalopods and derived products	35	9.4
Crustaceans and derived products	34	9.1
Bivalve molluscs and derived products	9	2.4
Total	373	100

Table 10. Notifications of rejections of the different categories of fishery products between 2008 and 2019

Product category	Number of rejection	Percentage
Fishery products	220	70.1
Fish and fish products	170	77.3
Cephalopods and derived products	33	15.0
Crustaceans and derived products	14	6.3
Bivalve molluscs and derived products	3	1.4
Other types of food	94	29.9
Total	314	100

Evolution of rejection notifications of fishery products from 2008 to 2019

During 2008-2010, the number of rejections contributes to 16.8% (n = 37) in fishery product rejections. Most of them involve the category of fish and fish products (n = 33) and the rest crustaceans and their products. The main reasons for rejections are parasite infestation and poor hygiene. The rejections between 2010 and 2019 constitute 83.2% of rejections of fishery products (n = 183). Regarding the rejections of fishery products exported via Morocco to the EU, the first three years had several rejections, especially in 2011 (23%) due to the large number of rejections resulted from parasites of the fish products. The number of rejections fell from 2013 and rise again in 2019 (11.4% of rejections). During this period, most of the rejections were due to the failure of the cold chain and parasitic infestation. This can be explained by the reinforced control of the competent authorities. The findings indicated that after 2011, there was a steady decrease in rejections year after year. The same observation, therefore, concerns the rejection of fishery products (Figure 6). In 2019, the number of rejections of fishery products was higher than the previous five years, reaching 21 out of 23.

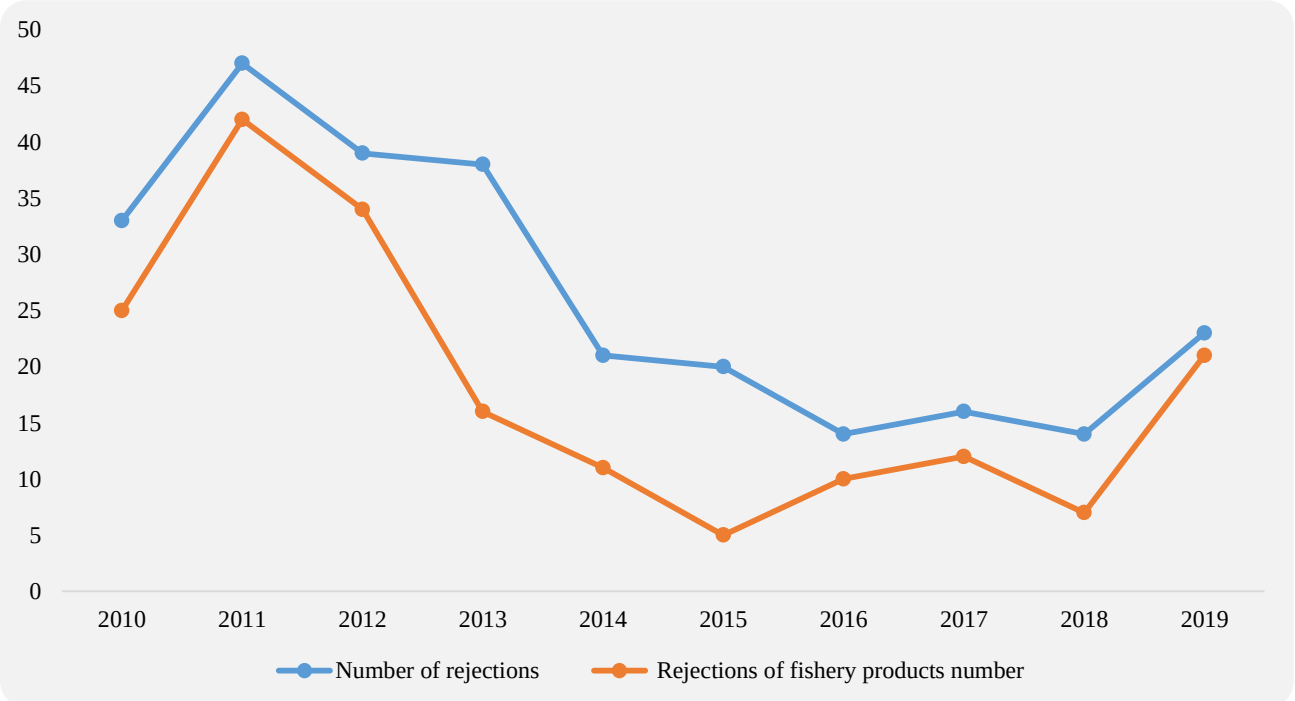

Figure 6. Rejections numbers of fishery products from 2010 to 2019

The decrease in the number of rejections notifications can be attributed mainly to strengthening the sanitary control of fishery products in terms of parasite research by the competent Moroccan authorities following the publication of law 28-07 underlying food safety, as well as by professionals within the framework of self-control (NFSO, 2010). The 2011 peak is attributed to the presence of rejections for parasitic hazards and also to notifications for the presence of high histamine levels. Following this increase, the Moroccan competent authority has put in place an action plan by

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strengthening controls along the food chain. Food business operators (FBOs) for their part have reviewed their health control plans. The collaboration and cooperation between the Moroccan competent authority and FBOs led to good results in the following years and which is confirmed in the results of this synthesis (Elhariri et al., 2017). In April 2010, the European Food Safety Authority (EFSA) adopted a scientific opinion on the assessment of the risk associated with parasites in fishery products. This scientific advice includes information for the health risk versus the presence of viable parasites in fishery products. According to EFSA opinion, all wild fish caught in seawater or freshwater is likely to contain parasites that pose a risk to human health. Fish to be used to produce raw or almost raw seafood products should be frozen prior to processing. However, the competent authorities may adopt national measures granting an exemption from the freezing treatment obligation for fishery products. These national measures must be notified to the committee (EFSA, 2010).

Analysis of rejections for parasitic infestation

During 1981-2019, notifications of rejections concerning fish and their derivatives for the presence of parasites constitute 37.6% of the notifications in this category or 64 rejections. The trend in rejections from 2010 to 2019 peaked in 2011 with 19 rejections accounting for 11%. Subsequently, the number of rejections gradually decreased with an average of 3 rejections per year (Figure 7). Scabbard fish is the species most affected by rejection due to parasitic infestation with 15 rejections for silver Scabbard fish and 2 for the common Scabbard fish. Anchovy registered the second position with 8 rejections in 2011 and 2014, followed by the Swordfish with 7 rejections. According to the obtained results, John Dory had 6 rejections, 5 of which have been recorded over the past three years, and hake had 4 border rejections (Table 11). Scabbard fish is the most infested species by parasites in Moroccan fishery products and hake is the most infested globally. This heavy infestation is mainly related to their diet and their large size. A positive correlation between the size of the fish and the number of parasites collected has already been observed in the Silver Scabbard fish (Bouchriti et al., 2015).

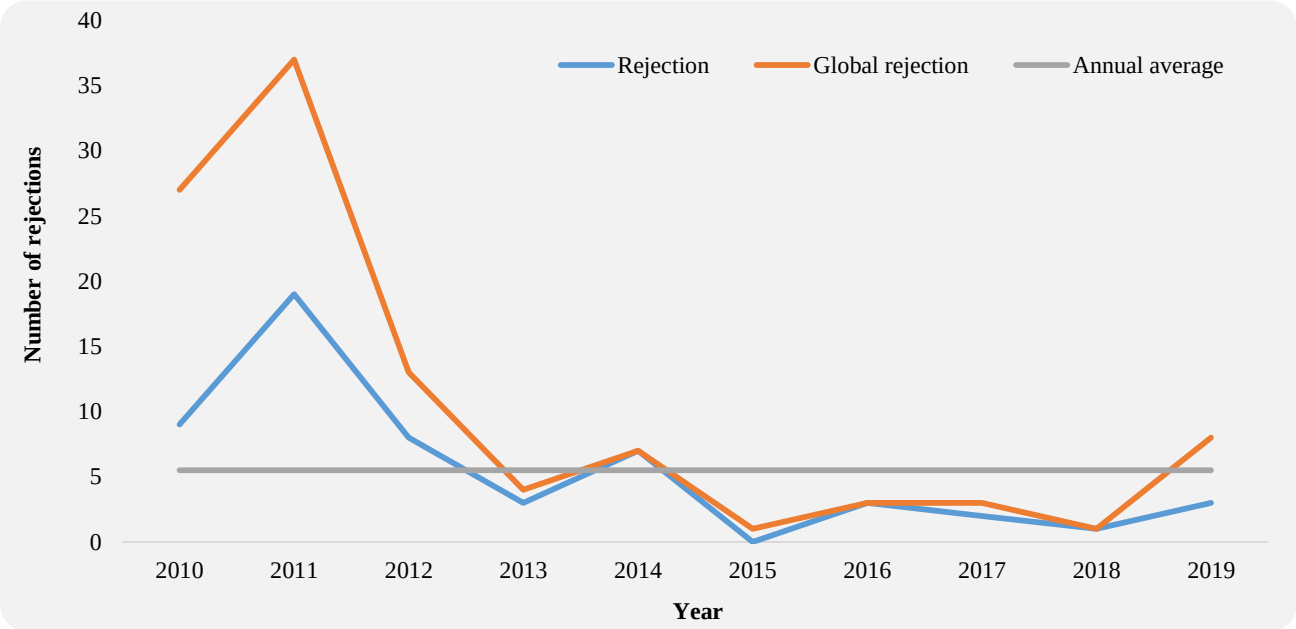

Figure 7. Rejection numbers of fish and derived products infested with parasites during 2010-2019

Based on the living environment of the fish, a possible difference was noticed. Bathypelagic species are the most prone to parasitism with a percentage of 47.1%. Pelagic fish represents 39.2% of rejections for parasitic reasons while benthic fish indicates only 13.7% (Table 12). The Silver Scabbard fish has a silvery, elongated, and compressed body, which is commonly 50 to 150 cm long and can reach up to 210 cm, with a maximum weight of 8 kg. *Lepidopus caudatus* is benthopelagic fish. It lives 60 meters on the continental shelf and beyond the slope up to 600 meters deep, generally on sandy and muddy bottoms from 100 to 300 m, but sometimes in coastal areas of the resurgence of deep water (Nakamura and Parin, 1993). The Scabbardfish are active predators that feed on fish, squid, and crustaceans that are intermediate hosts for the parasites. The hake is a benthopelagic or bathypelagic fish generally living at depths of 70-370 m on the edge of the continental slope, but it can be found in coastal waters up to about 1000 m depth. It measures up to 130 cm in length. Adult hake feeds mainly on fish (anchovies, sardines, herring, and cod) and squid. The young feed on crustaceans (especially euphausiids and amphipods, Cohen et al, 1990). Regarding parasite species, the two main species of parasite incriminated in fish infestations are *Anisakis* spp. (90.5%) in several species of fish and *Gymnorhynchus gigas* found in the Atlantic Pomfret (Table 13).

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Table 11. Number of rejections of fishery products based on the species of fish

Family	Scientific name	Common name	Number of rejections	Year
Bramidae	*Brama brama*	Atlantic Pomfret	4	2009
Trichiuridae	*Lepidopus caudatus*	Silver Scabbard fish	1	2009
			1	2010
			1	2011
			3	2012
			3	2013
			3	2014
			2	2016
			1	2017
	Trichiurus lepturus	Largehead Hairtail	1	2010
			1	2011
Xiphiidae	*Xiphias gladius*	Swordfish	4	2009
			3	2010
Engraulidae	*Engraulis encrasicholus*	European Anchovy	7	2011
			1	2014
Merlucciidae	*Merluccius merluccius*	European Hake	2	2011
			1	2016
Clupeidae	*Sardina pilchardus*	European Pilchard	1	2012
Scombridae	*Scomber* spp.	Atlantic Mackerel	1	2012
			2	2014
	Thunnus thunnus	Northern Bluefin Tuna	1	2012
Zeidae	*Zeus faber*	John Dory	1	2012
			1	2017
			1	2018
			3	2019
Congridae	*Conger conger*	European Conger	1	2014

Table 12. Occurrence of rejections of fishery products based on the living environment of fish types

Types of fish	Species	Number of rejections	Percentage (%)
Pelagic	European Anchovy Atlantic Mackerel European Pilchard Swordfish Northern Bluefin Tuna	20	39.2
Benthic	European Conger John Dory	7	13.7
Bathypelagic	Atlantic Pomfret European Hake Large head Hairtail Silver Scabbardfish	24	47.1

Table 13. Number of repressions depending on the species of parasite and fish

Species parasites	Species	Number of rejections	Percent
Anisakis spp.	Silver Scabbardfish, Largehead Hairtail (*Lepidopus caudatus*, *Trichiurus lepturus*)	15	23.4
	European Anchovy (*Engraulis* spp.)	8	12.5
	Swordfish (*Xiphias gladius*)	7	10.9
	John Dory (*Zeus faber*)	6	9.4
	Atlantic Mackerel (*Scomber* spp.)	3	4.7
	European Hake(*Merluccius merluccius*)	3	4.7
	European Pilchard (*Sardina pilchardus*)	1	1.6
	European Conger (*Conger conger*)	1	1.6
	Northern Bluefin Tuna (*Thunnus thunnus*)	1	1.6
	Not specified	13	20.2
Gymnorhynchus gigas	Atlantic Pomfret (*Brama brama*)	4	6.3
Not specified	Silver Scabbardfish (*Lepidopus caudatus*)	2	3.2

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It should be noted that the number of fishery products exported from Morocco during 2010-2019 was 5,363,674 metric tons (DPM, 2019). The total quantity of rejections due to parasite presence during these 10 years was 165 tons. The quantities rejected for the presence of parasites represent 0.003% of the total exported quantity, which is a very low rate. Despite the importance of parasitic infestation notifications, their economic impact is of a low magnitude nationwide. However, this impact may be severe for packaging the fishery products affected by the infestation.

According to previous field studies, nematodes (*anisakis* and acanthocephalic complex) are the parasites frequently found in seafood on the Moroccan coast, with a prevalence of 21.4% in the Atlantic coast and 24.9% in the Mediterranean (Benabbes and Boudakkou, 2019). The plerocercoïd larva of the Cestoda *Gymnorhynchus gigas* was exclusively found in the Atlantic Pomfret (*Brama brama*) with a prevalence of 89.5%. This internal parasite, found in the flesh of the fish, seems to have a certain predilection for this species. The silver Scabbardfish (*Lepidopus caudatus*) is known to be frequently and sometimes heavily infested by *Anisakis* spp. (Benabbes and Boudakkou, 2019).

A comparison of the research undertaken on the occurrence of parasites in Moroccan coastal waters revealed that this natural phenomenon of parasitism had slightly increased in the Mediterranean and decreased in the Atlantic. According to a study carried out between 1978 and 2015 on changes in the abundance of the two genera of nematodes (*anisakis* and *pseudoterranova*), it was found that the abundance of *Anisakis* spp. saw a significant 283-fold increase and no modification for *Pseudoterranova* spp. (Fiorenza et al., 2020). The worldwide increase in the abundance of *Anisakis* spp. may have faster consequences for human health, the health of marine mammals, and the performance of fisheries.

Fish parasitism is a worldwide phenomenon and is more commonly associated with marine environments than continental ones. The factors responsible for the re-emergence of parasites in different regions of the world are the intensification of fish production, environmental alteration, movement of animals and humans, and increased trade in fishery products (Chai et al., 2005).

The *Anisakis* complex, in particular, is expanding across the global ocean. Several answers can be advanced about the spread of this parasite in recent decades. Several potential factors may be involved, including climate change, increased nutrients, and increased marine mammal populations. As parasites with a complex life cycle, anisakidae can respond to changes in abundance of any definitive or intermediate host (marine mammal, crustacean, or fish) (Arneberg et al., 1998). However, it is almost impossible to know the most critical host in the abundance of anisakidae, that is, the bottleneck in the parasite's life cycle (Lafferty, 2012).

Since the adoption of the Marine Mammal Protection Act in 1972 and the adherence of many countries to the moratorium on commercial whaling imposed by the International Whaling Commission in 1982, the abundance of many species of marine mammals (definitive host) increased (Magera et al., 2013). This increase could lead to an increase in transmission of anisakidae in case the definitive mammalian hosts are the bottleneck of the life cycle (Lafferty, 2012). The replenishment of many populations of marine mammals may explain the increase in the abundance of *Anisakis*.

On the other hand, fish, crustaceans, and cephalopods are key intermediate and paratenic hosts for anisakidae, and their abundance has fluctuated widely over the past half-century (Christensen et al., 2014). Fishing has altered the abundance and density of many fish (Anderson et al., 2008). For example, Atlantic cod have experienced a decline on the northeastern coast of North America (Lilly et al., 2008) while cephalopods have increased in abundance (Doubleday et al., 2016).

Intensification of fish production or increased fishing pressure may lead to a decrease in the abundance of host fish and consequently, reduced transmission and abundance of *Anisakis* (Lafferty, 2012). However, if host fish are not the life cycle bottleneck, decreases in the abundance of intermediate hosts could lead to an increase in the concentration of parasites in the remaining hosts, resulting in an increase in the abundance of parasites (Wood et al., 2013). Long-term climate change could also influence the abundance of anisakid nematodes. The increase in temperature increases the susceptibility of hosts to disease and increases the pathogenicity of pathogens and parasites (Harvell et al., 2002; Burge et al., 2014). This could lead to an increased infestation of host fish, as these species lose their ability to resist infection when temperatures rise beyond the optimum. The temperature rise can also lead to the faster growth of Anisakis nematodes (Marcogliese, 2001).

On the other hand, the increased nutrient load of coastal ecosystems can produce phytoplankton blooms that can feed filter crustaceans (intermediate hosts) (Trainer et al., 2003). Increases in nutrients may facilitate increases in *Anisakis* spp., as crustacean hosts of Anisakid nematodes are sensitive to these environmental alterations.

CONCLUSION

The analysis of the data available on the rapid alert system for the food and feed portal has enabled us to draw some conclusions on the parasite profile of fishery products. The main hazard for fish and fish products border rejections is a parasitic infestation. Spain is the main reporting country as it is the gateway for fishery products to the EU. Since 2011, the number of rejections of fish and fish products has decreased steadily due to the strengthening control by the Moroccan health authority and professionals. Border control in the EU plays a crucial role in detecting and preventing

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nonconformities before the distribution of products in the market. The occurrence of parasites prevails in fresh fish. The silver Scabbardfish (*Lepidopus caudatus*) is the species most affected by parasitic infestations in Morocco and hake (*Merluccius* spp.) is the most infested by parasites worldwide because of their living environment and diet. The *Anisakis* complex is a parasite frequently implicated in the infestation of fish. The parasite *Gymnorhynchus gigas* seems to specifically infest the species *Brama brama*.

DECLARATIONS

Authors' contribution

All the authors contributed to the research in RASFF portal of the notification data, their analysis as well as the writing of the final manuscript. The authors confirmed the content of the final manuscript for publication in this journal.

Competing interests

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

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Ethical considerations

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

REFERENCES

Anderson NKC, Hsieh C, Sandin SA, Roger Hewitt R, Hollowed A, Beddington J, Robert M, May RM, and Sugihara G (2008). Why fishing magnifies fluctuations in fish abundance. Nature, 452: 835-839. DOI: https://www.doi.org/10.1038/nature06851.

Arneberg P, Skorping A, Grenfell B, and Read AF (1998). Host densities as determinants of abundance in parasite communities. Proceedings of the Royal Society of London B: Biological Sciences, 265(1403): 1283-1289. DOI: https://www.doi.org/10.1098/rspb.1998.0431.

Benabbes I, and Boudakkou A (2019). Occurrence des parasites dans les poissons collectés au niveau du littoral marocain. Thèse de doctorat vétérinaire, Institut Agronomique et Vétérinaire Hassan II, Rabat, Maroc, p. 222. Available at: https://eujournal.org/index.php/esj/article/view/12671

Bouchriti N, Triqui R, Lamane H, Hamouda A, and Karib H (2015). Parasitisme dans la filière des produits de la pêche au Maroc : Eléments d'évaluation et de gestion de risque. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 3: 12-18. Available at : https://www.agrimaroc.org/index.php/Actes_IAVH2/article/view/363.

Burge CA, Mark Eakin C, Friedman CS, Froelich B, Hershberger PK, Hofmann EE, Petes LE, Prager KC, Weil E, and Willis BL (2014). Climate change influences on marine infectious diseases: Implications for management and society. Annual Review of Marine Science, 6: 249-277. DOI: https://www.doi.org/10.1146/annurev-marine-010213-135029.

Chai JY, Darwin Murrell K, and Lymbery AJ (2005). Fish-borne parasitic zoonoses : Status and issues. International Journal for Parasitology, 35:1233-1254. DOI: https://www.doi.org/10.1016/j.ijpara.2005.07.013.

Christensen V, Coll M, Piroddi C, Steenbeek J, Buszowski J, and Pauly D (2014). A century of fish biomass decline in the ocean. Marine Ecology Progress Series, 512: 155-166. DOI: https://www.doi.org/10.3354/meps10946.

Cohen DM, Inada T, Iwamoto T, and Scialabba N (1990). FAO species catalogue. Vol. 10. Gadiform fishes of the world (Order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date. FAO Fisheries Synopsis, 125(10): 442. Available at : https://www.fishbase.se/Summary/SpeciesSummary.php?ID=30&AT=merlu.

Département de la Pêche Maritime (DPM) (2018). Rapport d'activité. Département de la Pêche Maritime relevant du Ministère de l'Agriculture, de la Pêche Maritime, du Développement Rural et des Eaux et Forêts. Available at: http://www.mpm.gov.ma/wps/wcm/connect/2a588972-0296-4455-ab6f-a5a6fce4de23/Rapport-Activit%C3%A9-DPM-2018-VF.pdf?MOD=AJPERES&CACHEID=2a588972-0296-4455-ab6f-a5a6fce4de23.

Département de la Pêche Maritime (DPM) (2019). La mer en chiffres. Département de la Pêche Maritime relevant du Ministère de l'Agriculture, de la Pêche Maritime, du Développement Rural et des Eaux et Forêts. Available at: http://www.mpm.gov.ma/wps/wcm/connect/4b46ced9-b6ed-47ef-b254-c671d8cc9c8f/MerChiffres2019VF.pdf?MOD=AJPERES&CACHEID=4b46ced9-b6ed-47ef-b254-c671d8cc9c8f.

Doubleday ZA, Prowse TA, Arkhipkin A, Pierce GJ, Semmens J, Steer M, Leporati SC, Lourenço S, Quetglas A, and Sauer W (2016). Global proliferation of cephalopods. Current Biology, 26(10): 406-407. DOI: https://www.doi.org/10.1016/j.cub.2016.04.002.

Elhariri O, Bouchriti N, and Bengueddour R (2017). Occurrence Et Evaluation Du Risque De L'histamine Dans Les Produits De La Pêche Commercialises Sur Le Marche Marocain. European Scientific Journal, 13(27): 225-250. DOI: https://www.doi.org/10.19044/esj.2017.v13n27p225.

European Commission (2002). Commission Regulation (European Union) n° 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Available at: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32002R0178.

European Commission (2011). Commission Regulation (European Union) n° 16/2011 of 10 January 2011 laying down implementing measures for the Rapid alert system for food and feed. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32011R0016.

European Food Safety Authority (EFSA) (2010). Scientific Opinion on risk assessment of parasites in fishery products. EFSA Journal, 8(4): 1543. DOI: https://www.doi.org/10.2903/j.efsa.2010.1543.

Fiorenza EA, Wendt CA, Dobkowoski KA, King TL, Pappaionou M, Rabinowitz P, Samhouri JF, and Wood CL (2020). It's a wormy world: Meta-analysis reveals several decades of change in the global abundance of the parasitic nematodes *Anisakis* spp. and *Pseudoterranova* spp. in marine fishes and invertebrates. Global Change Biology, 26(5): 2854-2866. DOI: https://www.doi.org/10.1111/gcb.15048.

Food and Agriculture Organization of the United Nations (FAO) (2018). La situation mondiale des pêches et de l'aquaculture 2018. Atteindre les objectifs de développementdurable. Rome, p. 254. Available at: https://reliefweb.int/sites/reliefweb.int/files/resources/i9540fr.pdf

Food and Agriculture Organization of the United Nations (FAO) (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome, P. 224. DOI: https://www.doi.org/10.4060/ca9229en.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, and Samuel MD (2002). Climate warming and disease risks for terrestrial and marine biota. Science, 296(5576): 2158-2162. DOI: https://www.doi.org/10.1126/science.1063699.

Huss HH (1998). Assurance de qualité des produits de la mer. FAO Document technique sur les pêches. N°. 334. Rome, FAO, p. 186. Available at: https://www.webgate.ec.europa.eu/rasffwindow/portal/?event=SearchForm&cleanSearch=1/.

Koepper S, Nuryati S, Palm HW, Theisen S, Wild C, Yulianto I, and Kleinertz S (2020). Parasite Fauna of the White-Streaked Grouper (*Epinephelus ongus*) from the Thousand Islands, Java, Indonesia. Acta Parasitoligia, In press. DOI: https://www.doi.org/10.1007/s11686-020-00312-0.

Lafferty KD (2012). Biodiversity loss decreases parasite diversity: Theory and patterns. Philosophical Transactions of the Royal Society B: Biological Sciences, 367(1604): 2814-2827. DOI: https://www.doi.org/10.1098/rstb.2012.0110.

Lilly GR, Wieland K, Rothschild BJ, Sundby S, Drinkwater K, Brander K, Ottersen G, Carscadden JE, Stenson GB, and Chouinard GA (2008). Decline and recovery of Atlantic cod (*Gadus morhua*) stocks throughout the North Atlantic. In Resiliency of Gadid Stocks to Fishing and Climate Change, Alaska Sea Grant College Program, AK-SG-08-01, pp. 39-66. Available at: https://www.imr.brage.unit.no/imrxmlui/bitstream/handle/11250/109356/Lilly+et+al+2008+AK-SG.pdf?sequence=1.

Magera AM, Flemming JEM, Kaschner K, Christensen LB, and Lotze HK (2013). Recovery trends in marine mammal populations. PLoS One, 8(10): e77908. DOI: https://doi.org/10.1371/journal.pone.0077908.

Marcogliese DJ (2001). Implications of climate change for parasitism of animals in the aquatic environment. Canadian Journal of Zoology, 79(8): 1331-1352. DOI: https://www.doi.org/10.1139/z01-067.

Nakamura I, and Parin NV (1993). FAO Species Catalogue. Vol. 15. Snake mackerels and cutlassfishes of the world (families Gempylidae and Trichiuridae). An annotated and illustrated catalogue of the snake mackerels, snoeks, escolars, gemfishes, sackfishes, domine, oilfish, cutlassfishes, scabbardfishes, hairtails, and frostfishes known to date. FAO Fisheries Synopsis, 125(15): 136. Available at: https://www.fishbase.se/Summary/SpeciesSummary.php?ID=645&AT=sabre

National Food Safety Office (NFSO) (2010). Law n° 28-07 relating to the safety of food products, promulgated by the Dahir N°. 1-10-08 of Safar 26 , 1431 (February 11, 2010). Available at: http://www.onssa.gov.ma/images/reglementation/transversale/LOI.28-07.FR.pdf.

Oshima MT **(**1987). Anisakiasis is the sushi bar guilty?. Parasitology Today, 3(2) : 44-48. DOI: https://www.doi.org/10.1016/0169-4758(87)90212-2.

Smith JW, and Wootten R (1978). Anisakis and anisakiasis. Advances in Parasitology, 16 : 93-163. DOI: https://www.doi.org/10.1016/S0065-308X(08)60573-4.

Teixeira Alves M, and Taylor Nick GH (2020). Models suggest pathogen risks to wild fish can be mitigated by acquired immunity in freshwater aquaculture systems. Scientific Reports, 10: Article number 7513. https://www.doi.org/10.1038/s41598-020-64023-2.

Trainer Vera L, EberhartBich-Thuy L, Wekell John C, Adams Nicaulos G, Linda H, Frank Cox, and Judy D (2003). Paralytic shellfish toxins in Puget Sound, Washington State. Journal of Shellfish Research, 22(1): 213-223. Available at: https://soundtoxins.org/papers/TrainerPSP03.pdf.

Wood CL, Micheli F, Fernández M, Gelcich S, Castilla JC, and Carvajal J (2013). Marine protected areas facilitate parasite populations among four fished host species of central Chile. Journal of Animal Ecology, 82(6): 1276-1287. DOI: https://www.doi.org/10.1111/1365-2656.12104.

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Role of Elastin Expression in Thickening the Postpartum Vaginal Wall in Virgin and Postpartum Rat Models

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ABSTRACT

Childbirth induces a number of alterations, including ligament weakening and increased vaginal distensibility. The occurrence of vaginal laxity or distensibility is associated with the vaginal wall and introitus overstretching during vaginal parturition while the pathophysiology is due to increased levator dimension and trauma to the levator ani muscle through avulsion (macrotrauma) or overdistension (microtrauma). Elastin is an extracellular matrix protein that confers elastic properties to organs and tissues, particularly those requiring elasticity. Elastin plays a vital role in the functioning of numerous tissues, such as the lungs, blood vessels, heart valves, ligaments, tendons, and skin. It is also a component of the vaginal mucosa. The aim of the present was to evaluate the role of elastin in the thickening of the postpartum vaginal wall composed of epithelial mucosa, and to understand the mechanism underlying vaginal laxity or distensibility within parous and nulliparous animal models. A total of 32 female white rats (*Rattus norvegicus*) were used in the present study. They were divided into two groups, each group consisting of 16 rats. The control group (C0) consisted of virgin nulliparous rats, which were sacrificed on the second day after vaginal parturition. Pregnant rats (group C1) were sacrificed on the second day after vaginal parturition. The median elastin expression in group C1 was higher (3 ± 0.56) than group C0 (2.85 ± 0.75). The mean thickness of the vaginal mucosal epithelium in group C0 (56,8 931µm) was greater than group C1 (44,98 349µm). The comparison of vaginal mucosal epithelium thickness between the two groups indicated a significant difference between groups C0 and C1. Elastin levels were significantly correlated with epithelial thickness. The expression of elastin significantly affects the vaginal wall thickness, which further affects vaginal laxity or vaginal distensibility.

Keywords: Distensibility, Elastin, Vaginal wall, Animals

INTRODUCTION

Pregnancy causes a number of alterations in the biomechanical behavior of both humans and animals, including ligament weakening and increased vaginal distensibility. The pelvic floor muscles in rats and humans support the protective mechanisms against perineal trauma by increasing stiffness and the extent of sarcomere during parturition. During vaginal delivery, the puborectalis muscle is subjected to several extreme stretching with an estimated stretch ratio of 1.5-3.5 cm long. The extent to which the muscle elongates varies is between 25% and 250% (Qureshi et al., 2018; Gachon et al., 2019).

The commonly associated mechanism with parturition involves overstretching of the vaginal wall and introitus during vaginal delivery, and its pathophysiology is related to increased elevator dimension and trauma to levator ani from avulsion (macrotrauma) and overextension (microtrauma), meaning that vaginal laxity or distensibility is linked to pregnancy and childbirth (Kamisan et al., 2015; Dietz et al., 2016; Abdool et al., 2018).

Shek and Dietz (2009) reported an Ultrasound study that examined the dimensional change of the levator hiatus in postpartum women with and without morphological abnormality, which was then associated with their type of childbirth. Vaginal birth could induce hiatal widening, particularly after an avulsion and even without macroscopic levator trauma, thus potentially enable increasing the hiatal distensibility. Vaginal laxity is the most undesirable symptom, reported by approximately 60.7% of women. Moreover, levator avulsion occurred in 15% of women who undergo vaginal delivery. Therefore, It can be inferred that significant changes in the pelvis and distension of the levator hiatus are followed by vaginal delivery (Shek and Dietz, 2009; Abdool et al., 2018).

The pelvic floor muscles play a critical role in female sexual function. A smaller vaginal dimension is linked to sexual dysfunction, particularly dyspareunia. Trauma to the levator ani muscle during childbirth is associated with an increase in vaginal hiatus, which in turn might affect sexual function and vaginal laxity (Roos et al., 2020).

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At the cellular level, vaginal muscles and pelvic supporter are sustained by the integrity of the connective tissue and the attachment between the vagina, sides of the pelvis, and levator ani muscle. The connective tissue as the base of the vagina and its surrounding structures contained collagen, elastin, glycoproteins, hyaluronan, and proteoglycans, which were actively redesigned throughout the woman's life, particularly during hormonal changes (Newman et al., 2018).

Elastin is an extracellular matrix protein that provides elastic properties to organs and tissues, mainly those that require elasticity or are involved in an elongation and shrinkage cycle. Elastinplays a vital role in the functions of numerous tissues, such as the lungs, vessels, heart valves, ligaments, tendons, and skin. Elastin is composed of 90% elastic fibers and forms an inner core that is surrounded by unbranched microfibrils. Elastin accounts for only two to four percent of the dry skin weight of a human, yet it has an important structural function in providing mechanical support and is involved in various cell signaling pathways. The rate of elastogenesis diminishes with age (Mithieux and Weiss, 2005; Rodríguez-Cabello et al., 2018).

A study by Zong et al. (2010) indicated that comparing elastin metabolism in the female vagina with and without Pelvic Organ Prolapse (POP) found increased levels of tropoelastin, mature elastin, pro Matrix Metalloproteinase (MMP) 9, and active MMP-9 in women with prolapse. The metabolism of elastin was altered in the vagina with prolapse. In addition, the shape of the vaginal tissue rapidly changed in response to mechanical stretching. It was also observed that elastin levels peaked in the absence of hormones. Damage to elastin fibers might be due to increased elastin-degradation enzymes, MMP-2, and MMP-9. Therefore, MMP-2 and MMP-9 levels would decrease along with an increase in the elastin levels in the vagina with POP. The thickening of the elastin fibers in the vaginal wall of patients with anterior POP was due to the remodeling of the extracellular matrix. Moreover, the proximal vagina contained more collagen level in total and less elastin level than its distal counterpart. The vagina is mostly composed of type I collagen, which gives it tensile strength (Zong et al., 2010; Zaki et al., 2016; Rynkevic et al., 2017). Accordingly, to understand the mechanism of elastin in the process of vaginal laxity or distensibility in parous and nulliparous animal models, the role of elastin in postpartum vaginal wall thickening was evaluated in the current study, which is composed of vaginal epithelial mucosa.

MATERIALS AND METHODS

The present research was an experimental study involving 32 female white rats (*Rattus norvegicus*). The subjects were allocated into two groups consisting of 16 rats each. Group 1 (C0) or the control group consisted of 16 female rats, nulliparous and virgin, aged 4-5 months with a body weight of 170-200 grams. that were sacrificed on day two, while group 2 (C1) entailed pregnant rats that were sacrificed on the second day following vaginal parturition. Rats were housed in individual rearing cages with dark lighting, monitored air temperature with a flow rate of 5 to 7.5 km/hours (gentle breeze), and local humidity with one-atmosphere pressure by inhalation and oxygen demand of 2.68 ml/gram/hour.

Ethical approval

Ethics approval of the present research project was obtained from the Ethics Committee of the Health Research Faculty of Veterinary Universitas Airlangga, Surabaya, Indonesia, with the certificate number 2.KE.116.12.2020. All research work was completed in the same institute.

Mating and breeding of female rats

Previous observations of the estrous cycles were carried out in female ratas using the vaginal swab. Swabs with small cotton swabs moistened with physiological NaCl were then checked on a glass slide. The cells were then fixed with methanol and stained with 10% Giemsa solution. Observation of the estrous cycle was carried out under a light microscope with a magnification of 100×.

Female rats were injected with Pregnant Mare Serum Gonadotropin (PMSG) to synchronize the estrous cycle and with human Chorionic Gonadotropin (hCG) to induce superovulation. The 10 IU PMSG was administered intraperitoneally, followed by 10 IU hCG after 48 hours. After the hCG administration, the rats were bred with male rats using a monomating method. Mating was confirmed for the next 17 hours using a vaginal plug. The vaginal plug was composed of coagulated gelatinous secretions that prevented spermatozoa from leaking. Successful copulation was assumed in the presence of a vaginal plug, and it was recorded as day 0 of pregnancy.

Vaginal tissue sampling

The rats were sacrificed under general anesthesia with ketamine and xylazine. Anesthetic ketamine n the range of 80-100 mg/kg mixed with xylazine in the range of 5-10 mg/kg. The intraperitoneal injection dose was 0.2 ml of ketamine-xylazine mixture for each rat. The entire vaginal tissue was swiftly dissected after disinfection with 70% alcohol. The

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tissues were then split and fixed in 10% formalin buffer. The collected samples were sent to the pathology laboratory for immunohistochemistry preparation and histopathological examination.

Hematoxylin Eosin staining procedure

Hematoxylin and eosin stains were used to observe and measure the thickness of the vaginal mucosa. The sliced tissue samples were initially deparaffinized. Then, these tissue slices were fixed in methanol at gradually decreasing concentrations (100%, 90%, 80%, 70%, and 30%) then washed in PBS. After immersion in ethanol, tissue slices were placed in hematoxylin for six minutes. After rinsing with water, the slices were consecutively dipped into ammonia and eosin solutions. Sample tissues were then dehydrated and re-fixated in methanol with gradually increasing concentration (80%, 90%, 95%, and ethanol absolute), allowed to dry, and thereafter evaluated under a microscope (Cardiff et al, 2014).

Immunohistochemistry evaluation technique

Immunohistochemical staining was performed to detect elastin expression. The vaginal wall samples were fixed to the object glasses with methanol containing 3% diluted hydrogen peroxide. Dakocytomation (peroxidase-blocking reagent) was applied to sample parts and then incubated at room temperature with primary antibodies against the monoclonal antibody anti-elastin (*monoclonal antibody elastin* (BA-4): sc-58756, Santa Cruz Biotechnology, Inc. (1: 300), California, USA). All samples were incubated with biotin-labeled secondary antibodies (Trekkie Universal Link) and incubated overnight with streptavidin-conjugated peroxidase (Trekavidin-HRP Label) and Diaminobenzidine (DAB) as the chromogen. The elastin was counterstained with Mayer's hematoxylin and eosin (Fedchenko and Reifenrath, 2014).

Measurement of vaginal wall thickness and elastin expression

Vaginal wall thickness was measured using the calibrated Image Raster 3 software. The measurements were performed in 10 microscopy fields at 200× magnification and counted in 10 different fields. The mean quantitative result (µm) of each HE-stained sample was recorded. The evaluation of elastin expression was based on the percentage of the elastin-expressing epithelium of the vaginal mucosa using the Semi-quantitative Immuno_Reactive Score (IRS) method. The mean percentage of the monoclonal antibody-positive vaginal mucosal epithelium was observed under a microscope at 400× magnification in 10 microscopy fields. The Remmele scale index was obtained by multiplying the positive cell percentage score by the color reaction intensity score. A positive cell percentage score was interpreted as score 0 indicating no positive cells, score 1 referring to less than 10% positive cells, score 2 showing 11-50% positive cells, Score 3 accounting for 51-80% positive cells, and Score 4 suggesting more than 80% positive cells. The color reaction intensity score was interpreted as score 0 meaning no color reaction, score 1 referring to low color intensity, score 2 denoting medium color intensity, and score 3 signifying strong color intensity (Fedchenko and Reifenrath, 2014).

Statistical analysis

The obtained data were tested for normality using the Kolmogorov-Smirnov test and analyzed using SPSS software (version 24). Furthermore, non-parametric intervariable data were verified using the Mann-Whitney-U Test to determine the differences in parameters between the groups, and statistical significance was set at $p \leq 0.05$. Parametric data were verified using an independent t-test. The correlation between the two groups was verified using Pearson's correlation test.

RESULTS AND DISCUSSION

The expression of elastin was monitored using immunohistochemistry and the vaginal wall thickness with hematoxylin and eosin staining. There were differences in elastin expression and vaginal wall thickness between the virgin nulliparous and parous rat groups. The data observed between the treated groups are provided in the following tables and figures.

The results of elastin expression in the vaginal mucosal epithelium are shown in Figure 1. Figure 1A represents the elastin expression in vaginal epithelium samples of group C0, exhibiting a score of 2 with the immunoreactive cells being in the range of 10-50% in all microscopic fields with a mean expression of 2.85 ± 0.75. Figure 1B represents the elastin expression in vaginal epithelial samples from group C1 with a score of 3 and around 51-80% immunoreactive cells in all microscopic fields, with a mean expression of 3.0 ± 0.56.

Diagram 1 indicates that the median value of elastin expression in group C1 was higher than that of group C0. Differential tests of both groups with the Mann Whitney U-test indicated that the elastin expression in the control group was significantly different from that of the treatment group C1 ($p = 0.032$; $\alpha < .05$, Table 1).

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Table 1. Mann-Whitney test results of elastin expression in female rats.

Group \ Elastin Expression	n	Median	Interquartile Deviation	Minimum	Maximum	P-*value*
C0	16	2.85	0.75	1.1	6.6	0.032*
C1	16	3.00	0.56	2.0	7.2	

(*) significantat p < .05, group C0: Nulliparous female rats, group C1: Postpartum female rats.

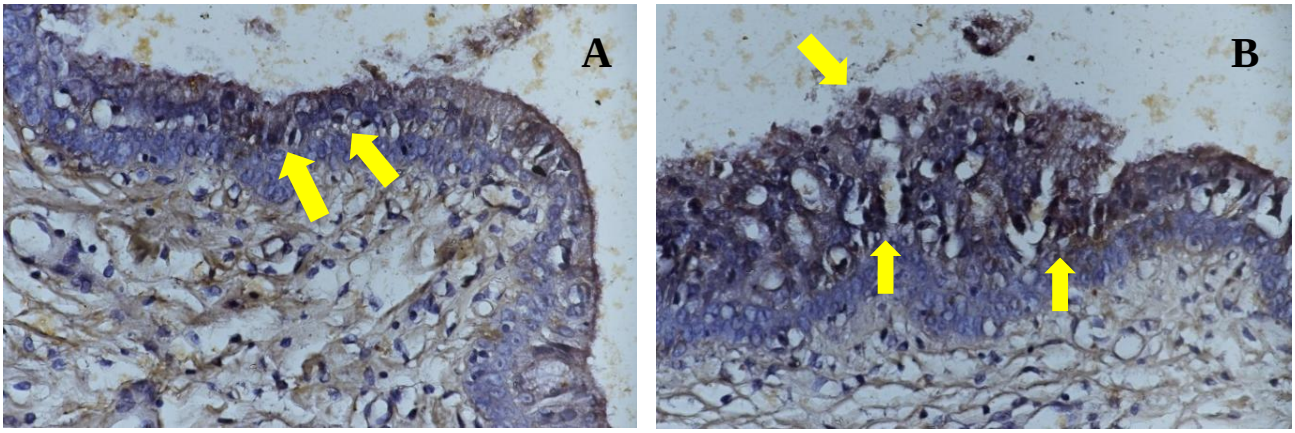

Figure 1. Immunohistochemical results of elastin in female rats. **A:** Expression of elastin with medium intensity on immunoreactive cells (arrows) in group C0, **B:** Positive expression with strong intensity is indicated by a brownish dark color on immunoreactive cells (arrows) in group C1, (Immunocytochemistry test; 400× magnification)

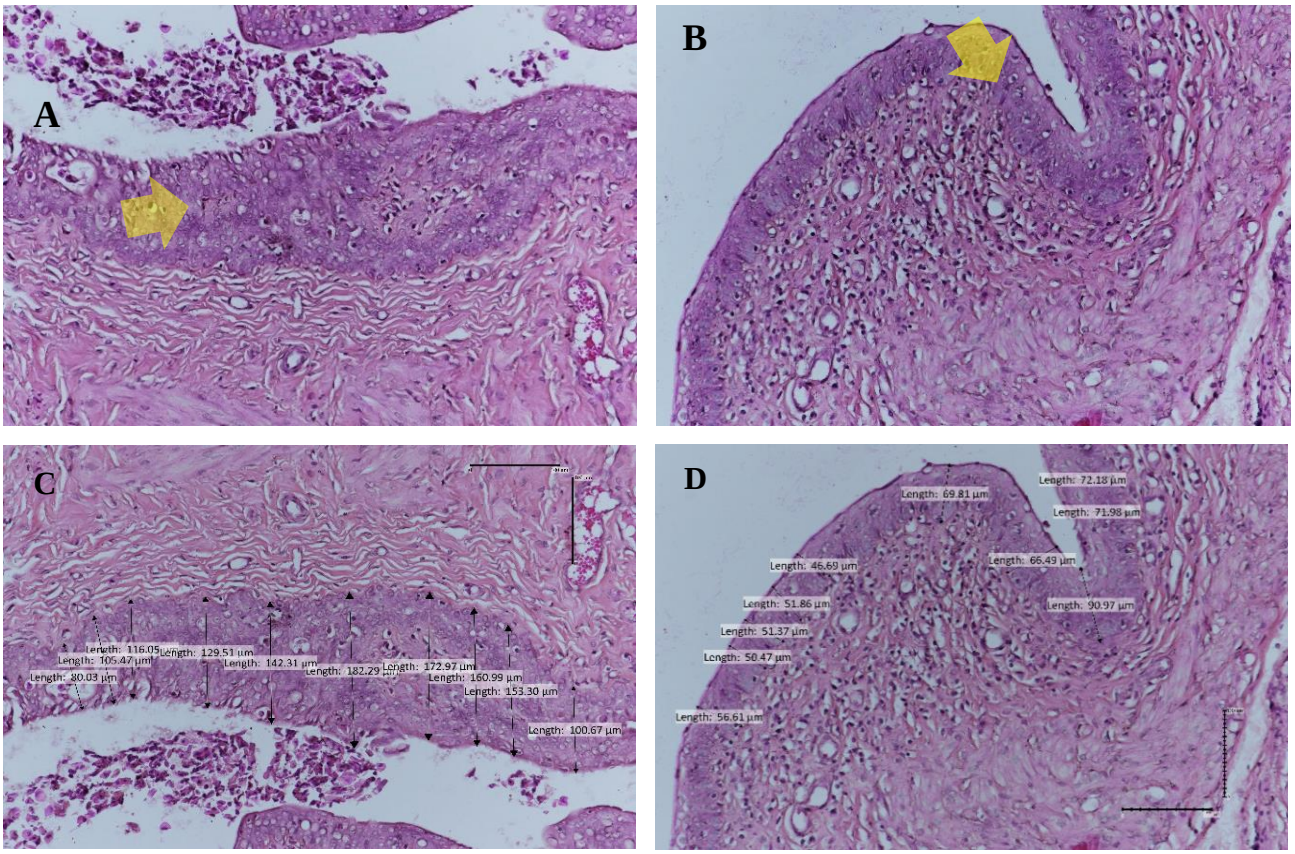

Figure 2. HE-stained samples for vaginal wall/mucosal epithelium thickness measurement. **A:** Vaginal mucosal epithelium layer (arrow) in group C0, **B:** Vaginal mucosal epithelium layer (arrow) in group C1, **C-D:** Measurement of vaginal mucosal epithelium thickness with Image Raster 3 software in groups C0 and C1 (Hematoxylin Eosin; 200× magnification)

Vaginal wall thickness results

Figure 2 shows a histological representation of vaginal wall thickness, indicated by the vaginal mucosal epithelium thickness. Figure 2A presents the thickness of the mucosal epithelium in group C0 while Figure 2B illustrates the thickness of the mucosal epithelium in group C1. Figures 2C and 2D show the measurement of the mucosal epithelial thickness of groups C0 and C1 with Image Raster 3. Remarkably, the majority of the mucosal epithelial thickness in

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group C0 was greater than that in group C1. Diagram 2 indicates that the mean value of vaginal mucosal epithelial thickness was greater in group C0 (56,8 931µm) than in group C1 (44,98349 µm). Table 2 shows a comparison of vaginal mucosal epithelium thickness between the groups using an independent t-test. As can be seen, groups C0 and C1 exhibited significant differences ($p = 0.033$; $\alpha < 0.05$, Table 2).

Table 2. Differentiation test of vaginal density in female rats.

Group	Vaginal Density					
	N	Mean	Standard Deviation	Minimum	Maximum	P
C0	16	56.8931	16.109954	38.205008	87.517398	0.033**
C1	16	44.98349	15.116408	27.482611	77.894783	

* Significant at $p < .05$, group C0: Nulliparous female rats; group C1: Postpartum female rats. N: number of samples. The correlation test between two variables of elastin and epithelial thickness indicated a significant correlation ($p = 0.385$; $\alpha < 0.05$).

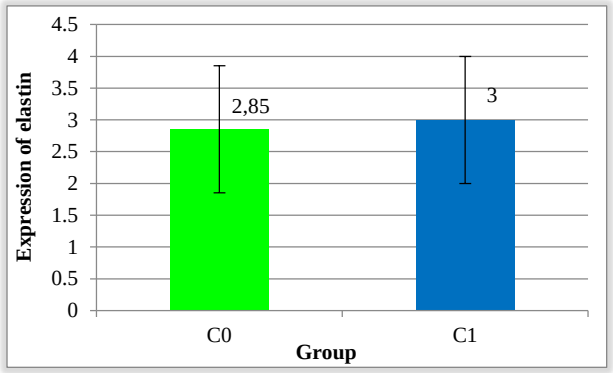

Diagram 1. Median value of expressed of elastin (Group C0: nulliparous female rats; group C1: postpartum female rats).

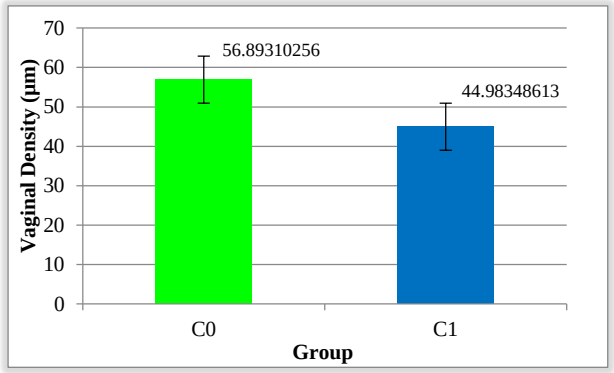

Diagram 2. Mean value of vaginal density (Group C0: nulliparous female rats; group C1: postpartum female rats).

The morphology and physiology of the vulva and vagina change with age, especially during puberty (when the menstrual cycle occurs), pregnancy, and menopause. Remodeling of the vaginal wall and pelvic floor connective tissues leads to a rapid increase in the weight of the uterus, as well as the size, due to the deposition of collagen and elastin. The studies in rats with multiple fetuses have shown that the increase in the weight of the uterus is six to eight-fold compared to non-pregnant uteri, while in humans it is 11-fold. It was then shown that collagen content reduced rapidly after parturition. The wet weight has completed 70% of the involution needed to restore to the baseline value. The collagen removal was 77% completed and elastin removal was 86% completed. The vaginal wall consisted of four layers, including stratified, non-keratinized, squamous epithelium, lamina propria, a dense connective tissue layer rich in fibrillar collagen and elastin, filled with fibroblasts, a muscular layer composed of internal circular and external longitudinal smooth muscle fibers, and tunica adventitia, an elastic tissue layer rich in fiber and collagen that supports the vaginal wall. The lamina propria and muscular layer are the two main layers that confer strength to the vaginal wall (De Landsheere et al., 2013; Dhital et al., 2016; Tadir et al., 2017).

In the present study, elastin was expressed more in the postpartum treatment group than in the nulliparous group, with a significant difference. Elastin was modulated by estrogen of the extracellular matrix and fibroblasts, which were responsible for collagen production. The mucosal epithelium functions according to the estrogen level, which naturally reacts to hormonal fluctuations throughout the woman's life, as well as during the menstrual cycle. Postmenopausal women reported having estrogen, whose estradiol levels averaged 14.1 ± 0.9 pg/ml and estrone levels averaged 27.5 ± 1.2 pg/ml. Physiological estradiol levels in prepubertal children in the range of approximately < 20 pg/ml, adolescent girls 20-300 pg/ml, adult mestrual women 30-800 pg/ml, and postmenopausal women < 20 pg/ml. During pregnancy, the average levels of estradiol in women were up to 20.000pg/ml. The ionized epithelium is rich in glycogen fermented by *Lactobacilli* to decrease vaginal pH levels. The lamina propria mainly consists of collagen fibers and elastin, and contains dense plexuses of small blood vessels, lymphatic vessels, and nerves. This layer is more populous toward the surface and less populous toward the muscular layer. The lamina propria papillae are scarce on the anterior vaginal wall and grow deeper and stronger toward the posterior wall (Tadir et al., 2017).

In the present study, elastin was more notably expressed in the postpartum treatment group than in the nulliparous group, with a significant difference. This result was in line with a study by Jallah et al. (2014), in which elastin production in nulliparous control animals was higher than in the four and eight weeks post-injury, supported by decreased smooth muscle bundles of the vaginal muscularis. Several other studies supported the fact that postpartum and vaginal wall prolapse samples exhibit diminished amounts of smooth muscle cells and their supporting tissues based on

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immunohistochemical evaluation, both quantitative and qualitative decrease in collagen and elastin (Farouk et al., 2013; Jallah et al., 2014; Kerkhof et al., 2014).

At the cellular level, the MMP-9 expression increased in the postpartum period due to its vital role in regulating type I and III collagen and elastin in vaginal elasticity. An imbalance in the proportion of MMP-9 and type I and III collagen carried the risk of prolapse. Furthermore, fibulin-5 expression played a critical role in elastin synthesis. Fibulin-5 was involved in the homeostasis of the extracellular elastin matrix in connective tissues. Moreover, elastin was genetically influenced by the mRNA gene LOX-1. It was also known that elastin synthesis was related to estrogen production. Estrogen participates in modulating the extracellular matrix, smooth muscles, and fibroblasts which are responsible for collagen production. Estrogen production gradually diminishes with age. Estrogen also contributes to childbirth. Elastin production is also vital during parturition to preserve vaginal wall elasticity and maintain structural integrity against the stretching of the vaginal wall. As Word et al. (2009) stated, elastin fibers in humans are synthesized in early life, reach their peak during the third trimester, and gradually decrease by the postnatal period. In a woman's reproductive organs, elastin fiber synthesis is accelerated, particularly during the postpartum period (Word et al., 2009; Downing et al., 2013; Novida, 2013).

In the present study, it has been found that the mean thickness of the vaginal mucosal epithelium was greater in the nulliparous animal model than in the postpartum group. This finding was in accordance with a study conducted by Hamner et al. (2020), who stated that the measured epithelial thickness in the nulliparous group was higher than the postpartum group on the third day. The muscular layer of the vaginal wall was significantly altered during the postpartum period due to uneven thinning of the smooth muscle and smooth muscle bundles. The proliferation of the vaginal mucosal epithelium was hormonally influenced by FBLN5, actionin, and estrogen. Downregulation of ERα correlated with a decreased efficacy of higher doses of estrogen in terms of collagen mRNA, total collagen content, distensibility, and activation of *TGFβ1* gene expression. The estrogen receptor RE-β on the stromal and epithelial layers could govern the mitotic activity of cells to differentiate and increase the mucosal epithelial thickness (Montoya et al., 2015; Hamner et al., 2020).

The thinner postpartum vaginal mucosal epithelium might be due to reduced estrogen levels during the postpartum period as well as mechanical factors. During parturition, intra-abdominal pressure increased sharply as part of the fetal expulsion process. These mechanisms have a direct effect on the vaginal wall. This increased mechanical force caused the connective tissue to stretch, which affected the thickness of the vaginal mucosal epithelium, including its extracellular matrix component. According to Young et al. (2017), pregnancy specifically induced the vagina and its supporting connective tissue causing vaginal distensibility. This distensibility reached its maximum point during vaginal childbirth, making the vaginal wall prone to injury, decreased elasticity, and weakness. Other physiological factors that affect the dilation of the vaginal wall and uterus include fetal size, mechanical contraction, and distension of uteri (Zaki et al., 2016; Young et al., 2017).

CONCLUSION

The tissues of the female reproductive tract are significantly remodeled and altered to allow the fetus to grow and give birth. Elastin production is a vital part of the extracellular matrix in the uterus and vaginal wall, which is remodeled during parturition and at various stages of pregnancy. This study highlights that the expression of elastin significantly influences the vaginal wall thickness, leading to vaginal distensibility that affects collagen structure in pregnancy and parity.

DECLARATIONS

Authors' contribution

Trisniartami Setyaningrum performed research concept and design, wrote the article, and approved the article. M. Yulianto Listiawan performed the collection and/or assembly of data. Brahmana Askandar Tjokroprawiro performed measurements and analyzes experimental data. Widjiati wrote the article and final approval of the article. Budi Santoso performed a critical revision of the article. Cita Rosita Sigit Prakoeswa performed a critical revision of the article. All authors approved the final draft of the manuscript for submission to this journal. 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.

Competing interests

The authors have not declared any conflict of interest.

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Ethical considerations

All authors should not submit manuscripts that are concurrently under consideration for publication in another journal or have already been published as a peer-reviewed publication. Duplicate submission and concurrent publication are highly unethical publishing behavior.

REFERENCES

Abdool Z, Lindeque BG, and Dietz HP (2018). The impact of childbirth on pelvic floor morphology in primiparous Black South African women: A prospective longitudinal observational study. International Urogynecology Journal, 29(3): 369-375. DOI: https://www.doi.org/10.1007/s00192-017-3530-1

Cardiff RD, Miller CH, and Munn RJ (2014). Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harbor Protocols, 6: 655-658. DOI: https://www.doi.org/10.1101/pdb.prot073411.

De Landsheere L, Munaut C, Nusgens B, Maillard C, Rubod C, Nisolle M, Cosson M, and Foidart JM (2013). Histology of the vaginal wall in women with pelvic organ prolapse: A literature review. International Urogynecology Journal, 24(12): 2011-2020. DOI: https://www.doi.org/10.1007/s00192-013-2111-1

Dhital B, Gul-E-Noor F, Downing KT, Hirsch S, and Boutis GS (2016). Pregnancy-induced dynamical and structural changes of reproductive tract collagen. Biophysical Journal, 111(1): 57-68. DOI: https://www.doi.org/10.1016/j.bpj.2016.05.049

Dietz HP, Wilson PD, and Milsom I (2016). Maternal birth trauma: Why should it matter to urogynaecologists? Current Opinion in Obstetrics and Gynecology, 28(5): 441-448. DOI: https://www.doi.org/10.1097/GCO.0000000000000304

Downing KT, Strobe FA, Mikhail MS, and Disanto ME (2013). Pregnancy with and without birth trauma modulates the gene expression of proteins involved in elastic fiber homeostasis in the rat vagina. Open Journal of Obstetrics and Gynecology, 3(08): 603-608. DOI: https://www.doi.org/10.4236/ojog.2013.38108

Farouk H, Fatah AA, Ibrahim K, and Helmy W (2013). Vaginal wall changes in muscles and connective tissues after vaginal birth. Life Science Journal, 10: 2816-2823. DOI: https://www.doi.org/10.7537/marslsj100113.339.

Fedchenko N, and Reifenrath J (2014). Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. Diagnostic pathology, 9: Article number 221. DOI: https://www.doi.org/10.1186/s13000-014-0221-9.

Gachon B, Nordez A, Pierre F, and Fritel X (2019). Tissue biomechanical behavior should be considered in the risk assessment of perineal trauma at child birth. Archives of Gynecology and Obstetrics, 300: 1821-1826. DOI: https://www.doi.org/10.1007/s00404-019-05369-5

Gunja-Smith Z, Lin J, and Woessner JF (1989). Changes in desmosine and pyridinoline crosslinks during rapid synthesis and degradation of elastin and collagen in the rat uterus. Matrix, 9: 21-27. DOI: https://www.doi.org/10.1016/s0934-8832(89)80014-9.

Hamner J, Florian-Rodriguez M, Acevedo J, Shi H, and Word RA (2020). Protease inhibition improves healing of the vaginal wall after obstetrical injury: Results from a preclinical animal model. Scientific Reports, 10(6358): 1-11. DOI: https://www.doi.org/10.1038/s41598-020-63031-6

Jallah ZC (2014). The role of vaginal smooth muscle in the pathogenesis of pelvic organ prolapsed. Thesis, Swanson School of Engineering, University of Pittsburgh, United States. p. 181. Available at: https://core.ac.uk/download/pdf/33561719.pdf

Kamisan AI, Gerges B, Shek KL, and Dietz HP (2015). The association between vaginal parity and hiatal dimensions: A retrospective observational study in a tertiary urogynaecological centre. International Journal of Obstetrics and Gynaecology, 122(6): 867-872. DOI: https://www.doi.org/10.1111/1471-0528.12920

Kerkhof MH, Ruiz-Zapata AM, Bril H, Bleeker MC, Belien JA, Stoop R, and Helder MN (2014). Changes in tissue composition of the vaginal wall of premenopausal women with prolapse. American Journal of Obstetrics and Gynecology, 210(2): 168.e1-168.e9. DOI: https://www.doi.org/10.1016/j.ajog.2013.10.881

Mithieux SM, and Weiss AS (2005). Elastin. Advances in Protein Chemistry, 70: 437-461. DOI: https://www.doi.org/10.1016/S0065-3233(05)70013-9

Montoya TI, Maldonado PA, Acevedo JF, and Word RA (2015). Effect of vaginal or systemic estrogen on dynamics of collagen assembly in the rat vaginal wall. Biology of Reproduction, 92(2): 1-9. DOI: https://www.doi.org/10.1095/biolreprod.114.118638

Newman R, Campbell P, Gooneratne M, Lowenstein L, Mu G, Qureshi A, and Krychman M (2018). Genito pelvic vaginal laxity: Classification, etiology, symptomatology, and treatment considerations. Current Sexual Health Reports, 10: 222-236. DOI: https://www.doi.org/10.1007/s11930-018-0168-z

Novida A (2013). Differences in expression of mmp-9 and timp-1 in nullipara and postpartum studies on the anal levator muscle, vaginal wall and sacrouterine ligament of rat rattus norvegicus. Magister thesis, Universitas Brawijaya, Indonesia. Available at: http://repository.ub.ac.id/158562/

Qureshi AA, Sharma K, Thornton M, Myckatyn TM, and Tenenbaum MM (2018). Vaginal laxity, sexual distress, and sexual dysfunction: A cross-sectional study in a plastic surgery practice. Aesthetic Surgery Journal, 38(8): 873-880. DOI: https://www.doi.org/10.1093/asj/sjx255

Rodríguez-Cabello JC, González de Torre I, Ibañez-Fonseca A, and Alonso M (2018). Bioactivescaff olds based on elastin-like materials for wound healing. Advanced Drug Delivery Reviews, 129: 118-133. DOI: https://www.doi.org/10.1016/j.addr.2018.03.003

Roos AM, Speksnijder L, and Steensma AB (2020). Postpartum sexual function; the importance of the levatorani muscle. International Urogynecology Journal, 31(11): 2261-2267. DOI: https://www.doi.org/10.1007/s00192-020-04250-3

Rynkevic R, Martins P, Hympanova L, Almeida H, Fernandes AA, and Deprest J (2017). Biomechanical and morphological

properties of the multiparous ovine vagina and effect of subsequent pregnancy. Journal of Biomechanics, 57: 94-102. DOI: https://www.doi.org/110.1016/j.jbiomech.2017.03.023

Shek KL, and Dietz HP (2009). The effect of child birth on hiatal dimensions. Obstetrics and Gynecology, 113(6): 1272-1278. DOI: https://www.doi.org/110.1097/AOG.0b013e3181a5ef23

Tadir Y, Gaspar A, Lev-Sagie A, Alexiades M, Alinsod R, Bader A,Calligaro A, Elias JA, Gambaciani M, Gaviria JE et al. (2017). Light and energy based therapeuticsfor genitourinary syndrome of menopause: Consensus and controversies. Lasers in Surgery and Medicine, 49: 137-159. DOI: https://www.doi.org/110.1002/lsm.22637

Word RA, Pathi S, and Schaffer JI (2009). Pathophysiology of pelvic organ prolapse. Obstetrics and Gynecology Clinics of North America, 36(3): 521-539. DOI: https://www.doi.org/110.1016/j.ogc.2009.09.001

Young N, Rosamilia A, Arkwright J, Lee J, Davies-Tuck M, Melendez J, Werkmeister J, and Gargett C (2017). Vaginal wall weakness in parous ewes: A potential preclinical model of pelvic organ prolapses. International Urogynecology Journal, 28(7): 999-1004. DOI: https://www.doi.org/110.1007/s00192-016-3206-2

Zaki A, Mardian K, and Mustokoweni S (2016). Increased thickness of elastin fibre on vaginal wall of aiunterior pelvic organ prolapse. Majalah Obstetri and Ginekologi, 24: 31-36. DOI: https://www.doi.org/10.20473/mog.V24I12016.31-36

Zong W, Stein SE, Starcher B, Meyn LA, and Moalli PA (2010). Alterationof vaginal elastin metabolism in women with pelvic organ prolapse. Obstetrics and Gynecology, 115: 953-961. DOI: https://www.doi.org/110.1097/AOG.0b013e3181da7946

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Molecular Characterization of Chicken Anaemia Virus Circulating in Commercial Poultry Flocks in Egypt during 2020

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ABSTRACT

Chicken Anemia Virus (CAV) is an extremely contagious immunosuppressive disease causing high economic losses in poultry production. In the present study, tissue samples (bone marrow, thymus, and spleen) were collected from 86 different broiler chicken farms located in fourteen governorates in Egypt during 2020. They suffered from retard growth, weakness, and a drop in egg production with an observed mortality rate ranged 5-15%. A total of 26 samples were positive for CAV using PCR in six governorates in Lower Egypt with a 30% incidence rate, especially in Sharkia (78%), Ismailia (62.5%), and Alexandria (60%). The viral protein1 (VP1) gene of CAV was genetically characterized by sequencing of 10 selected viruses in six governorates. revealing that all Egyptian strains were clustered into two groups (A, B) that was distinct from vaccine strains (Del-Ros, Cux-1, and 26PA) which were clustered in group C. The seven Egyptian viruses in this study (A-Egypt-AN1-2020 to A-Egypt-AN7-2020) were clustered with the viruses from Japan, Argentina, and Malaysia in group A, and the other three viruses (A-Egypt-AN8-2020, A-Egypt-AN9-2020, A-Egypt-AN10-2020) were clustered with the viruses from Nigeria, and India in group B. The Egyptian viruses in the current study acquired new specific mutations clustering them into new subgroups (2A, 2B). By mutation analysis comparing with Del-Rose reference strains, V75I, M97L, and K139Q, E144Q were recorded in all viruses in the group A and B. All Egyptian viruses in the current study had specific new mutations at Y13N, H22N. Moreover, mutation at G74E in Egyptian viruses recorded in the current study was related to sub group 2A, I83V in three strains (A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020), and S140A in the hypervariable region was found in four strains (A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and A/Egypt/AN5/2020) in subgroup 2A. Furthermore, Q139 and Q144 amino acid substitutions, which are important in viral replication, were observed in all viruses. The field viruses in the study were distinct from the vaccinal strains by phylogenetic analysis and A.A. identity. In conclusion, the CAV was continuously circulating in Egypt from different genotypes. It acquired new specific mutations clustering them in a new subgroup, and it was distinct from vaccinal strains. Therefore, it is important to conduct continuous monitoring on the genetic evolution of CAV and further studies on the pathogenicity of the virus and the vaccine efficacy.

Keywords: Chicken Anemia Virus, Egypt, Genetic evolution, Viral protein 1 gene

INTRODUCTION

Chicken Anaemia Virus (CAV) is an immunosuppressive pathogen leading to a barrage of economic losses for poultry breeders (Islam et al., 2002). The first detection of CAV was in Japan in 1978 (Yuasa et al., 1979). Then it was recorded and spread in different governorates in Egypt leading to high economic losses (Erfan et al., 2018).

The Chicken Anaemia Virus causes high economic losses, especially in broiler chickens. Chickens of all ages are vulnerable to infection, but the chickens less than two weeks old commonly represent the clinical diseases (Miller and Schat, 2004; Cheng et al., 2019). The infection is characterized mainly by anemia, weight loss, and lymphoid atrophy accompanied by immunosuppression that leads to an increase in the susceptibility of secondary bacterial and viral infection. The mortality and morbidity rates due to CAV infection reach 55-80% (Lai et al., 2018).

Chicken Anemia Virus (CAV) is a non-enveloped virus and has a negative-sense genome (Peters et al., 2006) that consists of three overlapping open reading frames encoding to three viral proteins (VP1, VP2, and VP3). The VP1 capsid protein is an important major structural protein. The VP2 protein is a dual-specificity protein that acts as a scaffold to phosphatase activity to assist the correct assemblage of VP1 protein (Craig et al., 2009; Rosario et al., 2017). The VP3 protein called apoptin is the primary factor of virulence of CAV inducing the apoptosis of the thymocytes and hemopoietic cells in infected chickens (Castaño et al., 2019).

The CAV's Amino Acid (A.A.) composition is extremely restrictive, with major differences in some regions in the VP1 gene called Hyper Variable Region (HVR) at 139-151 A.A. (Zhang et al., 2013). The VP1 gene is responsible for the genetic characterization of the virus, and It encodes a highly immunogenic protein responsible for the potential and

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virulence of infection (Lien et al., 2012). Three genotypes of CAV (I, II, and III) are genetically distinct and recognized based on the phylogeny of VP1 gene (Snoeck et al., 2012). Genotypes II and III are worldwide distributed, while genotype I consists alone of Australian isolates (Kim et al., 2010). Only minor mutations in amino acid sequences have been recorded in CAV viruses from various geographical positions (Ducatez et al., 2005; Kim et al., 2010). The Commercially used CAV vaccines (Cux-1N/Germany and CAV/Nobilis®P4) are derived from wild-type CAVs that are not completely attenuated; therefore, the virus can revert to virulent, and be transmitted horizontally and vertically causing clinical symptoms in young chickens (Tseng et al., 2019).

The objective of the present study was to disseminate the situation and molecular characterization of CAV circulating in Egypt during 2020 and to determine the relationship between current viruses and vaccine strains for improving the control of the virus.

MATERIALS AND METHODS

Tissue specimen

The samples were collected from 86 commercial broiler chicken farms (aged two to four weeks) located in 14 Egyptian Governorates (Beheira, Sharqia, Qalyubia, Dakahlia, Ismailia, Gharbia, Giza, Matruh, Cairo, Alexandria, Minya, Beni Suef, Sohag, Faiyum) during 2020 (Table 1 and Figure 1). Most tested broiler chicken farms were vaccinated. The samples were collected from different tissues (bone marrow, thymus, and spleen). The samples were homogenized by grinding. Twenty-five mg of various affected tissues were grounded with phosphate buffer saline (PBS) and the mixture of one mg streptomycin sulphate/ml and 1000 I.U. penicillin/ml using a mortar and pestle. the Freezing and thawing were carried out three times, and then it was centrifuged at 3000 rpm for 20 minutes. Next, the supernatant was collected in a new tube and stored at -20 till used (Hirai and Shimakura, 1974).

Ethical approval

The handling of chickens was based on the ethical role of the National Animal Health and Research Institute, Giza, Egypt. The tissue samples were collected from dead chickens suffering from an infection of CAV in commercial farms. Both handling of the chickens and sampling were performed with respect to animal rights, and immediately after the confirmation of death by a veterinarian.

Table 1. The location, number, and the result of evaluated samples by PCR for detection of chicken anemia virus

Governorate	No. of collected samples	No. of CAV positive farms
Beheira	10	5
Sharqia	9	7
Qalyubia	9	4
Dakahlia	8	0
Ismailia	8	5
Gharbia	7	0
Giza	7	0
Matruh	5	0
Cairo	5	2
Alexandria	5	3
Minya	5	0
Beni Suef	3	0
Sohag	3	0
Faiyum	2	0
Total	86	26 (30%)

CAV: Chicken anemia virus, No: Number

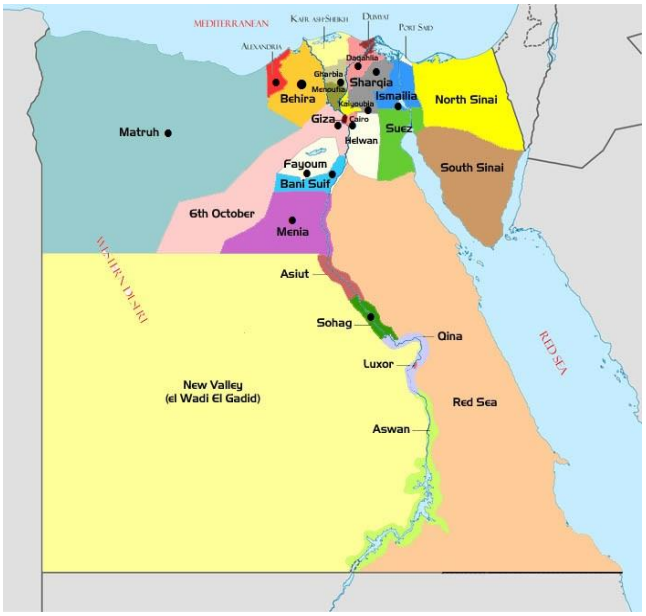

Figure 1. Distribution of chicken anemia virus in Egyptian map in 2020

Detection of chicken anemia virus by PCR

DNA was extracted from tissue homogenate by QIAmp DNA mini kit (Qiagen Inc., Valencia, Calif., USA) following the manufacturer's instructions. Amplification of partial VP1 gene was carried out by using EmeraldAmp® GT PCR kit (TaKaRa Bio, Inc., Shiga, Japan), gene-specific oligonucleotide primers (5-GAC TGT AAG ATG GCA AGA CGA GCT C-3 and 5-GGC TGA AGG ATC CCT CAT TC-3) were used (Todd et al., 1991) according to manufacturer's instructions. The specific amplified PCR product was detected by agarose gel electrophoresis.

Sequencing of partial VP1 gene

Ten positive samples were selected for partial sequencing of the VP1 gene (Table 2). The positive amplified PCR product was purified using QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The sequence reaction was carried

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out by using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), then it was purified by using Centrisep spin column, (Thermo Fisher, USA), and the sequence collection was performed by ABI 3500 Genetic Analyzer (Life Technologies, USA).

Genetic and phylogenetic studies

The DNA sequence of the partial VP1 gene for Egyptian CAV in this study was aligned with 23 different viruses from different countries and vaccine strains (Del-Ros, Cux-1, and 26PA) from the National Centre in Biotechnology Information (NCBI) by using Bio-Edit software clustal W (Hall, 1999). The Phylogenetic tree was made by using the Neighbor-Joining method in MEGA 6 with 1,000 bootstrap replications (Tamura et al., 2013). The genetic distance among different viruses was carried out using DNASTAR Lasergene 9 (Madison, WI, USA). The viruses in the present study were published in National Center for Biotechnology Information (NCBI) under accession number (Table 2).

Table 2. Governorates and Genbank accession number of partial VP1gene sequence of 10 chicken anemia virus selected samples

No.	Code	Governorate	Accession number
1	A-Egypt-AN1-2020	Beheira	MW286460
2	A-Egypt-AN2-2020	Sharqia	MW286461
3	A-Egypt-AN3-2020	Alexandria	MW286462
4	A-Egypt-AN4-2020	Cairo	MW286463
5	A-Egypt-AN5-2020	Ismailia	MW286464
6	A-Egypt-AN6-2020	Sharqia	MW286465
7	A-Egypt-AN7-2020	Qalyubia	MW286466
8	A-Egypt-AN8-2020	Beheira	MW286467
9	A-Egypt-AN9-2020	Beheira	MW286468
10	A-Egypt-AN10-2020	Sharqia	MW286469

RESULTS

Clinical signs and gross pathology

The gross examination showed that there were infected flocks suffering from stunting, depression, poor growth, weakness, mild drop in egg production with mortality rates ranging from 5% to 15%. While the complete necropsy examinations revealed paleness in bone marrow and liver, atrophy of bursa of Fabricius and thymus. Subcutaneous hemorrhage was also observed in some cases. It was similar to clinical signs findings of Swayne et al. (2019).

Detection of chicken anemia virus by PCR

Out of 86 tested tissue samples, 26 samples were CAV positive at the correct size band of 675 bp with an incidence rate of 30% (Table 1). All positive samples were recorded in six governorates (Beheira, Sharqia, Alexandria, Cairo, Ismailia, Qalyubia) in Lower Egypt with the highest incidence in Sharqia, Ismailia, and Alexandria (78%, 62.5%, and 60%, respectively), and the lowest incidence in Cairo (40%).

Genetic characterization of VP1 gene of chicken anemia virus

The nucleotide and amino acid sequence of VP1 gene of selected 10 positive samples of CAV located in six governorates (Beheira, Sharqia, Alexandria, Cairo, Ismailia, Qalyubia) were aligned with different CAV viruses and vaccine strains from the National Centre in Biotechnology Information (NCBI) By using Bioedit clustal W software (Hall, 1999), and The Phylogenetic tree was made by using the Neighbor-Joining method in MEGA 6 with 1,000 bootstrap replications (Tamura et al., 2013). The phylogenetic tree showed that VP1 gene of CAV was divided into two groups (A, B) distinct from vaccine strains (Del-Ros, Cux-1, and 26PA) that were clustered in group C. The seven Egyptian viruses (A/Egypt/AN1/2020 to A/Egypt/AN7/2020) were clustered with G6/Japan/AB119448.1, ArgA0021-3/Argentina/EU871783.1 and SMSC-1/Malaysia/AF285882.1 in group A in a new subgroup 2A, but the other three viruses (Egypt/AN8/2020, Egypt/AN9/2020 and Egypt/AN10/2020) were clustered with NIE/19.04/118/Nigeria and CAV-B/India in the group B in a new subgroup 2B as shown in Figure 2.

By mutation analysis of partial VP1 gene sequence including the hypervariable region (139-151) comparing with Del-Rose reference strains, the V75I, M97L, K139Q, and E144Q mutations were recorded in all viruses related to group A and B. The last two mutations were included in the hypervariable region. All Egyptian viruses in the current study had specific new mutations at Y13N and H22N. In addition, the seven Egyptian viruses related to group 2A had G74E and I83V in A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and S140Ain in A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and A/Egypt/AN5/2020. The last one is included in the hypervariable region as shown in Table 3.

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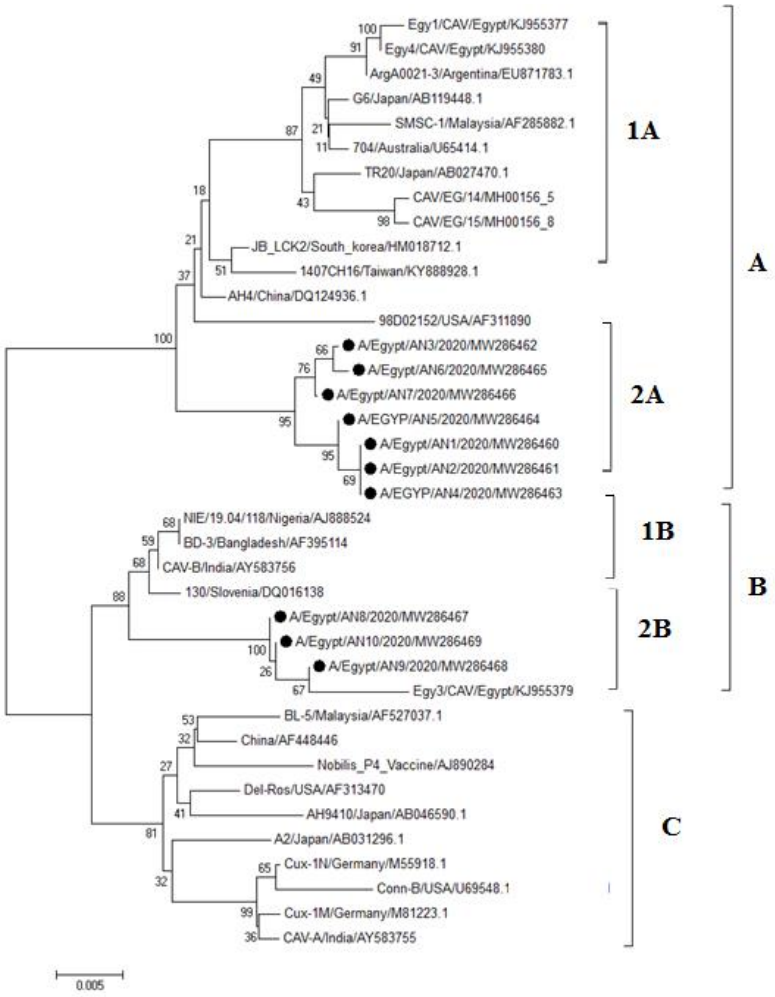

Figure 2. Phylogenetic tree of the partial VP1 gene sequence of chicken anemia virus. FN: The figure shows the phylogenetic analysis of VP1 gene of CAV revealing that all Egyptian viruses were clustered into two groups (A, B) in a new subgroup (2A, 2B) that was distinct from vaccine strains that were clustered in group C. The CAV viruses in the present study are indicated with a black dot.

Table 3. Numbers of amino acid substitution of partial VP1 gene sequence comparing with Del-Rose reference strains

Strain name	Genotype	Number of amino acid in VP1 gene								
		13	22	74	75	83	97	139	140	144
Del-Ros/USA/AF313470 (Refrence strain)	C	Y	H	G	V	I	M	K	S	E
SMSC-1/Malaysia/AF285882.1	1A	-	-	-	I	-	L	Q	-	Q
AH9410/Japan/AB046590.1	1A	-	-	-	I	-	L	Q	-	Q
ArgA00213/Argentina/EU871783.1	1A	-	-	-	I	-	L	Q	-	Q
A/Egypt/AN1/2020	2A	N	N	E	I	V	L	Q	A	Q
A/Egypt/AN2/2020	2A	N	N	E	I	V	L	Q	A	Q
A/Egypt/AN3/2020	2A	N	N	E	I	-	L	Q	-	Q
A/Egypt/AN4/2020	2A	N	N	E	I	V	L	Q	A	Q
A/Egypt/AN5/2020	2A	N	N	E	I	-	L	Q	A	Q
A/Egypt/AN6/2020	2A	N	N	E	I	-	L	Q	-	Q
A/Egypt/AN7/2020	2A	N	N	E	I	-	L	Q	-	Q
CAV-A/India/AY583755	1B	-	-	-	I	-	L	Q	-	Q
BD-3/Bangladesh/AF395114	1B	-	-	-	I	-	L	Q	-	Q
NIE/19.04/118/Nigeria/AJ888524	1B	-	-	-	I	-	L	Q	-	Q
A/Egypt/AN8/2020	2B	N	N	-	I	-	L	Q	-	Q
A/Egypt/AN9/2020	2B	N	N	-	I	-	L	Q	-	Q
A/Egypt/AN10/2020	2B	N	N	-	I	-	L	Q	-	Q
Nobilis P4 Vaccine/AJ890284	C	-	-	-	-	-	-	-	-	-

A: Alanine, E: Glutamic acid, G: Glycine, H: Histidine, I: Isoleucine, K: Lysine, L: Lysine, M: Methionine, N: Asparagines, Q: Glutamine, S: Serine, V: Valine, Y: Tyrosine. The A.A. identity showed that the seven Egyptian viruses (A/Egypt/AN1/2020 to A/Egypt/AN7/2020) had a high identity percent (97 to 97.9%) with G6/Japan/AB119448.1, ArgA0021-3/Argentina/EU871783.1, and SMSC-1/Malaysia/AF285882, and the other three Egyptian viruses (A/Egypt/AN8/2020, A/Egypt/AN9/2020, A/Egypt/AN10/2020) had a high identity percent (97.3-98.7%) with BD-3/Bangladesh and NIE/19.04/118/Nigeria. The A.A. identity percent among Egyptian viruses in the subgroup 2A and 2B in the current study and vaccine strains used in Egypt (Cux-1, Del-Ros, and 26PA) was 94.8 to 95.7% A.A. and 97-97.8% A.A. respectively as shown in Figure 3.

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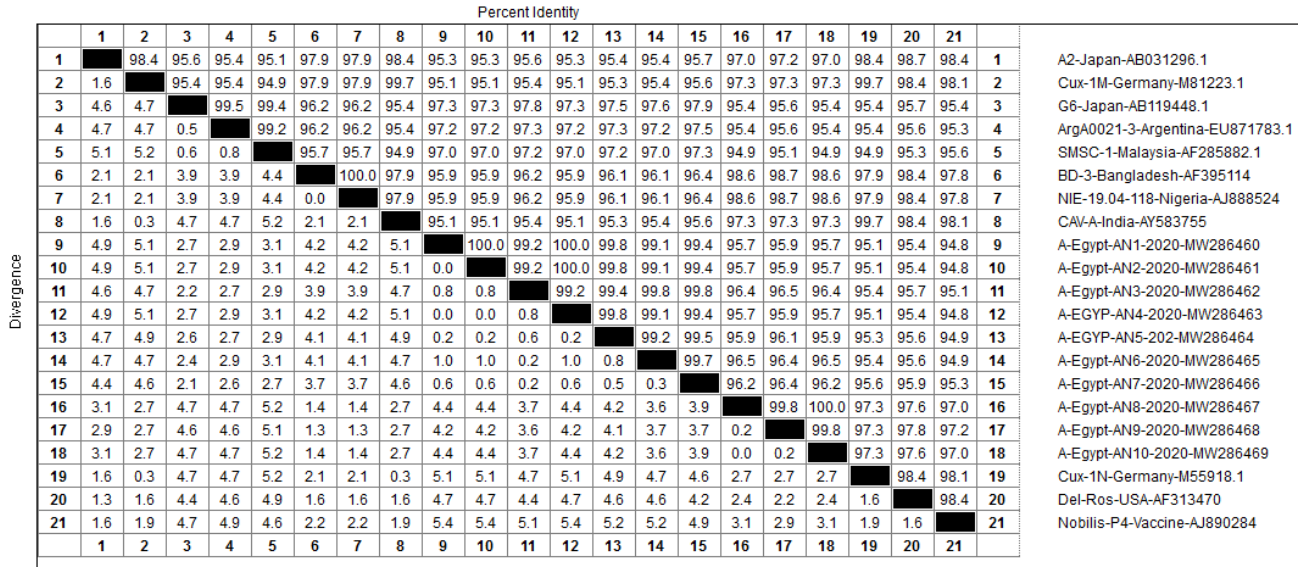

Figure 3. Amino acid identities of VP1 gene of CAV compared to other selected viruses and vaccines' strains. FN: The A.A. identity percent among Egyptian viruses in the subgroup 2A and 2B in the current study and vaccine strains used in Egypt (Cux-1, Del-Ros, and 26PA) was 94.8 to 95.7% A.A. and 97-97.8% A.A. respectively.

DISCUSSION

Chicken anemia virus worldwide is an immunosuppressive disease that circulates in several countries (Natesan et al., 2006; Zhang et al., 2013). In Egypt, the CAV was spread in different governorates causing high mortality and economic losses (Erfan et al., 2018). In the current study, 26 positive samples out of 86 samples were collected from different broiler chicken farms located in six out of 14 governorates in Egypt with a high incidence rate (30%) in Lower Egypt, especially in Sharkia (78%), Ismailia (62.5%), and Alexandria (60%) and most of them were vaccinated. It is an indicator for the progressive exacerbation of the disease in vaccinated broiler chicken farms in Egypt during 2020 with a high mortality rate that ranged from 5 to 15 percent.

The VP1 gene sequence is extremely important to determine the virulence of the virus, cell infection ability, viral replication, and the relationship among CAV viruses especially the N-terminal half of VP1 gene that contains the hypervariable region from 139 to 151 A.A. (Renshaw et al., 1996; Islam et al., 2002; Negasi et al., 2008). These hypervariable regions are the most variable region in the VP1 gene of chicken anemia virus as previously mentioned by Islam et al. (2002). Phylogenetically, the VP1 gene was divided into many groups in the world that can easily identify the field and vaccines' strains (Eltahir et al., 2011)

As previously recorded the CAV circulated in Egypt from different genotypes may be due to multiple introductions (AboElkhair et al., 2014; Abdel-Mawgod et al., 2018; Erfan et al., 2018). In the current study, ten viruses were selected from six different governorates to be sequenced for partial VP1 gene and to be compared with viruses isolated from many countries, such as Germany, India, Japan, Nigeria, Bangladesh, USA, Korea, Taiwan, and China (Schat, 2009). All Egyptian viruses in the current study related only to field viruses. The vaccine strains were not recorded. In the present study, the seven viruses were related to the viruses from Japan, Argentina, and Malaysia as previously recorded (AboElkhair et al., 2014; Abdel-Mawgod et al., 2018; Erfan et al., 2018). but the Egyptian viruses in the study were acquired new mutations cluster them in a new subgroup 2A and the other three Egyptian viruses were related to the viruses from Nigeria, India with new mutations cluster them in a new subgroup 2B. These findings indicated continuous circulation of multiple genotypes of CAV in Egypt with multiple evolutions in the VP1.

By A.A. mutation analysis, the viruses related to group A and B had A. A mutation at V75I, M97L, K139Q, E144Q when comparing with DEL-Rose vaccine strain as previously described by AboElkhair et al. (2014) and Erfan et al. (2018). The 139Q and 144Q could decrease the efficacy of the replication in the cell culture (Renshaw et al., 1996). In the current study, the Egyptian viruses had a specific new mutation. The Y13N, H22N were recorded in all Egyptian strains in our study and G74E, were recorded in subgroup 2A and I83V in A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020, and S140Ain in A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and A/Egypt/AN5/2020. The H22N was recoded by AboElkhair et al. (2014) in one Egyptian virus that was important in distinguishing the strain of CAV. Further studies are needed to work on the effect of these mutations in the pathogenicity of the virus and they may be the cause of outbreaks in vaccinated farms.

Recently, multiple vaccines are used (mainly Del-Ros, Nobilis P4, and Cux-1 strains) for breeder flocks producing maternal antibodies to newly born chickens which give protection till two to three weeks old (Haridy et al., 2009; Prezotto et al., 2016). The phylogenetic analysis and A.A. identity showed that the field viruses were distinct from the

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vaccines' strains as previously recorded by AboElkhair et al. (2014) and Erfan et al. (2018). It explains the occurred infection of the vaccinated flocks by CAV as mentioned by Abdel-Mawgod et al. (2018) and Erfan et al. (2018). Further study is needed to determine the vaccine efficacy against the field stains.

CONCLUSION

The chicken anemia virus was widely circulated with a high incidence in Lower Egypt in six governorates. The phylogenetic tree of the partial VP1 gene during the present study indicated the Egyptian virus were clustered in two groups (A, B) with a newly acquired mutation specific to Egyptian viruses forming new subgroups (2A, 2B). The field virus in the current study was genetically distinct from vaccine strains. Continuous monitoring on the genetic evolution of CAV and further studies are required to work on the effect of acquired mutations on the pathogenicity of the virus and the vaccine efficacy.

DECLARATIONS

Competing interests

The authors declare that they have no conflict of interest.

Authors' contribution

Ahmed Abd Elhalim Mohammed and Abdelhafez Samir carried out tissue specimen collection from the affected flocks, detection of the DNA of Chicken anemia virus and analysis of the data, and wrote the manuscript. Nahed Yehia carried out the sequencing of partial VP1 gene, genetic and phylogenetic analysis, and analysis of the data. All authors read and approved the final manuscript.

Ethical consideration

Ethical issue including plagiarism, consent to public misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy has been checked by the authors

REFERENCES

Abdel-Mawgod S, Adel A, Arafa AS, and Hussein HA (2018). Full genome sequences of chicken anemia virus demonstrate mutations associated with pathogenicity in two different field isolates in Egypt. Virus Disease, 29(3): 333-341. DOI: https://www.doi.org/10.1007/s13337-018-0467-z.

Aboelkhair M, El-Razak A, and Metwally A (2014). Molecular characterization of chicken anemia virus circulating in chicken flocks in Egypt. Advances in Virology, Article ID 797151. DOI: https://www.doi.org/10.1155/2014/797151

Castaño P, Benavides J, Lee MS, Fernández M, Fuertes M, Royo M, Fernández JM, Pérez V, and Ferreras MC (2019). Tissue tropism of chicken anaemia virus in naturally infected broiler chickens. Journal of Comparative Pathology, 167: 32-40. DOI: https://www.doi.org/10.1016/j.jcpa.2018.11.008

Cheng JH, Lai GH, Lien YY, Sun FC, Hsu SL, Chuang PC, and Lee MS (2019). Identification of nuclear localization signal and nuclear export signal of VP1 from the chicken anemia virus and effects on VP2 shuttling in cells. Virolology Journal, 16(1): Article number 45. DOI: https://www.doi.org/10.1186/s12985-019-1153-5

Craig MI, Rimondi A, Delamer M, Sansalone P, König G, Vagnozzi A, and Pereda A (2009). Molecular characterization of chicken infectious anemia virus circulating in Argentina during 2007. Avian Disease, 53(3): 331-335.DOI: https://www.doi.org/10.1637/8478-100808-Reg.1.

Ducatez MF, Owoade AA, Abiola JO, and Muller CP (2005). Molecular epidemiology of chicken anemia virus in Nigeria. Archieve Virology, 151: 97-111. https://www.doi.org/10.1007/s00705-005-0609-7

Eltahir YM, Qian K, Jin W, and Qin A (2011). Analysis of chicken anemia virus genome: evidence of intersubtype recombination. Virolology Journal, 8: 512-512. DOI: https://www.doi.org/10.1186/1743-422X-8-512.pdf.

Erfan AM, Selim AA, and Naguib MM (2018). Characterization of full genome sequences of chicken anemia viruses circulating in Egypt reveals distinct genetic diversity and evidence of recombination. Virus Research, 251: 78-85 DOI: https://www.doi.org/10.1016/j.virusres.2018.05.008.

Hall TA (1999). Bio Edit: A user-friendly biological sequence alignment editor and analysis for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95-98. Available at: https://www.sid.ir/en/journal/JournalList.aspx?ID=5696

Haridy M, Goryo M, Sasaki J, and Okada K (2009). Pathological and immunohistochemical study of chickens with co-infection of Marek's disease virus and chicken anaemia virus. Avian Pathology, 38(6): 469-483. DOI: https://www.doi.org/10.1080/03079450903349162.

Hirai K, and Shimakura S (1974). Structure of infectious bursal disease virus. Journal of Virology, 14: 957-964. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC355603/#:~:text=The%20buoyant%20density%20of%20infectious,diameter%20and%20had%20no%20envelope.

Islam MR, Johne R, Raue R, Todd D, and Müller H (2002). Sequence analysis of the full length cloned DNA of a chicken anaemia virus (CAV) strain from Bangladesh: Evidence for genetic grouping of CAV strains based on the deduced VP1 amino acid

sequences. Journal of Veterinary Medicine Series B, 49(7): 332-337. DOI: https://www.doi.org/10.1046/j.1439-0450.2002.00581.x.

Kim HR, Kwon YK, Bae YC, Oem JK, and Lee OS (2010). Molecular characterization of chicken infectious anemia viruses detected from breeder and broiler chickens in South Korea. Poultry Science, 89(11): 2426-2431. DOI: https://www.doi.org/10.3382/ps.2010-00911.

Lai GH, Lin MK, Lien YY, Cheng JH, Sun FC, Lee MS, Chen HJ, and Lee MS (2018). Characterization of the DNA binding activity of structural protein VP1 from chicken anaemia virus. BMC Vetrinary Reseach, 14: Article number 155. DOI: https://www.doi.org/10.1186/s12917-018-1465-5.

Lien YY, Huang CH, Sun FC, Sheu SC, Lu TC, Lee MS, Hsueh SC, Chen HJ, and Lee MS (2012). Development and characterization of a potential diagnostic monoclonal antibody against capsid protein VP1 of the chicken anemia virus. Journal of Veterinary Science, 13: 73-79. DOI: https://www.doi.org/10.4142/jvs.2012.13.1.73.

Miller MM, and Schat KA (2004). Chicken Infectious Anemia virus: An example of the ultimate host-parasite relationship. Avian Disease, 48: 734-745. DOI: https://www.doi.org/10.1637/7271-090304R.

Natesan S, Kataria JM, Dhama K, Rahul S, and Baradhwaj N (2006). Biological and molecular characterization of chicken anaemia virus isolates of Indian origin. Virus Research, 118: 78-86. DOI: https://www.doi.org/10.1016/j.virusres.2005.11.017.

Negasi Z, Omar AR, Hair-Bejo M, and Giap TC (2008). Detection and characterization of chicken anemia virus from commercial broiler breeder chickens. Virology Journal, 5: Article number 128. DOI: https://www.doi.org/10.1186/1743-422X-5-128.

Peters MA, Crabb BS, Washington EA, and Browning GF (2006). Site-directed mutagenesis of the VP2 gene of chicken anemia virus affects virus replication, cytopathology and host-cell MHC class I expression. 1996. Journal of General Virology, 87(4): 823-831. DOI: https://www.doi.org/10.1099/vir.0.81468-0.

Prezotto C, Marin S, Araújo T, Barbosa F, Barrios P, Gomes A, Peconick A, Resende M, Sousa R, and Martins N (2016). Experimental coinfection of chicken anemia virus and mycoplasma gallisepticum vaccine strains in broiler chicks. Brazilian Journal of Poultry Science, 18: 475-480. DOI: https://www.doi.org/10.1590/1806-9061-2016-0235

Renshaw RW, Soiné C, Weinkle T, O'Connell PH, Ohashi K, Watson S, Lucio B, Harrington S, and Schat KA (1996). A hypervariable region in VP1 of chicken infectious anemia virus mediates rate of spread and cell tropism in tissue culture. Journal of Virology, 70: 8872-8878. DOI: https://www.doi.org/10.1128/JVI.70.12.8872-8878.1996.

Rosario K, Breitbart M, and Harrach B (2017). Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. Archives of Virology, 162(5):1447-1463. DOI: https://www.doi.org/10.1007/s00705-017-3247-y

Schat KA (2009). Chicken anemia virus. Current Topics in Microbiology and Immunology, 331: 151-183. DOI: https://www.doi.org/10.1007/978-3-540-70972-5_10.

Snoeck CJ, Komoyo GF, Mbee BP, Nakouné E, Le Faou A, Okwen MP, and Muller CP (2012). Epidemiology of chicken anemia virus in Central African Republic and Cameroon. Virology Jornal, 9(1): Article number 189. DOI: https://www.doi.org/10.1186/1743-422X-9-189

Swayne DE, Boulianne M, Catherine ML, Larry RM , Venugopal N, Suarez DL, Sjaak W, and Grimes T (2019). Disease of Poultry. 4th edition. Chapter 8. Available at: https://dokumen.pub/diseases-of-poultry-fourteenth-edition-9781119371168-1119371163.html

Tamura G, Stecher D, Peterson A, Filipski, and Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30(12): 2725-2729. DOI: https://www.doi.org/10.1093/molbev/mst197.

Todd D, Niagro FD, Ritchie BW, Curran W, Allan GM, Lukert PD, Latimer KS, Steffens WL, and McNulty MS (1991). Comparison of three animal viruses with circular single-stranded DNA genomes. Archieve Virology, 117: 129-135. DOI: https://www.doi.org/10.1007/BF01310498

Tseng TY, Liu YC, Hsu YC, Chang PC, Hsieh MK, Shien JH, and Ou SC (2019). Preparation of chicken anemia virus (CAV) virus-like particles and chicken interleukin-12 for vaccine development using a baculovirus expression system. Pathogens, 8(4): Article number 262. DOI: https://www.doi.org/10.3390/pathogens8040262.

Yuasa N, Taniguchi T, and Yoshida I (1979). Isolation and some characteristics of an agent inducing anemia in chicks. Avian Disease, pp. 366-385. Available at: https://www.jstor.org/stable/1589567?origin=crossref&seq=1#metadata_info_tab_contents

Zhang X, Liu Y, Wu B, Sun B, Chen F, Ji J, Ma J, and Xie Q (2013). Phylogenetic and molecular characterization of chicken anemia virus in southern China from 2011 to 2012. Scientific Reports, 3: Article number 3519. DOI: https://www.doi.org/10.1038/srep03519

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Effect of Agro-ecological Zone, Age, and Sex on Prevalence and Intensity of Gastrointestinal Parasites in Donkeys in Maseru District, Lesotho

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ABSTRACT

Gastrointestinal parasites are considered to be silent killers of animals. The objective of the current study was to determine the effect of the agro-ecological zone, age, and sex on the parasite prevalence and fecal egg/oocyst count in donkeys residing in Lesotho. A total number of 720 fecal samples were collected rectally from 120 indigenous donkeys that were randomly selected from the highlands, foothills, and lowlands of Maseru district, Lesotho. The fecal samples were collected every two months for one year and examined using the floatation technique. The overall prevalence for nematodes, coccidia, and cestodes in donkeys were 87.78%, 4.31%, and 1.53%, respectively. The highest nematode prevalence and intensity were detected in the donkeys of highlands. The coccidian infection was lower in the lowlands while cestodes infection was more prevalent in the foothills. Donkey's age had an impact on the nematode fecal egg load but did not affect the prevalence of nematodes in donkeys. Age did not significantly affect the prevalence and fecal egg/oocyst count of cestodes and coccidia. Male donkeys had a higher prevalence and fecal egg count of cestodes. In conclusion, the nematodes were found to be the major gastrointestinal parasites of donkeys in the Maseru district. Therefore, there is a need to design a sustainable strategy aimed at controlling the gastrointestinal parasites in donkeys.

Keywords: Agro-ecological zone, *Eimeria*, Fecal egg count, Helminth, Prevalence

INTRODUCTION

In many countries, equines have a great contribution to the national economy; however, gastrointestinal parasites (GIPs) are the major constraints that hinder their probable output (Enigidaw et al., 2015). These parasites hinder the maximum working capacity of donkeys (Nakayima et al., 2017). The poor nutritional and immune status of equines also makes the equine to be susceptible to parasitic diseases, which negatively influences their health and working performance (Zerihun et al., 2011; Belay et al., 2016). Tesfu et al. (2014) observed that among the helminths, nematodes, especially strongyles, are one of the most common and prevalent GIPs in donkeys. Factors such as temperature and moisture provide a favorable environment for the development of larvae to the infective stage which is ingested during animal grazing (Saeed et al., 2019). The infection occurs from the ingestion of infective larvae or eggs during grazing (Andarge et al., 2017). Takele and Nibret, (2013) stated that open grazing promotes ingestion of the helminth eggs. According to Upjohn et al. (2010), the intensity of infection can be affected by climatic conditions, grazing practices, and anthelmintic use. Waqas et al. (2015) indicated a high likelihood of GIP infection occurrence to grazing equines. In addition, Molla et al. (2015) reported that communal grazing serves as a favorable factor for the survival of strongyles.

Tedla and Abichu (2018) indicated that lack of sufficient veterinary services and poor management of equines contribute to higher parasitic infections. Donkeys depend on rangelands for feeding, therefore, the chance of being infected with parasites is very high. In Lesotho, owners do not follow appropriate animal health management practices in the donkey population (Demelash et al., 2016), leading to high incidences of parasite infections. In addition, there is a lack of information on the prevalence of GIPs among donkeys in Lesotho. Therefore, the objective of the current study was to determine the effect of Agro-ecological Zone (AEZ), age, and sex on the prevalence and intensity of GIP infections in donkeys. The findings of the present study would help donkey owners and other relevant stakeholders to design the holistic deworming strategy for donkeys. The welfare of donkeys would be enhanced and hence the lives of the rural communities would improve due to the enhanced performance of donkeys.

MATERIALS AND METHODS

Ethical approval

The scientific and ethics committee of the Department of Animal Science of the National University of Lesotho approved the study protocol. The use of animals was in accordance with the international animal welfare standards laid

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down in the 1964 Declaration of Helsinki and its later amendments.

Study area

The study was conducted for 12 months from May 2018 to April 2019 in the three AEZs of Maseru district in Lesotho, including the lowlands, foothills, and highlands named Koro-koro, Matsieng, and Marakabei, respectively. The lowlands have an altitude ranging from 1500-1800 m above sea level, foothills are in the middle between 1800-2200 m above sea level and it covers 10-15% of the total area while the highlands covers over 2/3 of the country and rises to the elevation of 2200-3000 m above the sea level (FAO, 1996). The lowlands have the annual rainfall ranging from 600-900 mm, foothills between 900-1000 mm while 1000-1300 mm is experienced in the highlands. The temperatures vary from 11 to 30 $^{\circ}$C (Olaleye et al., 2016).

Sample size and fecal egg counting

A total number of 120 indigenous donkeys were randomly selected from the three AEZs. The selection of villages in the three AEZs was done through the help of Agricultural Technical Officers. The data was collected with respect to AEZ, sex, and age. A total number of 40 donkeys were selected per AEZ, consisting of 20 males and 20 females, and 5 young and 15 adults were selected as experimental units from each gender group. The age estimation of donkeys was performed through dentition whereby the young animals consisted of the donkeys less than two years old while adults were defined as those above two years old.

A total number of 720 fecal samples was directly collected rectally from 120 indigenous donkeys with disposable gloves into airtight bottles. The fecal samples were collected every two months for a period of one year (six samples per animal). The bottles containing samples were kept in the cool box to prevent hatching the eggs before Fecal Egg Counting (FEC), then examined by using the floatation technique (Zajac and Conboy, 2012). The collected fecal samples were refrigerated in the Animal Science Laboratory and the laboratory analyses were done within 48 hours. In the laboratory, each fecal sample weighing 4 g was thoroughly mixed with 56 mL of sodium chloride (floatation solution). The solution was sieved into the beaker and a few drops of amyl alcohol were added to treat the bubbles. The pipette was used to draw some milliliters of the solution to fill the two chambers of the McMaster slide and then viewed under a light microscope (Olympus, Japan). All eggs/oocyst of GIPs were counted and identified based on their morphology. The number of eggs/oocysts per grams of feces was calculated using the following formula (Nakayima et al., 2017):

Fecal egg/oocyst count = (number of eggs/oocysts in chamber 1 + number of eggs/oocysts in chamber 2) × 50

The prevalence of GIPs was calculated as follows:

Prevalence = [(number of positive samples) / (number of examined samples)] × 100

Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 16.0) was used for data analysis. The general linear model was used to evaluate the effect of AEZ, sex, and age on the GIPs prevalence and fecal egg/oocyst count in donkeys. The data was also subjected to binary logistic regression under General Estimating Equations (GEE) to observe the likelihood of change in prevalence. The GEE was also used to analyze fecal egg/oocyst count data adopting a negative binomial regression model expressing the results in terms of Beta Exponential (in percentages). Based on Duncan's multiple range test, the confidence interval was held at 95% and p-value less than 0.05 was considered statistically significant.

RESULTS

Overall prevalence of gastrointestinal parasites infection

The results from the examination of fecal samples indicated that donkeys were positive for different GIPs. The identified fecal eggs/oocysts were from nematodes, cestodes, coccidia with the prevalence of 87.78%, 1.53%, and 4.31%, respectively (Table 1).

Prevalence and intensity of gastrointestinal parasites infection in different agro-ecological zones

Nematode eggs were most observed in donkeys in the study areas (Table 2). The highest prevalence of nematodes was observed in donkeys reared in the highlands (90.40%), followed by lowlands (89.20%), and foothills (83.80%). However, the difference was not significant between the donkeys in the highlands and lowlands ($p > 0.05$). However, the fecal egg count in donkeys in the highlands was significantly higher than those kept in the lowlands ($p < 0.05$). In the foothills, the number of donkeys infected with nematodes was significantly lower than those in the highlands ($p < 0.05$), but the fecal egg count was not affected by AEZs. In the case of coccidia, the prevalence was higher in donkeys found in the highlands (6.20%) than those in the foothills (4.20%) but not statistically significant ($p > 0.05$). The prevalence of

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coccidia in donkeys in the lowlands (2.50%) was not statistically different from those in the foothills but significantly different ($p < 0.05$) from donkeys found in the highlands. The difference in fecal oocyst counts was non-significant across the three AEZs (Table 2). There was a non-significant difference ($p > 0.05$) in the prevalence and intensity of cestode infection among donkeys in different agro-ecological zones (Table 2).

Prevalence and intensity of gastrointestinal parasites infection in different age groups

As presented in Table 3, there was no significant difference ($p > 0.05$) in the prevalence of gastrointestinal nematodes between young and adult donkeys while the fecal egg count was significantly different ($p < 0.05$) between these two groups. The adult donkeys were more heavily infected with gastrointestinal nematodes. Regarding cestodes and coccidia, the obtained results indicated that the prevalence and intensity of infection did not differ significantly in adult and young donkeys ($p > 0.05$, Table 3).

Prevalence and intensity of gastrointestinal parasites infection according to sex in donkeys

As presented in Table 4, the prevalence and intensity of nematode infection were higher in the female donkeys than male donkeys but the effect was not statistically significant ($p > 0.05$). The prevalence of coccidia infection was higher in male donkeys but had a lower fecal oocyst count than female donkeys. However, the differences were insignificant ($p > 0.05$, Table 4). The cestodes prevalence and fecal egg count were significantly higher in males than those in females ($p < 0.05$, Table 4).

Table 1. Prevalence of gastrointestinal parasites in fecal samples collected from 120 indigenous donkeys in Maseru District, Lesotho, based on fecal egg/oocyst counts.

Gastrointestinal parasites	Number of samples examined	Number of positive samples	Prevalence (%)
Nematodes	720	632	87.78
Coccidia	720	31	4.31
Cestodes	720	11	1.53

Table 2. Effect of the ago-ecological zone on the gastrointestinal parasites prevalence and fecal egg/oocyst count in donkeys in Maseru District, Lesotho.

Agro-ecological zone		Prevalence		Fecal egg/oocyst count	
		%	Standard error	EMM	Standard error
Nematodes	Lowlands	89.20[ab]	0.020	528.12[a]	40.609
	Foothills	83.80[a]	0.024	639.17[ab]	36.843
	Highlands	90.40[b]	0.019	673.96[b]	51.511
Coccidia	Lowlands	2.50[a]	0.010	4.79[a]	2.163
	Foothills	4.20[ab]	0.0.13	5.00[a]	2.259
	Highlands	6.20[b]	0.016	6.67[a]	2.108
Cestodes	Lowlands	0.80[a]	0.060	1.04[a]	0.843
	Foothills	2.10[a]	0.090	2.92[a]	1.574
	Highlands	1.70[a]	0.050	1.46[a]	1.058

[ab] Means within a column with a common superscript do not differ significantly ($p>0.05$). EMM: Estimated Marginal Means. Exp.B: Exponential Beta.

Table 3. Effect of age group on the gastrointestinal parasites prevalence and fecal egg/oocyst count in donkeys in Maseru District, Lesotho.

Age		Prevalence		Fecal egg/oocyst count	
		%	Standard error	EMM	Standard error
Nematodes	Young (<2 y)	86.70[a]	0.024	492.22[a]	27.365
	Adults (>2 y)	88.10[a]	0.014	654.26[b]	31.444
Coccidia	Young (<2 y)	4.40[a]	0.015	6.67[a]	2.528
	Adults (>2 y)	4.30[a]	0.090	5.09[a]	1.450
Cestodes	Young (< 2y)	0.00[a]	0.090	0.00[a]	1.295
	Adults (> 2y)	2.00[a]	0.050	2.41[a]	0.748

[ab] Means within a column with a common superscript do not differ significantly ($p>0.05$). EMM: Estimated Marginal Means. Exp. B: Exponential Beta.

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Table 4. Effect of sex on gastrointestinal parasites prevalence and fecal egg/oocyst count in donkeys in Maseru District, Lesotho.

Sex		Prevalence		Fecal egg/oocyst count	
		%	Standard error	EMM	Standard error
Nematodes	Male	86.10[a]	0.018	592.92[a]	40.347
	Female	89.40[a]	0.016	634.58[a]	30.650
Coccidia	Male	4.40[a]	0.11	4.86[a]	1.704
	Female	4.20[a]	0.11	6.11[a]	1.852
Cestodes	Male	2.50[a]	0.060	2.92[a]	1.255
	Female	0.60[b]	0.050	0.69[b]	0.566

[ab] Means within a column with a common superscript do not differ significantly (p>0.05). EMM: Estimated Marginal Means. Exp.B: Exponential Beta.

DISCUSSION

The higher prevalence of nematodes recorded in the present study was in accordance with the findings of Asefa and Dulo (2017) who observed an overall prevalence of 83.70% in Bishoftu town, Ethiopia. Different studies conducted in several countries observed a range of 2- 80% *E. leuckarti* widespread, for instance, the prevalence ranged 4.5- 5.88% in Turkey (Studzińska et al., 2008), which is close to the overall prevalence of coccidia (4.31%) observed in the present study. In line with the observations of the current study, Nakayima et al. (2017) found the occurrence of gastrointestinal cestodes to be less in donkeys. However, Belay et al. (2016) recorded a prevalence of 3.7% of cestodes as compared to 1.53% observed in the current study.

The higher nematode prevalence and fecal egg count in the donkeys of highlands might be due to grazing on wetlands which have the potential of promoting nematode development due to high moisture content since the annual mean rainfall is between 1000 to 1300 mm in this region (Olaleye et al., 2016). Raza et al. (2007) also showed that most green pastures that are waterlogged increase the spread of helminths. Similarly, higher mean egg per gram of feces was recorded in donkeys in the highlands (Sheferaw and Alemu, 2015). Development and survival of the nematode eggs and larvae are influenced mainly by climatic conditions, such as temperature and humidity, which differ greatly according to geographical locations (Belete and Derso, 2015).

The high prevalence and level of infection of coccidia in the highlands might be due to poor animal husbandry. Other researchers noted that the occurrence of coccidiosis is associated with undernourishment, poor sanitation, overstocking, poor management practices, and stress (Sudan et al., 2013). Therefore, the lower fecal oocyst count in the lowlands could be attributed to an unfavorable environment for the development of the oocysts because the lowlands are very dry with high temperatures.

The gastrointestinal cestodes were the lowest prevalence in the current study. Tolossa (2016) explained that low prevalence could be due to the seasonality of oribatid mites as vectors. However, in this study, the higher prevalence and intensity of cestodes in the foothills could be due to the longer grazing periods which result in the ingestion of more mites and hence increased cestodes prevalence (IhIer et al., 1995). Moisture is of great importance in increasing the mite population which favors the cestodes infection (Tomczuk et al., 2017). Given the meteorological data, the higher rainfall (900–1000 mm) in the foothills (Olaleye et al., 2016) results in high moisture which contributes to oribatid mites and hence the prevalence of cestodes.

The GIPs affect equines of all ages, although the older equines are likely to develop resistance and act as the source of infection by contaminating the pastures (Shite et al., 2015). There was a significant association between age and the nematode fecal egg load whereby high intensity was in adults probably due to poor body condition. The poor body condition and decreased immunity in adult equines are the results of extensive work, overload, and poor nutritive feeds (Takele and Nibret, 2013; Belay et al., 2016). These adult working donkeys sometimes remain the source of infection to young donkeys because most of them are heavily infected with GIPs without showing any signs of infection. In addition, the lower level of nematode infection in young donkeys may be due to the fact that they are kept in stables most of the time while adults work as pack animals moving from one area to another and this exposes adult donkeys to different parasites. However, Andarge et al. (2017) observed that in terms of susceptibility to nematodes there was no significant difference between young and adult donkeys. On the other hand, the prevalence of *Parascaris equorum* (nematode) was found to be significantly higher in younger equines (Sheferaw and Alemu, 2015).

The findings of the current study were in accordance with Dubey and Bauer (2018) who observed a higher prevalence of coccidia in foals than that in adult animals and further indicated that foals can acquire infection on the day of birth due to a contaminated environment than being infected from oocysts excreted by their mares. It is emphasized

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that *Eimeria leuckarti* is more prevalent among young foals though it can be infrequently detected in adult animals (De Souza et al., 2009). Nakayima et al. (2017) stated that the infection of coccidia is related more to young donkeys.

Concerning cestodes, Getachew (2006) also showed that there is no association between age and tapeworm infection prevalence in horses. Sori et al. (2017) indicated that sex was not associated with the prevalence of equine strongylosis, possibly because the management is the same regardless of the sex class (Tone et al., 2016). The insignificant difference in nematode egg counts between male and female donkeys could be due to the fact that they were reared and grazed on the same pasture (Tone et al., 2016). However, Sheferaw and Alemu (2015) stated that *P. equorum* was highly prevalent in female equids than males. Females have a close relationship to their foals, so this makes the females more susceptible as the parasites recycle between dams and foals. Moreover, the possible explanation for non-significant differences in terms of GIP infection prevalence and intensity between male and female donkeys could be attributed to the mutual grooming behavior of donkeys that could play a role in transmitting the infection between members of a herd. On the other hand, there are reported cases of the high prevalence of strongylosis in males compared to females despite the fact that the differences were not significant (Sori et al., 2017). The possible reason might be due to the workload done by males than females, which causes stress and consequent immune-suppression which results in parasitic burden (Regassa and Yimer, 2013). In accordance with the results of the present study, other researchers observed equal prevalence of *E. leuckarti* (coccidia) in both males and females (Ghahfarrokhi et al., 2014). On the other hand, *E. leuckarti* infection was found to be prevalent in females (De Souza et al., 2009). This explains why there was a higher mean fecal oocyst count in females than males in the current study. However, the obtained results of the present study were in contradiction with that of Nakayima et al. (2017) who found higher mean fecal oocysts count in males than females.

In agreement with the results of the present study, Tomczuk et al. (2017) reported a higher prevalence of cestodes infection in females than that in males. However, Getachew (2006) reported the non-significant difference in the prevalence of cestodes between donkeys from different sexes.

CONCLUSION

Nematodes were the main gastrointestinal parasites affecting donkeys in the Maseru district. The donkeys in the highlands and adult donkeys were more heavily infected with nematodes. The coccidia prevalence and fecal oocyst load were not affected by the age and sex of the donkeys. The cestodes were more prevalent in male donkeys than females. Therefore, there is a need to develop a deworming strategy addressing specific gastrointestinal parasites for different age and sex groups of donkeys in different agro-ecological zones of Lesotho.

DECLARATIONS

Authors' contribution

Masara Elizabeth Nts'aoana conducted the study, analyzed the data, performed laboratory experiments, and wrote the manuscript. Setsumi Mots'oene Molapo conceptualized the study, reviewed, and edited the manuscript. Paseka Kompi analyzed the data and performed laboratory experiments. All authors read and approved the results of study and final manuscript.

Competing interests

The authors declare no conflict of interest.

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REFERENCES

Andarge B, Muhammed C, and Tibesso G (2017). Prevalence of major intestinal nematodes of equines in Jimma Town, South Western Ethiopia. International Journal of Veterinary Sciences and Research, 3(2): 69-73. DOI: http://www.dx.doi.org/10.17352/ijvsr.000024

Asefa S, and Dulo F (2017). A Prevalence of gastrointestinal nematode parasitic infections in horses and donkeys in and around Bishoftu Town, Ethiopia. Middle-East Journal of Applied Sciences, 3(3): 38-43. DOI: https://www.doi.org/10.5829/idosi.mejas.2017.38.43

Belay W, Teshome D, and Abiye A (2016). Study on the prevalence of gastrointestinal helminths infection in equines in and around Kombolcha. Journal of Veterinary Science and Technology, 7(5): 367-372. DOI: https://www.doi.org/10.4172/2157-7579.1000372

Belete S, and Derso S (2015). Prevalence of major gastrointestinal parasites of horses in and around Mekelle (Quiha and Wukro).

World Journal of Animal Science Research, 3(3): 1-10. Available at: http://wjasr.com/WJASR_Vol.%203,%20No.%203,%20May%202015/PREVALENCE.pdf

Demelash K, Abebaw M, Negash A, Alene B, Zemene M, and Tilahun M (2016). A review on diagnostic techniques in veterinary helminthology helminthlogy. Nature and Science, 14(7): 109-118. DOI: http://www.doi.org/10.14202/vetworld.2018.1298-1306

De Souza PNB, Bomfim TCB, Huber F, Abboud LCS, and Gomes RS (2009). Natural infection by *Cryptosporidium sp*., *Giardia sp.* and *Eimeria leuckarti* in three groups of equines with different handlings in Rio de Janeiro, Brazil. Veterinary Parasitology, 160: 327-333. DOI: https://www.doi.org/10.1016/j.vetpar.2008.10.103

Dubey JP, and Bauer C (2018). A review of *Eimeria* infections in horses and other equids. Veterinary Parasitology, 256: 58-70. DOI: https://www.doi.org/10.1016/j.vetpar.2018.04.010

Enigidaw S, Assefa A, Mekonnen N, and Belete S (2015). Prevalence of gastro intestinal nematode parasitic infections of horses and donkeys in and around Kombolcha town. American-Eurasian Journal of Scientific Research, 10: 228-234. DOI: https://www.doi.org/10.5829/idosi.aejsr.2015.10.4.95266

Food and Agricultural Organisation (FAO) (1996). Plant genetic resources, Ministry of Agriculture Lesotho. Available at: http://www.fao.org/fileadmin/templates/agphome/documents/PGR/SoW1/africa/LESOTHO.PDF

Getachew MA (2006). Endoparasites of working donkeys in Ethiopia: Epidemiological study and mathematical modelling [Doctoral dissertation]. University of Glasgow. Available at: http://theses.gla.ac.uk/id/eprint/1444

Ghahfarrokhi EK, Ahmadi AMIN, Shahraki SG, and Azizi H (2014). *Eimeria leuckarti* (Flesch, 1883; Reichenow, 1940) from worker horses and donkeys of Shahrekord, Iran. International Journal of Advanced Biological and Biomedical Research, 2(6): 1980-1984. Available at: http://www.ijabbr.com/article_7391_8fa5dc3f64f8ce910bb04674472f8685.pdf.

IhIer CF, Rootwelt V, Heyeraas A, and Dolvik NI (1995). The prevalence and epidemiology of *Anoplocephala perfoliata* infection in Norway. Veterinary Research Communications, 16(6): 487-494. DOI: https://www.doi.org/10.1007/BF01839337

Molla B, Worku Y, Shewaye A, and Mamo A (2015). Prevalence of strongyle infection and associated risk factors in equine in Menz Keya Gerbil District, North-Eastern Ethiopia. Journal of Veterinary Medicine and Animal Health, 7(4): 117-121. DOI: https://www.doi.org/10.5897/JVMAH2014. 0354

Nakayima J, Kabasa W, Aleper D, and Okidi D (2017). Prevalence of endo-parasites in donkeys and camels in Karamoja sub-region, North-eastern Uganda. Journal of Veterinary Medicine and Animal Health, 9(1): 11-15. DOI: https://www.doi.org/10.5897/JVMAH2016.0499

Olaleye AO, Tambi E, Bangali S, and Odularu GOA (2016). Likoti farming under changing climate in Lesotho: Agronomic Grain Yield versus Technical Efficiency. Journal of Ecosystem and Ecography, 5: 2-7. DOI: https://www.dx.doi.org/10.4172/2157-7625.S5- 001

Raza MA, Iqbal Z, Jabbar A, and Yaseen M (2007). Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. Journal of Helminthology, 81(3): 323-328. DOI: https://www.doi.org/10.1017/S0022149X07818554

Regassa A, and Yimer E (2013). Gastrointestinal parasites of equine in South Wollo zone, North Eastern Ethiopia. Global Veterinaria, 11(6): 824-830. DOI: https://www.doi.org/10.5829/idosi.gv.2013.11.6.76207

Saeed MA, Beveridge I, Abbas G, Beasley A, Bauquier J, Wilkes E, Jacobson C, Hughes KJ, El-Hage C, O'Handley R et al. (2019). Systematic review of gastrointestinal nematodes of horses from Australia. Parasites and Vectors, 12(1): Article number 188. DOI: https://www.doi.org/10.1186/s13071-019-3445-4

Sheferaw D, and Alemu M (2015). Epidemiological study of gastrointestinal helminths of equines in Damot-Gale district, Wolaita zone, Ethiopia. Journal of Parasitic Disease, 39(2): 315-320. DOI: https://www.doi.org/10.1007/s12639-013-0352-z

Shite A, Admassu B, and Abere A (2015). Large strongyle parasites in equine: A review. Advances in Biological Research, 9(4): 247-252. DOI: https://www.doi.org/10.5829/idosi.abr.2015.9.9559

Sori G, Bekele T, Geso G, Ibrahim H, Gobena F, Jarso G, Melaku M, and Shumet A (2017). Prevalence of equine strongyle infection and its associated risk factors in Jimma Town, Southwest Ethiopia. International Journal of Livestock Production, 8(11): 187-191. DOI: https://www.doi.org/10.5897/IJLP2016.0325

Studzińska MB, Tomczuk KR, and Sadzikowski AB (2008). Prevalence of *Eimeria leuckarti* in young horses and usefulness of some coproscopical methods for its detection. Bulletin - Veterinary Institute in Pulawy, 52: 541-544. Available at: http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.agro-5b70c8bf-156b-4ce0-95ae-8025679948bd

Sudan V, Sharma RL, Gupta SR, and Borah MK (2013). Successful therapeutic management of concurrent subclinical *Eimeria leukarti* and *Babesia (Theileria) equi* infection in a mare. Journal of Parasitic Diseases, 37(2): 177-180. DOI: https://www.doi.org/10.1007/s12639-012-0156-6

Takele B, and Nibret E (2013). Prevalence of gastrointestinal helminthes of donkeys and mules in and around Bahir Dar, Ethiopia. Ethiopian Veterinary Journal, 17(1): 13-30. DOI: https://www.doi.org/10.4314/evj.v17i1.2

Tedla M, and Abichu B (2018). Cross-sectional study on gastro-intestinal parasites of equids in South-western Ethiopia. Parasite Epidemiology and Control, 3(4): e00076. DOI: https://www.doi.org/10.1016/j.parepi.2018.e00076

Tesfu N, Asrade B, Abebe R, and Kasaye S (2014). Prevalence and risk factors of gastrointestinal nematode parasites of horse and donkeys in hawassa town, Ethiopia. Journal of Veterinary Science and Technology, 5(5): 2157-7579. DOI: https://www.dx.doi.org/10.4172/2157-7579.1000210

Tolossa YH (2016). Gastro-intestinal parasites of equines in Tiyo district of Oromia Region, Ethiopia. Global Veterinaria, 17(2): 154-160. DOI: https://www.doi.org/10.5829/idosi.gv.2016.17.02.10468

Tomczuk K, Grzybek M, Szczepaniak K, Studzińska M, Demkowska-Kutrzepa M, Roczeń-Karczmarz M, Abbass ZA, Kostro K, and Junkuszew A (2017). Factors affecting prevalence and abundance of A. perfoliata infections in horses from south-eastern Poland. Veterinary Parasitology, 246: 19-24. DOI: https://www.doi.org/10.1016/j.vetpar.2017.08.027

Tone T, Basaznew B, and Shimels T (2016). Prevalence of major gastrointestinal nematode parasites of donkeys in selected districts

To cite this paper: Nts'aoana ME, Molapo SM, and Kompi P (2021). Effect of Agro-ecological Zone, Age, and Sex on Prevalence and Intensity of Gastrointestinal Parasites in Donkeys in Maseru District, Lesotho. *World Vet. J.*, 11 (2): 242-248.

of Wolaitazone, Southern Ethiopia. Advances in Biological Research, 10(6): 360-364. DOI: https://www.doi.org/10.5829/idosi.abr.2016.360.364

Upjohn MM, Shipton K, Lerotholi T, Attwood G, and Verheyen KL (2010). Coprological prevalence and intensity of helminth infection in working horses in Lesotho. Tropical Animal Health and Production, 42(8): 1655-1661. DOI: https://www.doi.org/10.1007/s11250-010-9617-z

Waqas M, Nawaz M, Sajid M, Ahmad Z, Mushtaq A, Jabbar A, and Zubair M (2015). Strongylosis (red worms' infestation); a potential threat to donkey's health and performance. Global Veterinaria, 14(3): 345-350. DOI: https://www.doi.org/10.5829/idosi.gv.2015.14.03.9340

Zajac AM, and Conboy GA (2012). Veterinary clinical parasitology. John Wiley and Sons. 8th Edition, UK, pp. 8-11. Available at: https://www.wiley.com/en-us/Veterinary+Clinical+Parasitology%2C+8th+Edition-p-9780813820538

Zerihun A, Bersissa K, Bojia E, Ayele G, Tesfaye M, and Etana D (2011). Endoparasites of donkeys in Sululta and Gefersa districts of central Oromia, Ethiopia. Journal of Animal and Veterinary Advances, 10(14): 1850-1854. DOI: https://www.doi.org/10.3923/javaa.2011.1850.1854

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Effects of Genotype and Weaning Age Interaction on Growth Traits in Rabbits

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ABSTRACT

Weaning age is an important factor that affects the growth and health of weaned animals. Therefore, the current experiment was conducted to study genotype (G) and weaning age (WA) interaction (G×WA) effects on growth traits of the animals belonged to two lines of rabbits (APRI and V line) reared under Egyptian conditions. Multiparous doe rabbits were serviced to obtain 225 litters with 1800 young rabbits at weaning. The weaning ages ranged from 26 to 43 days where the young rabbits were weaned at different ages (≥28 days, WA1; 28 < Treatment ≥ 35 days, WA2; 35 < Treatment ≥ 40 day, WA3 and 40< Treatment, WA4). Body weight (BW) from 4 to 16 weeks of age and corresponding average daily gain (ADG_{t1-t2}) were measured. The BW significantly increased in APRI rabbits, compared to those in V line at the different ages where at the end of the fattening period, the difference was 105 g per animal with higher ADG. Regarding the weaning age effects, positive effects were observed where the highest BW was observed at the fattening period. The ADG of rabbits weaned in late weaning was higher than in early weaning with significant differences. The observed results suggest the existence of relevant G×WA interaction for the investigated traits. Therefore, the weaning age of 29-35 days is recommended for young APRI rabbits while it is suggested to wean the V rabbits after 35 days. The study confirmed that early weaning is not preferable for the rabbit under Egyptian conditions and it is better to wean young rabbits at the minimum age of 30 days to achieve the best BW and growth rate.

Keywords: Fattening period, Genotype, Growth traits, Rabbit, Weaning age

INTRODUCTION

In recent years, interest in rabbit production has increased due to its economic and health importance for humans, as it is considered an ideal solution to the growing protein shortage in developing countries (Dalle Zotte 2002; Petracci et al., 2009; Ebeid et al., 2013). The weaning of rabbit kits is very critical during the life of doe rabbits since it can affect the health status and the growth performance of the weaning kits during the fattening period, particularly during the first post-weaning weeks. Moreover, weaning age has a significant effect on the body condition of the doe rabbits, such as energy deficit and body lipid depots by limiting the duration of lactation and reproduction rhythm (Xiccato et al., 2004; Arias-Alvarez et al., 2009). The health and mortality of weaned rabbits are affected by weaning age (Savietto et al., 2016; Rebollar et al., 2009). With this in mind, it is important to determine the appropriate weaning age when the litter can technically be separated from the does. The results of the weaning age effects on the mortality and the yields of the kits during the fattening are contradictory. Lebas (1993) recommended late weaning to reduce post-weaning mortality. Also, Gidenne and Fortun-Lamothe (2002) found higher mortality between 32 and 45 days in kittens weaned at 23 days than in those weaned at 32 days (17.2 versus 9.2%).

Other properties that are affected by weaning age include body weight (BW) and gut microbiota (Bennegadi et al., 2003; Gallois et al., 2004). De Blas et al. (1981) found that in 35-day weight of the kits weaned at 25 days was lower than that of animals weaned at 35 days (750 vs. 870 g, respectively) although all finished the bait with the same weight (2.0 kg) due to the compensatory growth of first ones.

The APRI line was established from Egyptian Baladi Red (BR) and a Spanish line (V) rabbits started in 2002 at the Sakha experimental rabbitry, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The APRI line was founded by crossing Baladi Red bucks with V line does to produce F1 (½B½V) stock, followed by two generations of inter se matings to achieve performance stability (Youssef et al., 2008 and Abou Khadiga et al., 2010). The V line was established from four different synthetic maternal populations in 1984, crossing crossbred males of two types with crossbred females of two other types. Selection candidates were also genetically

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evaluated for prolificacy at weaning using a repeatability animal model, obtaining Best Linear Unbiased Prediction (BLUP) predictions of their additive genetic value (Estany et al., 1989). A replicate of the V lines was established in 2002 in Sakha and the selection criterion was changed to litter weaning weight as in the APRI line (Youssef et al., 2008). The objective of the current study was to study the effects of genotype and weaning age interaction (G×WA) on growth traits of the animals belonged to two lines of rabbit on kits performance during the fattening period under Egyptian conditions.

MATERIALS AND METHODS

Ethics approval

All experimental procedures were approved by the Committee of Ethics and Animal Welfare of Animal Production Research Institute, Sakha, Kafr El-Sheikh Governorate, Egypt.

Animals

The present study was conducted involving a synthetic Egyptian line (APRI) and a Spanish maternal line (V line). Rabbits were raised at the Experimental Rabbitry of Animal Production Research Institute, Sakha, Kafr El-Sheikh Governorate, Egypt. The analysis included the growth data recorded for APRI and V lines in 2019.

The APRI is a maternal line founded in 2008 at the Animal Production Research Institute, Egypt (Youssef et al., 2008). This line was founded as a synthetic line from the cross of Baladi Red bucks with V line does to get F1. After its foundation, it was selected for litter weaning weight. The V line was established from four specialized maternal lines in 1984 into a composite synthetic line for which the method of evaluating the animals is by Best Linear Unbiased Prediction (BLUP) under a repeatability animal model (Ragab and Baselga, 2011).

The multiparous rabbit does were serviced 12-14 days post-kindling and a pregnancy test was carried out by abdominal palpation on day 14 after mating. Litters born were examined and recorded as a total number of born and the number of those born alive. Litters were reared by their dams until weaning. A total of 1800 rabbits were chosen to be weaned from a litter equalized at birth in 8 kits of 225 litters. At weaning, the young rabbits were individually identified by a number tattooed on the ear. The first age for weaning of kids was ≥28 days (WA1). The second weaning age was 28 < T ≥ 35 days included rabbit weaned at 29, 30,31, 32, 33, 34, and 35 days of age (WA2). The third age of weaning was 35 < T ≥ 40 days included rabbit weaned at 36, 37,38, 39, and 40 days of age (WA3). The fourth age of weaning included rabbit weaned at 41, 42, 43 days (40<T days, WA4). At weaning, young rabbits were raised in a semi-closed Rabbitry of commercial type wired cages with standard dimensions (60 × 50 × 35 cm, length × width × height) in pyramid-type batteries. The kids were placed in collective cages of about 5 rabbits until 16 weeks of age.

During the post-weaning period, rabbits were fed *ad libitum*, with a standard commercial pellet diet and fresh water. The diet was composed of 32% barley, 21% wheat bran, 10% soybean meal, 22% hay, 6% berseem straw, 3% molasses, 1% limestone, 0.34% table salt, 0.3 minerals and vitamins, 0.06 methionine, and 1.3% anti-coccidian. This diet included 16.3% crude protein, 13.2% crude fiber, 2.5% either extract, 0.6% minerals mixture. No serious health problems were observed throughout the experiment.

Traits

Individual rabbit weights were recorded weekly. Body weight (BW_t, g) was measured at 4 (BW_4), 6 (BW_6), 8 (BW_8), 10 (BW_{10}), 12 (BW_{12}), 14 (BW_{14}), and 16 (BW_{16}) weeks of age, which corresponds to age at weeks 4, 6, 8, and 10 (the week of slaughter) and the periods from 12 to 16 weeks of age, respectively. Individual average daily gain (ADG_{t1-t2}, g/d) during the study period **(**4-6 weeks (ADG_{4-6}), 6-8 weeks (ADG_{6-8}), 8-10 weeks (ADG_{8-10}), 10-12 weeks (ADG_{10-12}), 12-14 weeks (ADG_{12-14}), 14-16 weeks (ADG_{14-16}), overall fattening period (ADG_{4-10}), 6-10 week (ADG_{6-10}, after the critical period of age), 10-16 weeks (ADG_{10-16}) and 4 to 16 weeks (ADG_{4-16}) of age**)** were calculated.

Statistical analysis

The obtained data for the two lines were used in the analysis where univariate animal models were fitted to estimate the genetic parameters for all traits. A total of 2231 individuals were obtained from 371 parities for the two lines were analyzed using the following model:

$$y_{ijklmn} = YS_i + PO_j + \beta(NBA)_k + L_l + LP_m + L_l \times LP_m + e_{ijklmn}$$

where,

y_{ijklmn} is a record of growth traits, YS_i denotes a fixed effect, year-season of the parity (one year season every three months: 3 levels), PO_j signifies a fixed effect, parity order of the doe (3 levels), NBA is a covariate including the number of born alive in the litter in which the animal was born, being β the regression coefficient, L_l refers to a fixed effect, line effect (2 levels), LP_m stands for a fixed effect, lactation-length (4 levels), $L_l \times LP_m$ is the effect of line-weaning age interaction, and e_{ijklmn} is a random effect, residual of the model.

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To test the significance of the used effects in the model, factorial ANOVA was applied using the GLM procedure of SAS 9.2 (SAS, 2012). The different levels of each effect included in the models were compared using Duncan's multiple range test. A probability of $p \leq 0.05$ was required for statements of significance.

RESULTS AND DISCUSSION

Table 1 shows descriptive statistics of the analyzed BW traits, including their number, mean, standard deviation, and minimum and maximum values which take into account the entire data. The actual means of post-weaning BW are within the ranges of the study conducted on the same lines under Egyptian (Youssef et al., 2008; Galal Galal et al., 2013).

Means of BW traits at different weeks of ages for APRI and V lines in the current experiment are presented in Table 2. Differences in weaning weight are economically important where the observed results showed that APRI line was superior over V line in all BW traits during the whole period of the experiment with significant differences. The difference in BW at 4 weeks of age was around 80 g in favor of line APRI with a significant difference because fattened rabbits of the APRI line came from litters with the lowest number of kits born alive and the lowest number of rabbits at weaning. Orengo et al. (2004) reported that higher BWs at weaning were obtained when litter size at birth was lower. Moreover, previous studies confirmed that that BW at weaning is associated with milk production (Lukefahr et al., 1983; McNitt and Lukefahr, 1990)

Regarding BW after weaning, the BW differences were in favor of the line APRI and the difference increased with V line after the 6 weeks of age. At the end of the fattening period (BW_{10}), the BW was higher in the APRI line group, compared to the V line (1706.94 vs. 1601.41). Moreover, the differences at the end of the study period reached to be more than 100 g per animal favoring the APRI line. This could be partially attributed to the fact that the APRI rabbit could be still affected by its foundation where theoretically containing 50% of its constituents from Egyptian strain (Red Baldi) genes leading to a higher adaptability level to the Egyptian climatic conditions. APRI rabbits have also benefited from the selection program, which has resulted in genetic improvements in litter weight traits. (Abou Khadiga et al., 2010).

Table 1. Basic statistics for body weight traits at different ages, mean, standard deviation, and extreme values

Traits (g)	N	Mean	SD	Minimum	Maximum
BW_4	1799	472.05 ± 2.65	112.78	226	991.5
BW_6	1799	793.40 ± 3.18	135.07	365	1531.10
BW_8	1799	1194.87 ± 3.55	150.84	605	1950.45
BW_{10}	1799	1655.09 ± 3.42	145.20	1205	2450.00
BW_{12}	1799	2057.86 ± 3.78	160.35	1480.9	2871.85
BW_{14}	1720	2399.94 ± 4.09	170.00	1770.9	3238.60
BW_{16}	1693	2712.53 ± 4.26	175.49	2055	3563.90

BW: Body weight at 4, 6, 8, 10, 12, 14, 16 weeks of age. N: Number of observation, SD: Standard deviation.

Table 2. Effect of rabbit lines (APRI and V lines) on body weight at different weeks of age

Triats (g) / Lines	BW_4	BW_6	BW_8	BW_{10}	BW_{12}	BW_{14}	BW_{16}
APRI	511.17±4.07^a	838.01±4.80^a	1241.04±5.06^a	1706.94±4.78^a	2138.62±5.12^a	2497.33±5.19^a	2818.19±5.12^a
V	431.55±2.79^b	747.23±3.53^b	1147.07±4.45^b	1601.41±4.20^b	1974.26±3.94^b	2300.49±4.19^b	2604.21±4.39^b

BW_{4-16}: Body weight at 4,6,8,10,12,14,16 weeks of age. $^{a-d}$: Means within columns with no common superscript differ significantly ($p < 0.05$). Values are least-squares means.

Table 3. Effect of weaning age on body weight of rabbits at different weeks of age

Triats / WA (g)	BW_4	BW_6	BW_8	BW_{10}	BW_{12}	BW_{14}	BW_{16}
WA1	471.71±6.64^a	730.78±7.62^d	1109.73±7.95^d	1580.60±8.42^c	1998.13±8.96^d	2331.66±10.12^c	2637.99±10.77^b
WA2	467.63±5.23^a	767.42±6.20^c	1177.51±7.07^c	1648.92±6.52^b	2053.67±7.96^c	2403.77±8.81^b	2731.20±9.28^a
WA3	475.81±4.17^a	821.95±4.52^b	1220.33±5.48^b	1686.09±5.07^a	2072.47±5.79^b	2422.11±5.66^a	2737.74±5.62^a
WA4	473.32±5.14^a	849.12± 5.44^a	1264.83±5.75^a	1698.36±5.89^a	2101.74± 6.47^a	2434.90±6.92^a	2734.81±7.02^a

BW_{4-16}: Body weight at 4,6,8,10,12,14,16 weeks of age. WA: Weaning ages 1, 2, 3, and 4 mean weaning ages at less or equal than day 28, between 29 and 35 days, between 35 and 40 days, and more than day 40 respectively. $^{a-d}$: Means within columns with no common superscript differ significantly ($p < 0.05$).

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Table 3 shows the effects of age at weaning on BW from 4 weeks up to 16 weeks of age. The mean weights at 4 weeks of age were similar among the different weaning ages due to the experimental design. The results indicated that at 6 weeks of age, the differences in BW were economically important with significant differences. The BW of the kits weaned older than 35 days of age was significantly higher than those weaned before 35 days at BW16 ($p > 0.05$).

Moreover, the previous result continued until were increased until 8 weeks to be 98.96 g per animal and the lowest weight achieved by the animals that were weaned early (WA1). At the end of the fattening period (BW_{10}), the animals of WA3 and WA4 were the heaviest; moreover, there were no significant differences between the rabbit of WA3 and WA4. While rabbits of WA1 were still affected by weaning age with economically relevant differences of 68.32, 105.49, and 117.76 g per animal at WA2, WA3, and WA4, respectively. The negative effects of early weaning on BW were compensated after 8 weeks of age where the observed differences in BW decreased between kits weaned at different ages and these differences were non-significant between the rabbits that weaned after 35 days. At 16 weeks, the only significant differences were observed between these animals weaned at a younger age (before 30) than those weaned after 30 days of age (at least 94 g per kit).

Similar results observed by Gallois et al. (2004) indicated the live weight of rabbits weaned at an early age remained lower than late-weaned rabbits. Forthermore, Kovács et al. (2012) and El-Sabrout and Aggag (2017) found that, at market age, rabbits weaned early (≤28 days) had significantly lower BW than those weaned later (35 d). In addition, McNitt and Moody (1992) and Ferguson et al. (1997) found that kits weaned at 14 days had lower growth and mortality than kits weaned at 28 days. Weaning early (less than 28 days) has shown a series of disorders related to the replacement from consumption of the milk to the granulated diet, which leads to contradictory results. The early weaning may be less problematic with the intake of solid food as Gidenne and Fortun-Lamothe (2002) found that kits weaned at an early age did not ingest any feed for 1 or 2 days. Xiccato et al. (2000, 2003) compared kits weaned at different ages (21, 25, 28, and 32 days) and observed that kits weaned early (21 and 25 days) had a lower weight at 32 days (678 and 679 g, respectively) than those weaned at 28 and 32 days (704 and 719 g, respectively). Moreover, Gabr et al. (2017) reported that the weaning of young rabbits is a complex process with many impacts of dietary, environmental, and psychological stress, which results in inconsistent weight gain, weight loss, and possible total cessation of growth, and even death. Similar results have recently been obtained by Gidenne and Fortun-Lamothe (2002) although with higher mortality at the beginning of the fattening period (32 to 45 days) for the kits weaned early despite using a specific weaning diet (17.2 and 9.2% mortality for rabbits weaned at 23 and 32 days, respectively). Furthermore, late weaning has the advantage of reduced stress in young rabbits (Marongiu and Gulinati, 2008) because this stress could create some serious health problems within the young rabbits whose gut microbiota is still undeveloped. With increasing age, the gut microbe population increases continuously (Bennegadi et al., 2003). The obtained results of a study conducted by Gallois et al. (2007) showed the protective effect of milk intake in the young rabbit challenged with diseases of the intestine which are frequently seen during the post-weaning period.

As can be seen in Table 4, Genotype × weaning age interaction is analyzed regarding BW of the different ages. There are clear indications for Genotype × weaning age interactions for BW at the end of the fattening period (BW_{10}) as well as the consecutive BWs. The superior BWs in APRI were achieved when the weaning was carried out after 30 days while the higher weight for the V line was when the weaning age was after 35 days. So, as these results illustrate, the rabbit breeder should wean the young rabbits of V line at late ages (at least 35 days of age) to obtain the highest levels of weight under Egyptian conditions and the those in APRI line could be weaned after 30 days of age.

Table 4. Effect of rabbit lines (APRI and V lines) and weaning ages on body weight during the fattening period

Strain	WA	Triats (g)						
		BW_4	BW_6	BW_8	BW_{10}	BW_{12}	BW_{14}	BW_{16}
APRI	WA1	509.28±10.97^{a}	780.07±11.94^{d}	1172.84±12.11^{c}	1646.37±12.13^{b}	2090.09±12.45^{d}	2454.59±12.45^{b}	2779.22±12.30^{c}
	WA2	505.88±7.30^{a}	821.10±9.57^{c}	1234.69±10.24^{b}	1713.38±8.99^{a}	2154.00±10.36^{b}	2532.86±10.78^{a}	2865.32±11.13^{a}
	WA3	518.74±5.58^{a}	850.02±6.51^{b}	1247.50±7.24^{b}	1730.50±6.30^{a}	2124.33±8.40^{c}	2470.91±8.23^{b}	2797.58±7.17^{c}
	WA4	511.22±8.19^{a}	898.21±8.06^{a}	1305.02±8.41^{a}	1733.93±9.08^{a}	2181.35±8.46^{a}	2526.48±8.56^{a}	2827.16±8.80^{b}
V line	WA1	430.51±5.77^{b}	676.75±7.50^{f}	1040.53±7.43^{e}	1508.49±9.21^{d}	1897.30±8.25^{g}	2191.94±8.17^{f}	2478.03±8.19^{g}
	WA2	428.08±6.60^{b}	711.92±5.95^{e}	1118.39±8.12^{d}	1582.27±7.27^{c}	1949.94±7.63^{f}	2274.67±7.01^{e}	2597.69±7.76^{f}
	WA3	433.08±4.71^{b}	794.00±5.72^{d}	1193.28±7.83^{c}	1641.88±6.76^{b}	2020.84±6.32^{e}	2374.21±6.26^{c}	2677.90±6.47^{d}
	WA4	434.57±4.99^{b}	798.95±5.60^{d}	1223.76±6.86^{c}	1662.02±6.66^{b}	2020.37±6.22^{e}	2344.56±6.61^{d}	2643.31±6.49^{e}

$BW_{4\text{-}16}$: Body weight at 4,6,8,10,12,14,16 weeks of age. WA: Weaning ages 1, 2, 3, and 4 mean weaning ages at less or equal than day 28, between 29 and 35 days, between 35 and 40 days, and more than day 40 respectively. $^{a-g}$: Means within columns with no common superscript differ significantly ($p < 0.05$). Values are least-squares means.

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It seems that rabbits in the V line are affected by weaning age more than those in the APRI line where the differences between WA1 and WA4 in V and APRI lines were 153.53 and 87.56, respectively. Later weaning is better for the kits of V line, compared to other lines, such as Spanish ones, since less milk is produced (El Nagar et al., 2014). Moreover, El-Sabrout and Aggag (2017) found that rabbits from the V line weaned later (at 33 days) had significantly higher BW at 63 days (market age) than rabbits weaned earlier (at 23 and 28 d of age). Identical results were found by Marongiu and Gulinati (2008) when they compared different rabbit genotypes and found that rabbits of California were heavier than New Zealand White ones at the same weaning age which suggested an interactive effect of genotype with weaning age. At the end of the study period, there was a clear line-weaning age interaction where the rabbits of APRI line in WA2 were the heaviest while the heaviest rabbits of V line were at WA3.

The statistical results for average daily gain traits are shown in Table 5. The phenotypic means of ADG (g/d) during the fattening period (until 10 weeks) and the rest of the study period for the different lines are presented in Table 6. It should be noted that in the whole fattening period (ADG_{4-10}), the ADG values at the initial period (4-6 weeks) were lower than the rest of the fattening period and after the 6 weeks of age while the ADG increased to achieve the maximum growth rate during the period from 8-10 weeks of age. Line APRI was growing faster for the whole fattening period (4-10 wk) with respect to the V line (28.47 versus 27.85). Regarding the ADG after the fattening period, APRI lines significantly gained higher weights per day than V line in whole the period (10-16 weeks) (2.26 g/day per rabbit). Moreover, during the entire period from 4 to 16 weeks of age, the observed difference was 1.49 g/d favoring the APRI line.

The obtained results of weaning age effects on ADG traits can be observed in Table 7. The pattern of weaning age effects in the first 2 weeks of growth was different from the pattern for the whole period. The ADG_{4-6} values in all weaning ages were lower than the rest of the whole period. For the whole fattening period (4 -10 weeks), a negative effect of early weaning was significant when the weaned rabbits after 28 d of age had a higher daily gain than rabbits weaned at 28 d to be at least 1.72 g/d per rabbit. At the end of the fattening period (ADG_{8-10}), the inverse situation was found where the differences in favor of rabbits weaned at late age were compensated, and finally, ADG_{8-10} for WA1 was the highest.

Considering the weaning age, it is better to wean the rabbit after 35 days under Egyptian conditions, and the weaning age of 28 days is not recommended in such a condition. After the fattening period, the ADG values were similar. Although these differences were statistically significant, these values did not exceed 0.5 g/d in WA1, WA3, and WA4. The lowest growth rate was in rabbits weaned late and the highest ADG was reported in rabbits in WA2. Similarly, Cesari et al. (2007) and Kovács et al. (2012) observed that the growth of rabbits weaned later was higher than those weaned early

Table 8 shows the line-weaning age interaction effects on ADG during the studied period. The V line seems to be more affected by weaning age than the APRI line. The line APRI had a 7.53% increased daily gain in late weaned rabbits than early weaning while the V line was affected by 13.87%. After 6 weeks of age, the ADG values in WA1, WA2, and WA3 were higher than ADG in WA4. In contrast, line V had the lowest ADG after 6 weeks of age, being significant among early weaning periods (WA1, WA2, and WA3). The highest values were obtained in WA2 and WA3 for the APRI line while for the V line the highest values were for WA3 and WA4. Also, the effect of WA on ADG in V line was more significant than that in APRI line from 4 to 16 weeks.

Table 5. Basic statistics for average daily gain traits of rabbits at different ages

Traits (g)	N	Mean	SD	Minimum	Maximum
ADG_{4-6}	1799	22.95 ± 0.13	5.93	1.20	46.07
ADG_{6-8}	1798	28.68 ± 0.08	3.43	2.49	45.28
ADG_{8-10}	1799	32.87 ± 0.12	5.47	10.28	43.71
ADG_{10-12}	1794	28.87 ± 0.11	4.98	1.6	42.50
ADG_{12-14}	1715	24.40 ± 0.06	2.67	4.28	40.28
ADG_{14-16}	1692	22.30 ± 0.06	2.47	10	40.35
ADG_{6-10}	1799	30.77 ± 0.06	2.66	13.74	48.14
ADG_{4-10}	1799	28.16 ± 0.05	2.40	15.75	36.11
ADG_{10-16}	1692	25.05 ± 0.04	2.00	11.42	34.04
ADG_{4-16}	1692	26.63 ± 0.03	1.45	19.24	31.76

*ADG: Average daily gain (g/d), N: Number of observation, SD: Standard deviation.

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Table 6. Effect of rabbit lines (APRI and V lines) on average daily gain traits between different ages

Lines \ Triats	ADG_{4-6} (g/d)	ADG_{6-8} (g/d)	ADG_{8-10} (g/d)	ADG_{4-10} (g/d)	ADG_{6-10} (g/d)	ADG_{10-12} (g/d)	ADG_{12-14} (g/d)	ADG_{14-16} (g/d)	ADG_{10-16} (g/d)	ADG_{4-16} (g/d)
APRI	23.34±0.16^{a}	28.79±0.08^{a}	33.27±0.14^{a}	28.47±0.05^{a}	31.03± 0.06^{a}	30.98 ± 0.12^{a}	25.52± 0.06^{a}	22.81± 0.09^{a}	26.17± 0.04^{a}	27.36± 0.92^{a}
V	22.54± 0.22^{b}	28.55± 0.14^{b}	32.45± 0.21^{b}	27.85± 0.09^{b}	30.50± 0.10^{b}	26.69± 0.16^{b}	23.26 ± 0.10^{b}	21.78± 0.06^{b}	23.91± 0.06^{b}	25.87± 0.50^{b}

ADG: Average daily gain (g/d). $^{a–b:}$ Means within columns with no common superscript differ significantly ($p < 0.05$). Values are least-squares means.

Table 7. Effect of weaning age on average daily gain traits between different ages.

WA \ Traits	ADG_{4-6} (g/d)	ADG_{6-8} (g/d)	ADG_{8-10} (g/d)	ADG_{4-10} (g/d)	ADG_{6-10} (g/d)	ADG_{10-12} (g/d)	ADG_{12-14} (g/d)	ADG_{14-16} (g/d)	ADG_{10-16} (g/d)	ADG_{4-16} (g/d)
WA1	18.50± 0.27^{d}	27.08± 0.17^{c}	33.63± 0.23^{b}	26.40± 0.13^{d}	30.35± 0.15^{c}	29.82± 0.25^{a}	23.41± 0. 14^{c}	21.64± 0.08^{c}	25.05± 0.12^{b}	25.80± 0.08^{b}
WA2	21.41± 0.27^{c}	29.29± 0.15^{a}	33.67± 0.18^{a}	28.12± 0.11^{c}	31.48± 0.09^{a}	28.91± 0.19^{b}	24.72± 0.14^{a}	23.12± 0.11^{a}	25.62± 0.10^{a}	26.89± 0.08^{a}
WA3	24. 72 ± 0.20^{b}	28.45 ± 0.15^{b}	33.26 ± 0.33^{c}	28.81 ± 0.06^{b}	30.86 ± 0.13^{b}	28.02 ± 0.24^{c}	24.93 ± 0.05^{a}	22.48 ± 0.16^{b}	25.01 ± 0.08^{b}	26.91 ± 0.04^{a}
WA4	26.84 ± 0.18^{a}	29.69 ± 0.14^{a}	30.96 ± 0.24^{c}	29.1 ± 0.08^{a}	30.33 ± 0.11^{c}	28.81 ± 0.23^{b}	24.43 ± 0.12^{b}	21.87 ± 0.07^{c}	24.51 ± 0.06^{c}	26.81 ± 0.04^{a}

ADG: Average daily gain (g/d). WA: Weaning ages 1, 2, 3, and 4 mean weaning at less or equal day 28, between 29 and 35 days, between 35 and 40 days and more than 40 days respectively. $^{a–d}$: Means within columns with no common superscript differ significantly ($p < 0.05$). Values are least-squares means.

Table 8. Effects of rabbit lines (APRI and V lines) and weaning ages on average daily gain traits between different ages

Lines	WA	Traits									
		ADG_{4-6} (g/d)	ADG_{6-8} (g/d)	ADG_{8-10} (g/d)	ADG_{4-10} (g/d)	ADG_{6-10} (g/d)	ADG_{10-12} (g/d)	ADG_{12-14} (g/d)	ADG_{14-16} (g/d)	ADG_{10-16} (g/d)	ADG_{4-16} (g/d)
APRI	**WA1**	19.34± 0.21^{f}	28.09± 0.11^{d}	33.82± 0.13^{ab}	27.07± 0.08^{d}	30.93± 0.07^{c}	31.69± 0.12^{a}	25.81± 0.06^{b}	22.89± 0.06^{a}	26.85± 0.04^{b}	27.02± 0.03^{c}
	WA2	22.51± 0.30^{d}	29.54± 0.15^{b}	34.19± 0.29^{ab}	28.75± 0.10^{b}	31.86± 0.13^{a}	31.47± 0.23^{a}	26.44± 0.15^{a}	23.26± 0.16^{a}	27.16± 0.10^{a}	27.99± 0.07^{a}
	WA3	23.66± 0.20^{c}	28.39± 0.20^{d}	34.50± 0.34^{a}	28.85± 0.08^{ab}	31.44± 0.10^{ab}	28.72± 0.35^{b}	24.86± 0.09^{d}	23.31± 0.31^{a}	25.40± 0.08^{c}	27.10± 0.05^{c}
	WA4	27.64 ± 0.28^{a}	29.05 ± 0.16^{bc}	30.63 ± 0.24^{d}	29.11 ± 0.12^{ab}	29.84 ± 0.13^{de}	31.95 ± 0.22^{a}	24.92 ± 0.10^{c}	21.77 ± 0.07^{b}	25.25 ± 0.06^{c}	27.30 ± 0.05^{b}
V-Line	**WA1**	17.58 ± 0.51^{g}	25. 98 ± 0.31^{e}	33.42 ± 0.46^{b}	25.66 ± 0.25^{e}	29.70 ± 0.30^{e}	27.77 ± 0.47^{c}	20.67 ± 0.13^{f}	20.22 ± 0.08^{c}	23.00 ± 0.17^{g}	24.42 ± 0.11^{g}
	WA2	20.27 ± 0.44^{e}	29.03 ± 0.26^{bc}	33.13 ± 0.20^{b}	27.48 ± 0.19^{c}	31.08 ± 0.13^{cb}	26.26 ± 0.20^{d}	22.99 ± 0.17^{e}	22.99 ± 0.15^{a}	24.08 ± 0.08^{e}	25.80 ± 0.10^{f}
	WA3	25.78 ± 0.33^{b}	28.51 ± 0.23^{cd}	32.04 ± 0.56^{c}	28.78 ± 0.10^{b}	30.28 ± 0.24^{d}	27.33 ± 0.34^{c}	25.01 ± 0.14^{c}	21.64 ± 0.06^{b}	24.62 ± 0.13^{d}	26.71 ± 0.06^{d}
	WA4	26.02 ± 0.24^{b}	30.34 ± 0.23^{a}	31.30 ± 0.41^{cd}	29.22 ± 0.10^{a}	30.82 ± 0.16^{c}	25.59 ± 0.29^{d}	23.96 ± 0.21^{d}	21.96 ± 0.12^{b}	23.79 ± 0.08^{f}	26.33 ± 0.04^{e}

ADG: Average daily gain (g/d). WA: Weaning ages 1, 2, 3, and 4 mean weaning at less or equal than day 28, between 29 and 35 days, between 35 and 40 days and more than 40 days respectively. $^{a-g}$: Means within columns with no common superscript differ significantly ($p = 0.05$). Values are least-squares means.

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CONCLUSIONS

Considering Egyptian conditions, the early weaning had negative effects on growth traits during the fattening period, but these negative effects were compensated after 8 weeks of age. Early weaning (before 28 d) is not recommended for Egyptian rabbits. Clear indications of Genotype × weaning age (G×WA) interactions were observed for growth traits where V line is affected by weaning age than the APRI line. It is recommended to wean the young rabbit of APRI between 29-35 days while in V line is recommended the weaning age after 35 days. However, comparison studies between APRI line and V line about weaning age and its effect on the incidences of pathology are required.

DECLARATIONS

Authors' contributions

M.R., K.H.E, L.M.R., A.E. and I.T.E. developed the concept of the manuscript. M.R. wrote the manuscript. All authors checked and confirmed the final revised manuscript.

Competing interests

None of the authors have any conflict of interest to declare

Ethical considerations

M.R., K.H.E, L.M.R., A.E., and I.T.E. had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis as well as ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy). All authors confirmed the final edition of the article and declared that they did not use any related data of this article on any other publications.

REFERENCES

Abou Khadiga G, Youssef YMK, Saleh K, Nofal RY, and Baselga M (2010). Genetic trend in selection for litter weight in two maternal lines of rabbits in Egypt. World Rabbit Science, 18: 27-32. DOI: http://www.doi.org/10.4995/wrs.2010.18.04

Arias-Alvarez M, Garcia-Garcia RM, Rebollar PG, Nicodemus N, Revuelta L, Millán P, and Lorenzo PL (2009). Influence of metabolic status on oocyte quality and follicular characteristics at different postpartum periods in primiparous rabbit does. Theriogenology, 72: 612-623. DOI: https://www.doi.org/10.1016/j.theriogenology.2009.04.017

Bennegadi N, Fonty G, Ginnede T, and Licois D (2003). Effects of age and dietary fibre level on caecal microbial communities of conventional and specific pathogen-free rabbits. Microbial Ecology in Health and Disease, 5: 23-32. DOI: https://www.doi.org/ 10.1080/08910600310015574

Cesari V, Toschi I, Ferrazzi V, Cesari N, Grilli G, and Lavazza A (2007). Effect of weaning age and diet on growth performance, caecal characteristics and potential pathogenic microflora in rabbits. Italalian Journal of Animal Science, 6: 755-757. DOI: https://www.doi.org/ 10.4081/ijas.2007.1s.755

Dalle Zotte A (2002). Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Liveststock Prouction Science, 75: 11-32. DOI: https://www.doi.org/10.1016/S0301-6226(01) 00308-6.

De Blas JC, Perez E, Fraga M J, Rodriguez JM, and Galvez JF (1981). Effect of diet on feed intake and growth of rabbits from weaning to slaughter at different ages and weights. Journal of Animal Science, 52: 1225-1232. DOI: https://www.doi.org/ 10.2527/jas1981.5261225x

Ebeid TA, Zeweil HS, Basyony MM, Dosoky WM, and Badry H (2013). Fortification of rabbit diets with vitamin E or selenium affects growth performance, lipid peroxidation, oxidative status and immune response in growing rabbits. Livestock Science, 155: 323-331. DOI: https://www.doi.org/10.1016/j.livsci.2013.11.004.

El Nagar AG, Sánchez JP, Ragab M, Mínguez C, and Baselga M (2014). Genetic comparison of milk production and composition in three maternal rabbit lines. World Rabbit Science, 22: 261-268. DOI: https://www.doi.org/10.4995/wrs.2014.1917

El-Sabrout K, and Aggag S (2017). The gene expression of weaning age and its effect on productive performance of rabbits. World Rabbit Science., 25: 1-7. DOI: https://www.doi.org/10.4995/wrs.2017.4777

Estany J, Baselga M, Blasco A, and Camacho J (1989). Mixed model methodology for the estimation of genetic response to selection in litter size of rabbits. Livestock Production Science, 21: 67-76. DOI: https://doi.org/10.1016/0301-6226(89)90021-3

Ferguson FA, Lukefaahr SD, and Mcnitt JI (1997). A technical note on artificial milk feeding of rabbit kits weaned at 14 days. World Rabbit Science, 5: 65-70. DOI: https://www.doi.org/10.4995/wrs.1997.321

Gabr AA, Shalaby NA, and Rahma HM (2017). Effect of weaning age and using mixture of tracts with cow milk as a supplementation on growing rabbits performances. Asian Journal of Animal and Veterenary Advances, 2: 96-102. DOI: https://www.doi.org/10.3923/ajava.2017.96.102

Galal OA, Rehan M, and Abd El-Karim RE (2013). Analysis of genetic diversity within and among four rabbit genotypes using biochemical and molecular genetic markers. African Journal of Biotechenology, 12: 2830-2839 DOI: https://www.doi.org/10.5897/AJB2013.12332

Gallois M, Gidenne T, Fortun-Lamothe L, Le Huerou-Luron I, and Lalles JP (2004). Weaning age and development of the small intestinal mucosa in the young rabbit. In: Proc. 8th World Rabbit Congress, Puebla, Mexico, pp. 1079-1085. Available at: http://www.world-rabbit-science.com/WRSA-Proceedings/Congress-2004-Puebla/Papers/Physiology-Digestion/D-Gallois.pdf

Gallois M, Gidenne T, Tasca C, Caubet C, Coudert C, Milon A, and Boullier S (2007). Maternal milk contains antimicrobial factors that protect young rabbits from enteropathogenic Escherichia coli infection. Clinical and Vaccine Immunology, 14: 585-592. DOI: https://www.doi.org/10.1128/CVI.00468-06

Gidenne T, and Fortun-Lamothe L (2002). Feeding strategy for young rabbits around weaning: A review of digestive capacity and nutritional needs. Animal Science, 75(2): 169-184. DOI: https://www.doi.org/10.1017/S1357729800052942

Kovács M, Bónai A, Szendrő Z, Milisits G, Lukács H, Szabó-Fodor J, Tornyos G, Matics Z, Kovács F, and Horn P (2012). Effect of different weaning ages (21, 28 or 35 days) on production, growth and certain parameters of the digestive tract in rabbits. Animal, 6: 894-901. DOI: https://www.doi.org/10.1017/S1751731111002254

Lebas F (1993). Amélioration de la viabilité des lapereaux en engraissement par un sevrage tardif. Cuniculture, 20: 73-75. Available at: https://hal.inrae.fr/view/index/identifiant/hal-02701450

Lukefahr SD, Hohenboken WD, Cheeke PR, and Patton NM (1983). Characterization of strainghtbred and crossbred rabbits for milk production and associative traits. Journal of Animal Science, 57: 1100-1107. DOI: https://www.doi.org/10.2527/jas1983.5751100x

Marongiu ML, and Gulinati A (2008). Ultrasound evaluation of ovarian follicular dynamics during early pseudo pregnancy as a tool to inquire into the high progesterone (P+) syndrome of rabbit does. In: Proceedings of the 9th World Rabbit Congress; June 10-13, Verona, Italy. Castanet-Tolosan, France: World Rabbit Science Assoiation, pp. 393-397. Available at http://world-rabbit-science.com/WRSA-Proceedings/Congress-2008-Verona/Papers/R-Marongiu2.pdf

McNitt JI, and Lukefahr SD (1990). Effects of breed, parity, day of lactation and number of kits on milk production of rabbits. Journal of Animal Science, 68: 1505-1512. DOI: https://www.doi.org/10.2527/1990.6861505x

McNitt JI, and Moody GLA (1992). Method for weaning rabbit kits at 14 days. Journal of Appled Animal Research, 15: 661-665. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-1992-Corvallis/Communications-pdf/D10-MCNITT.pdf

Orengo J, Gomez EA, Piles M, Rafel O, and Ramon J (2004). Growth traits in simple crossbreeding among dam and sire lines. In: Proceeding of the 8th World Rabbit Congress, September 7-10, Puebla, Mexico, pp. 114-120. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2004-Puebla/Papers/Genetics/G-Orengo.pdf

Petracci M, Bianchi M, and Cavani C (2009). Development of rabbit meat products fortified with n-3 polyunsaturated fatty acids. Nutrients, 1: 111-118. DOI: https://www.doi.org/10.3390/nu1020111 PMID: 22253971.

Ragab M, and Baselga MA (2011). comparison of reproductive traits of four maternal lines of rabbits selected for litter size at weaning and founded on different criteria. Livestock Science,, 136: 201-206. DOI: https://www.doi.org/10.1016/j.livsci.2010.09.009

Rebollar PG, Pérez-Cabal MA, Pereda N, Lorenzo PL, Arias-Álvarez M, and Garcia-Rebollar P (2009). Effects of parity order and reproductive management on the efficiency of rabbit productive systems. Livestock Science, 123: 107-115. DOI: https://www.doi.org/10.1016/j.livsci.2008.06.018

SAS (2012). Statistical Analysis Software. Software for Statistical Analysis; Version 9.4., SAS Institute Inc.: Cary, NC, USA.

Savietto D, Marono S, Martínez I, Martínez-Paredes E, Ródenas L, Cervera C, and Pascual J (2016). Patterns of body condition use and its impact on fertility. World Rabbit Science, 24(1): 39-45. DOI: https://www.doi.org/10.4995/wrs.2016.4006

Xiccato G, Trocino A, Sartori A, and Queaque P (2003). Effect of weaning diet and weaning age on growth, body composition, and caecal fermentation of young rabbits. Animal Science, 77(1): 101-111. DOI: https://www.doi.org/10.1017/S1357729800053704

Xiccato G, Trocino A, Sartori A, and Queaque PI (2000). Early weaning of rabbits: Effect of age and diet on weaning and post-weaning performance. En: Proceeding of the 7th World Rabbit Congress, 4-7 July, 2000, Valencia, Spain, pp. 483-490. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2000-Valencia/Valencia-2000a.htm#management

Xiccato G, Trocino A, Sartori A, and Queaque PI (2004). Effect of parity order and litter weaning age on the performance and body energy balance of rabbit does. Livestock Production Science, 85: 239-251. DOI: https://www.doi.org/10.1016/S0301-6226(03)00125-8

Youssef YMK, Baselga M, Khalil MH, Gad-Alla S, and García ML (2008). Evaluation of litter traits in a crossing project of V-line and Baladi Red rabbits in Egypt. Liveststock Research for Rural. Development, 20: Article #135. Available at: http://www.lrrd.org/lrrd20/9/yous20135.htm

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The Effect of Dietary Inclusion of Whole Yeast, Extract, and Cell Wall on Production Performance and Some Immunological Parameters of Broiler Chickens

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ABSTRACT

A total number of 192 male one-day-old broilers chickens were randomly divided into four treatment groups of 48 chickens. Chickens of group one fed a plain diet without any supplement (control), while the diets ib groups two, three, and four were supplemented with Whole Yeast (WY, *Saccharomyces cerevisiae*, 0.1%), Yeast Cell Wall (YCW, 0.3 %), and Yeast Extract (YE, 0.07 %), respectively. At the end of the experimental period (35 days), the bodyweight of chickens and the feed intake of each cage were measured, and then the feed conversation ratio was calculated. Blood samples were also collected to measure the serum components and relative spleen, bursa of Fabricius, and thymus gland. The results obtained indicated that all productive performance parameters improved in response to the feeding supplementation. Blood parameters indicated that the treated groups had a significantly higher level of serum total protein and albumin as well as significantly lower serum total lipids and cholesterol. The enzyme activities of ALT, AST, and ALP were significantly reduced by WY, YCW, and YE supplementation. The relative organ weights of the spleen, bursa of Fabricius, and thymus increased significantly in broilers fed with WY, YCW, and YE, and the highest values were observed in the chickens fed with WY. It can be demonstrated that the supplementation of WY or its derivatives in the diet of broiler chickens improves the production performance as well as the physiological and immunological parameters, and consequently produce a healthier chicken.

Keywords: Broilers, Immunity, Yeast, Yeast cell wall, Yeast extract

INTRODUCTION

The use of antibiotics as growth promoters was completely banned in 1999 by the European Union (EU). This was due to increases in microbial resistance to antibiotics and residues in chicken meat products which might be harmful to consumers (Koc et al., 2010). Numerous Natural Growth Promoters are used or have been proposed as agents to suppress pathogens or to improve growth and feed conversion, including predominantly organic acids, probiotics, prebiotics, synbiotics, phytogenic, feed enzymes, and immune stimulants (Jaiswal et al., 2017). Probiotics is a live microbial feed supplement that benefits the host animal by enhancing its microbial intestinal balance (Gibson et al., 2017). Probiotics have a major effect on the efficiency of the broiler, such as intestinal microflora modification, pathogen inhibition, intestinal histological changes, immunomodulation, certain haemato-biochemical parameters, sensory characteristics of dressed broilers improving (Kabir, 2009).

The prebiotic is a carbohydrate derived from Yeast Cell Walls (YCW) that can block the proliferation of pathogenic bacteria and stimulate the non-specific immune system, thereby improving birds' health and growth efficiency (Gibson et al., 2017). Yeast Extract (YE) is a source of protein extracted from the live yeast's cell material. High levels of nucleotides, inositol, and glutamic acid (Zarei et al., 2016). Nucleotides, especially in infant diets, are traditionally used in human diets; they favor the development of the gastrointestinal tract and immune functions and preserve the gut flora (Alizadeh et al., 2016).

The objective of the present study was to investigate the effect of Whole Yeast (WY, *Saccharomyces cerevisiae*), YCW, and YE on the improvement of production performance and the immune response in commercial broiler chickens.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in Poultry Research Unit, Biological Application Department, Radioisotopes Applications Division, in the Inshas area, the Nuclear Research Center, Egyptian Atomic Energy Authority October to

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December 2017. The experimental protocols were approved and carried out according to the ordinance and guidelines of the Ethics Committee of Cairo University for the Care and Use of Experimental Animals in Education and Scientific.

Animals and housing

One day-old male Cobb 500 broiler chicken (n=192) were used in the present study. The test period extended from one day of age up to slaughter (35 days). The chickens were allotted into four equal groups, each consisting of 48 chickens, and assigned into four equal replicates of 12 chickens. All groups ran from one day of the set to 35 days of age simultaneously. Chickens in group one fed on a plain diet without any supplementation (control), while the diets of groups 2, 3, and 4 were supplemented with WY (0.1%), (YCW) (0.3 %), and YE (0.07 %), respectively.

Diets

Chickens fed *ad libitum* a commercial starter diet (23% crude protein and 3000 kcal ME/kg diet) during the first week of age, a commercial grower diet (22% crude protein and 3150 kcal ME/kg diet) from two to four weeks of age, and then commercial finisher diet (19% crude protein and 3200 kcal ME/kg diet) until the end of the experiment. The chickens had free access to water. The diet compositions are indicated in Table 1, while the vaccination program is shown in Table 2.

Probiotics and prebiotics

Viable yeast contains 10 billion live yeast cells (*Saccharomyces cerevisiae*) per gram that are used as a probiotic. As shown in Table 3, yeast cell walls, extracted from *Saccharomyces cerevisiae* and used as a prebiotic, are rich in manno-oligosaccharides and beta-glucans (Kwiatkowski et al., 2009). Yeast extract which is considered as a protein source derived from the cell content of live yeast and has high nucleotide, inositol, and glutamic acid levels used as another source for the prebiotics.

Measurements

Production performance

The body weight (BW) of the chickens and the feed intake of each cage were measured at the end of the experimental duration (35 days), and then the Feed Conversation Ratio (FCR) was estimated. After slaughtering the weight of the spleen, bursa, and thymus were individually recorded and presented as a percentage of living body weight.

Blood biochemical assay

Fasted blood samples were collected were obtained by cervical cutting from four birds per pen (n=16). Blood samples were centrifuged rapidly at 4000 rounds per minute (rpm) for 15 minutes. The serum was separated and stored in a freezer at -20ºC until the biochemical analysis. Total Protein (TP) and Albumin were determined calorimetrically, however, Globulin was calculated by subtracting serum albumin from total serum protein. Aspartate Aminotransferase (AST) and Alanine Transaminase (ALT) activities were colorimetrically determined (Bergmeyer et al.,1978), where alkaline phosphatase (ALP) activities were assayed using commercial kits (Bio-Merieux Co. Marcy. L. Eoile chorbonnieres, France). Total cholesterol and Total Lipids (TL) were determined according to Pesce and Bodourian (1976).

Immunological measurements

Haemagglutination test against sheep red blood cells

At 21 days of age, four chickens from each group were randomly selected and housed in separated cages. Sheep Red Blood Cells (SRBC) were collected from three egyption sheep using a hparenized syringe and washed three times using phosphate buffer saline (PBS). The saline was discarded and SRBC sample were diluted with normal saline solution to form a concentrate of 5% SRBC that was used immediately for injection. Each chicken was intravenously primary injected with 0.5 ml of 5 % SRBC suspension in the right or left brachial vein. Blood samples were collected from the brachial vein of each bird at four, seven, and 10 days after the primary injection to detect the primary antibodies titer according to the method described by Wegman and Smithies (1966).

Haemaggluttination inhibition test against newcastle disease virus

At 28 days of age, five chickens from each experimental group were randomly chosen and blood samples were collected from the brachial vein of each chicken at three, seven, and ten days post-vaccination. The Haemagglutination Inhibition (HI) antibody titer against Newcastle Disease Virus (NDV) was determined by the HI test according to the method proposed by Majiyagbe and Hitchner (1977).

Statistical analysis

The data obtained were statistically analyzed on a one-way design basis using the SAS® software statistical analysis program (SAS, 2001). The significant differences between the four treatment groups (Control, WY, YCW, and

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YE) for all parameters were analyzed by Duncan's multiple range test. The significance level was set at $p \leq 0.05$. The results are expressed as Least Square Means (LSM) ± SEM.

Table 1. Composition of the three-phase broilers diet and its calculated chemical composition (on as fed basis)

Ingredients	Starter (kg)	Grower (kg)	Finisher (kg)
Yellow corn	524.5	544.2	628.5
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Oil	30	43.8	40
Di-calcium phosphate	18	18	18
Lime stone	13	13	13
D.L. Methionine	2.2	2.1	2.3
Lysine hydrochloride	2.9	2.8	3.6
Sodium chloride	4	4	4
Premix*	3	3	3
Calculated analysis			
Crude protein (%)	23.0	22.0	19.0
Metabolizable energy (kcal/kg)	3000	3150	3200

*Each gram of mineral mixture contained: vitamin A (transretinyl acetate) 9,000 IU; vitamin D3 (cholecalciferol) 2,600 IU; vitamin E (dl-α-tocopheryl 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; 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.

Table 2. Vaccination program of broiler chickens in Poultry Research Unit, Egyptian Atomic Energy Authority

Age (days)	Vaccines	Method used
8	IB + MA5 vaccine	Eye drop
10	Newcastle disease + H5N2 (oil killed vaccines) 0.5 cm	Subcutaneously into the lower back part of the neck
14	Infection bursal disease (Gumboro)	Eye drop
19	Newcastle disease vaccine (clone 30)	Drinking water

Table 3. The components of yeast cell wall

Component	Cell wall mass (%, dry weight)
(1→3)-β-D-glucan	50-55
(1→6)-β-D-glucan	5-10
(1→4)-α-(1→3)-β-D-glucan	3-7
* Mannoprotein complex	35-40
Chitin	2

RESULTS AND DISCUSSION

Production performance

The improvement in broilers' productive performance affected by dietary WY, YCW, and YE supplementation is shown in Table 4. The WY group harvested the highest BW, body weight gain, and feed intake as well as the best FCR, followed by those fed 0.07 % YE, and 0.03 % YCW. The control group, on the other hand, had inferior values. Koc et al. (2010), who indicated that the addition of *Saccharomyces cerevisiae* at 0.2 percent increased the broiler chickens' weight gain and feed intake, agreed with the results obtained in broiler chickens, the beneficial effects of YC on efficiency have also been shown (Zhang et al., 2005). However, other studies reported that yeast products did not affect the production performance in turkeys (Bradley and Savage, 1995). Differences in animal response can be correlated with differences in the formulations of product. Yeast products are categorized as active dried yeast, live YC, or fermented YC interchangeably, rendering comparisons between studies difficult.

Blood biochemical assays

The current results explained that the WY group had the significantly highest total protein concentration while the control group had the lowest. However, the total globulin of treated groups did not differ from one another but was significantly higher, compared to the control group (Table 5). This finding is in agreement with Abou El- Naga (2012), who found a significantly higher total serum protein and serum albumin in the WY group than the control group.

The enzyme activities of ALT, AST, and ALP were significantly reduced by WY, YCW, and YE supplementation ($p < 0.05$, Table 5). The lowest values for ALT and ALP were observed in chickens fed a diet with WY, followed by those fed YE and YCW. However, the lowest values of AST were recorded in chickens fed a diet with yeast, followed by those fed YCW and then YE. These results agreed with Nikpiran et al. (2013), who showed that the activity of ALP in males depressed by probiotic (yeast) consumption ($p < 0.05$). The present results indicated that the WY group had a

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significantly ($p < 0.05$) lower total serum cholesterol, whereas the control group had the significantly highest value (Table 5). The YCW and YE groups, on the other hand, had almost identical values and were in the center, slightly different from the other two groups.

The results also revealed that the total serum lipids concentration of the WY group was the lowest while the control group had the highest value and was significantly different from the WY and YE groups. Similar observations were recorded by Abou El- Naga (2012). Fukushima and Nakano, (1995) indicating that probiotic microorganisms were known to inhibit hydroxymethylglutaryl coenzyme A, an enzyme that is involved in the cholesterol synthesis pathway, thereby decreasing cholesterol synthesis.

Immunological parameters

The data presented in Table 6 indicate that the spleen, bursa, and thymus weights in broiler chickens that were fed either WY, YCW, or YE diets were significantly higher, compared to the control group ($p \leq 0.05$). Abou-Zeid et al. (2019) found that the relative weights of bursa of fabricius and thymus were significantly ($P \leq 0.05$) improved in groups fed 2g yeast containing diet and they found that in conclusion, yeast (*Saccharomyces cerevisiae*) could be safely used in broiler feeding as natural feed additives at 2g /kg feed with superior effects on their productive and immune response.

Antibody titer

The findings in Table 6 also showed that on post-immunization days, either WY, YCW, or YE were almost the highest antibody titers against SRBCs and NDV. The present findings were in line with those studies by Yalçın et al. (2010), who found that in laying hens fed diets containing 2, 3, or 4 g/kg of yeast autolysate had a higher antibody titer. Furthermore, Mohiti-Asli et al. (2007) stated that laying hens with multi-strain probiotic supplementation and yeast supplementation had a greater immune response than the control group.

These indicators relating to the weight of the lymphoid organs and the humoral immune response reflect the immunologic system status of broilers. The bursa of Fabricius is the primary site of B-cell development in chickens (Ratcliffe, 2006). In accordance with the findings of the present research, Gheisari and Kholeghipour (2006) observed a significant increase in relative bursa weight in broilers fed a diet containing yeast powder, compared to other treatments. Moreover, Wang et al. (2017) reported that KM (yeast: *Kluyveromyces marxianus)* had a positive effect on the broiler's immune organs.

Macrophages have receptors (CR3) for β1, 3/1, 6 branched glucans. By recognizing specific sugars found in glycoproteins of the epithelial surface, prebiotics would bind to macrophage reception sites, causing a cascading reaction that would activate macrophages and release cytokines, thus triggering the immune response acquired and causing higher antibody responses to antigens (Bohn and BeMiller, 1995). Following that, innate and acquired responses are increased by T immune lymphocyte proliferation (Swiatkiewicz et al., 2014). These results might be used as an indicator of the good health status of chickens fed dietary yeast or its derivatives supplementations.

Table 5. Effect of whole yeast, cell wall, or extract supplementations on some blood serum parameters of broiler chickens (mean ± standard error).

Treatments	Control	WY	YCW	YE	p Value
Total protein (g / dL)	3.75 ± 0.043^{d}	4.27 ± 0.037^{a}	3.98 ± 0.038^{c}	4.10 ± 0.122^{b}	0.0001
Total albumin (g / dL)	1.78 ± 0.025^{c}	2.01 ± 0.173^{a}	1.82 ± 0.041^{bc}	1.88 ± 0.038^{b}	0.001
Total globulin (g / dL)	1.97 ± 0.065^{b}	2.26 ± 0.024^{a}	2.16 ± 0.018^{a}	2.22 ± 0.043^{a}	0.002
ALT (Iμ / L)	131.75 ± 0.66^{a}	124.33 ± 1.55^{b}	127.45 ± 1.23^{b}	126.31 ± 1.07^{b}	0.005
AST (Iμ / L)	121.15 ± 0.88^{a}	110.33 ± 0.35^{b}	119.37 ± 0.79^{a}	120.49 ± 1.24^{a}	0.0001
ALP (Iμ / L)	308.22 ± 2.72^{a}	283.89 ± 3.53^{c}	297.42 ± 3.03^{b}	286.00 ± 3.41^{c}	0.001
Total lipids, (mg /dl)	634.87 ± 15.25^{a}	452.03 ± 6.81^{c}	478.88 ± 20.32^{ab}	510.28 ± 3.22^{b}	0.0001
Total cholesterol, (mg /dl)	156.43 ± 2.86^{a}	135.37 ± 1.72^{c}	144.28 ± 1.65^{b}	148.58 ± 1.15^{b}	0.0001

[a, b, c]: The mean values, which are followed by different superscripts, within trait within age are significantly different ($P \leq 0.05$).

Table 4. Effect of whole yeast, cell wall, or extract supplementations on broiler performance at 35 days of age (mean ± standard error).

Treatments	Control	WY	YCW	YE	p Value
Initial body weight (1 day) (g)	52.87 ± 0.48	53.19 ± 0.52	53.08 ± 0.45	53.15 ± 0.42	0.9618
Final Body weight (35) (g)	1769.10 ± 6.26^{d}	1991.08 ± 23.42^{a}	1838.791 ± 18.56^{c}	1928.10 ± 17.26^{b}	0.0001
Body weight gain (1-35 day) (g)	1716.24 ± 6.40^{d}	1937.89 ± 23.58^{a}	1785.71 ± 18.58^{c}	1874.95 ± 17.42^{b}	0.0001
Feed intake (1- 35) (g)	2883.66 ± 4.50^{c}	2972.41 ± 17.52^{a}	2913.31 ± 9.33^{bc}	2935.29 ± 2.62^{b}	0.0001
Feed conversion ratio (1- 35) (g/g)	1.68 ± 0.007^{a}	1.53 ± 0.028^{c}	1.63 ± 0.032^{ab}	1.57 ± 0.012^{bc}	0.003

[a, b, c, d]: The mean values, which are followed by different superscripts, within the age characteristic between treatments, are significantly different ($p \leq 0.05$). Where WY is whole yeast, YCW is weast cell wall and YE is yeast extract.

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Table 6. Effect of whole yeast, cell wall, or extract supplementations on immunological parameters of broiler chickens

Treatments	Control	WY	YCW	YE	P Value
Spleen	0.1203 ± 0.0029 ^{d}	0.1988 ± 0.0052 ^{a}	0.1390 ± 0.0012 ^{c}	0.1602 ± 0.0077 ^{b}	0.0001
Bursa	0.0601 ± 0.0325 ^{d}	0.1146 ± 0.0525 ^{a}	0.0730 ± 0.0128 ^{c}	0.0932 ± 0.0463 ^{b}	0.0001
Thymus	0.2730 ± 0.0187 ^{c}	0.6106 ± 0.0354 ^{a}	0.3885 ± 0.0141 ^{b}	0.4198 ± 0.0386 ^{b}	0.0001
HA titer against SRBCs (days post-immunization)					
3	4.03 ± 0.125 ^{c}	6.20 ± 0.163 ^{a}	5.60 ± 0.248 ^{ab}	5.48 ± 0.309 ^{b}	0.0001
7	6.88 ± 0.14 ^{c}	9.23 ± 0.20 ^{a}	8.73 ± 0.30 ^{a}	7.73 ± 0.36 ^{b}	0.0001
10	4.28 ± 0.18 ^{b}	5.45 ± 0.20 ^{a}	4.38 ± 0.13 ^{b}	4.73 ± 0.30 ^{b}	0.008
HI titer against NDV (days post-immunization)					
3	3.90 ± 0.14 ^{c}	6.95 ± 0.38 ^{a}	5.90 ± 0.19 ^{b}	5.63 ± 0.18 ^{b}	0.0001
7	5.88 ± 0.18 ^{c}	8.50 ± 0.27 ^{a}	7.58 ± 0.25 ^{b}	7.35 ± 0.12 ^{b}	0.0001
10	5.20 ± 0.11 ^{c}	8.25 ± 0.34 ^{a}	7.08 ± 0.27 ^{b}	6.60 ± 0.21 ^{b}	0.0001

*Means followed by different superscripts, between treatments, within trait within age are significantly different ($p \leq 0.05$). Where WY is whole yeast, YCW is weast cell wall, YE is yeast extract, HA is haemagglutination, SRBBCs is sheep red blood cells, HI is haemagglutination inhibition and NDV is Newcastle disease virus.

CONCLUSION

It can be demonstrated that the supplementation of whole yeast at 0.1 percent or yeast cell wall at 0.03 percent and yeast extract at 0.07 percent increased the broiler chickens' body weight and feed conversion. Also, the inclusion of 0.1 whole yeast or its drevatives improved the immunological parameters, such as the antibody titer and immunological organ weights.

DECLARATIONS

Authors' contribution

Engy Youssry Abd El-Salam collected the data, performed the data analysis, and wrote the draft of manuscript. A. M. Atta designed the study, and revised the manuscript. M. A. EL-Mannawy was responsible for the scientific material collection that was used in the experiment and revised the manuscript. A. M. Abo-Taleb was responsible for the laboratory analysis. All authors have read and approved the final data and manuscript. 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.

Competing interests

The authors declared that they have no competing interests.

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Ethical considerations

Athours took responsibility for the integrity of the data and the accuracy of the data analysis as well as ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy). All authors confirmed the final edition of the article and declared that they did not use any related data of this article on any other publications.

REFERENCES

Abou El- Naga MK (2012). Effect of dietary yeast supplementation on broiler performance. Egyption Poultry Science, 32: 95-106. Available at: https://www.cabdirect.org/cabdirect/abstract/20123164745

Abou-Zeid AE, EL-Damarawy SZ, Mariey YA, and Kayed AS (2019**)**. Effect of using yeast (*Saccharomyces cerevisiae*) and mushroom (pleurotus ostreatus) as natural feed additives on productive and immune response of broiler chicks. Journal of Animal and Poultry Production, 10: 149-155. Available at: https://jappmu.journals.ekb.eg/issue_6398_10849_.html

Alizadeh M, Rodriguez-Lecompte JC, Rogiewicz A, Patterson R, and Slominski BA (2016). Effect of yeast-derived products and distillers dried grains with solubles (DDGS) on growth performance, gut morphology, and gene expression of pattern recognition receptors and cytokines in broiler chickens. Poultry Science, 95: 507-517. DOI: https://www.doi.org/10.3382/ps/pev362.

Bohn JA, and BeMiller JM (1995). (1-3)-β-D-glucans as biological response modifiers: A review of structure-functional activity relationships. Carbohydrate Polymers, 28: 3-14. DOI: https://www.doi.org/10.1016/0144-8617(95)00076-3.

Bergmeyer, HU, Scheibe P, and Wahlefeld AW (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clinical Chemistry, 24(1):58–73. https://doi.org/10.1093/clinchem/24.1.58.

Bradley GL, and Savage TF (1995). The effect of autoclaving a yeast culture of *Saccharomyces cerevisiae* on turkey poult GAO ET AL.1384 performance and the retention of gross energy, and selected minerals. Animal Feed Science and Technology, 55: 1-7. DOI: https://www.doi.org/10.1016/0377-8401(95)00800-3

Fukushima M, and Nakano M (1995). The effect of probiotic on faecal and liver lipid classes in rats. British Journal of Nutrition, 73: 701-710. DOI: https://www.doi.org/10.1079/BJN19950074

Gheisari A, and Kholeghipour B (2006). Effect of dietary inclusion of live yeast (*Saccharomyces cerevisiae*) on growth performance, immune responses and blood parameters of broiler chickens. 7 European Poultry Conference, Verona, Italia, 10(2): 17. Available at: https://www.cabdirect.org/cabdirect/abstract/20093210426

Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD et al. (2017). The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology and Hepatology, 14: 491-502. DOI: https://www.doi.org/10.1038/nrgastro.2017.75

Jaiswal SK, Chaturvedani AK, Raza M, Dilliwar L, Dhruw K, and Sahu V (2017). Review: Natural growth promoters, alternative to antibiotic growth promoters on poultry. International Journal of Science, Environment and Technology, 6: 254-259. Available at: https://www.ijset.net/journal/1536.pdf.

Kabir SML (2009). The role of probiotics in poultry industry. International Journal of Poultry Science, 10: 3531-3546. DOI: https://www.doi.org/10.3390/ijms10083531

Koc F, Samli H, Okur A, Ozduven M, Akyurek H, and Senkoylu N (2010). Effects of *Saccharomyces cerevisiae* and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. Bulgarian Journal of Agricultural Science, 16: 643-650. Available at: https://www.agrojournal.org/16/05-15-10.pdf

Kwiatkowski S, Thielen U, Glenny P, and Moran C (2009). A study of *Saccharomyces cerevisiae* cell wall glucans. The Institute of Brewing and Distilling, 115: 151-158. DOI: https://wwwdoi.org/10.1002/j.2050-0416.2009.tb00361.x

Majiyagbe KA, and Hitchner SB (1977). Antibody response to strain combinations of Newcastle disease virus as measured by haemagglutination inhibition. Avian Disease, 21: 576-584. Available at: https://pubmed.ncbi.nlm.nih.gov/606219/

Mohiti-Asli M, Hosseini SA, Lotfollahian H, and Shariatmadan F (2007). Effect of probiotics, yeast, Vitamin E and Vitamin C supplements on performance and immune response of laying hen during high environmental temperature. International Journal of Poultry Science, 6: 895-900. DOI: https://wwwdoi.org/10.3923/ijps.2007.895.900

Nikpiran H, Vahdatpour T, Babazadeh D, and Vahdatpour S (2013). Effects of *Saccharomyces cerevisiae*, thepax and their combination on blood enzymes and performance of Japanese quails (coturnix japonica). Journal of Animal and Plant Sciences, 23: 369-375. Available at: http://www.thejaps.org.pk/docs/v-23-2/06.pdf .

Pesce MA and Bodourian SH (1976). Enzymatic rate method for measuring cholesterol in serum.. Clinical Chemistry, 22(12):2042-2045. https://doi.org/10.1093/clinchem/22.12.2042a.

Ratcliffe MJ (2006). Antibodies, immunoglobulin genes and the bursa of Fabricius in chicken B cell development. Developmental and Comparative Immunology, 301: 101-118. DOI: https://wwwdoi.org/10.1016/j.dci.2005.06.018.

Swiatkiewicz S, Arczewska-Wlosek A, and Jozefiak D (2014). Immunomodulatory efficacy of yeast cell products in poultry: A current review. World's Poultry Science Journal, 70: 57-68. DOI: https://www.doi.org/10.1017/S0043933914000051

Wang W, Li Z, Lv Z, Zhang B, Lv H, and Guo Y (2017). Effects of Kluyveromyces marxianus supplementation on immune responses, intestinal structure and microbiota in broiler chickens. PloS one, 127: e0180884. DOI: https://www.doi.org/10.1371/journal.pone.0180884

Wegman TG, and Smithies O (1966). A simple hemagglutination system requiring small amounts of red cells and antibodies. Transfusion, 6: 67-73. DOI: https://www.doi.org/10.1111/j.1537-2995.1966.tb04696.x

Yalçın S, Yalçın S, Çakın K, Eltan Ö, and Dağaşan L (2010). Effects of dietary yeast autolysate (*Saccharomyces cerevisiae)* on performance, egg traits, egg cholesterol content, egg yolk fatty acid composition and humoral immune response of laying hens. Journal of the Science of Food and Agriculture, 90: 1695-1701. DOI: https://www.doi.org/10.1002/jsfa.4004

Zarei O, Dastmalchi S, and Hamzeh-Mivehroud M (2016). A simple and rapid protocol for producing yeast extract from *Saccharomyces cerevisiae* suitable for preparing bacterial culture media. Iranian Journal of Pharmaceutical Research, 15: 907-913. Available at: https://pubmed.ncbi.nlm.nih.gov/28243289/

Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB, and Lee CH (2005). Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Science, 84: 1015-1021. DOI: https://www.doi.org/10.1093/ps/84.7.1015.

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The Effect of Essential Amino Acid (Lysine) in Commercial Feed of Patin Catfish (*Pangasius* sp.)

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ABSTRACT

The Patin catfish (*Pangasius* sp.) is a species of fish that is widely cultivated both in quarantine and in ponds. The success of Patin catfish cultivation is influenced by several factors, one of them is the feed. Patin catfish need essential amino acids to meet their needs. The addition of the amino acid (lysine) in the commercial feed not only affects the metabolism of the fish but also the content of Omega-3 and Omega-6 would be found in the fish**.** This study was conducted to observe the influences of essential lysine on the content of Omega-3 and Omega-6 of Patin catfish. This was an experimental study with a completely randomized design method, consisting of four treatments and five replications. The treatment which was given to experimental fish was commercial feed with the addition of lysine at different doses. The result indicated that the addition of lysine in commercial feed for 30 days of this research had a significant effect ($p < 0.05$) on the increase in the content of Omega-3 and Omega-6 in Patin catfish meat (*Pangasius* sp.). Based on the results of the current study, Patin catfish can be a good source of Omega-3 and omega 6 if the feed that is used in the cultivation process, contains lysine as an amino acid source.

Keywords: Cultivation, Lysine, Omega-3, Omega-6, *Pangasius* sp.

INTRODUCTION

The Patin catfish's need for essential amino acids is an important factor to be fulfilled in order to increase the Catfish quality. Essential amino acids are those amino acids that cannot be synthesized by animals or plants to generate maximum growth (Tom, 1998). The addition of essential amino acids to the commercial fish feed and how it affects the Patin catfish Omega-3 and Omega-6 content are interesting things to be explored and studied further.

The high consumption level of Patin catfish which is with the Latin name of *Pangasius* sp. Has become an interesting object to be observed. Among the many types of freshwater fish, Patin catfish is a fish with the highest level of proteins, with nutritional content of 16.08% protein, about 5.75% fat content, 1.5% carbohydrate, 0.97% ash, and 75.7% water (Almunadi et al., 2001). When compared with the fat content of other freshwater fish such as Snakehead fish and Goldfish, Patin catfish has a higher fat content. Patin catfish have the potential to meet the food and nutrition needs of the community. The production of Patin catfish in Indonesia has increased from 2010 to 2013, which was 147,890 tons in 2010 and reached 972,779 tons in 2013 (Almunadi et al., 2001).

This increase in production raises the question of how the quality of the Patin catfish was produced by farmers. In this case, the quality of the content of Omega-3 and Omega-6 in Patin catfish which is widely consumed by the community has become an interesting factor to be discussed further. Omega-3 and Omega-6 belong to the group of essential fatty acids. Omega-3 has derivatives from ALA (α-Linolenic Acid), EPA (Eicosapentaenoic Acid), and DHA (Docosahexaenoic Acid). While Omega-6 is derived from LA (Linolenic Acid), and ARA (Arachidonic Acid, Panagan et al., 2012). Omega3 and Omega-6 which are contained in Patin catfish are influenced by the cultivation process itself, one of them is feeding (Oktavianawati et al., 2016).

Patin catfish need essential amino acids in their feed to meet their needs. One of the essential amino acids is lysine. The role of lysine is important in metabolism because lysine is used for protein synthesis as well as compiling other important components used for metabolism. Lysine is one of the essential amino acids needed by Patin catfish which plays a role in growth (Millamena et al., 2019). Lysine can increase fish growth because it can improve the balance of utilization of other amino acids (Alam et al., 2005). Therefore, research on the effect of the addition of essential amino acids like lysine in commercial feed with considering the content of Omega-3 and Omega-6 (essential fatty acids that have derived from Linoleic Acid (LA)) in Catfish needs to be done.

MATERIALS AND METHODS

Ethical approval

The present study was conducted at the Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia in 2019. The current study was carried out in correspondence with the research principles based on basic

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principles of ethics of respect, beneficence, non-maleficence, and justice. All experimental protocols and procedures were approved by the Institutional Animal Care of Indonesia

Experimental fish

The experimental animals used in the study were Patin catfish with a size of 6-8 cm per head. The Patin catfish were randomly allotted to four treatment groups with five replicates per treatment, and 10 Patin catfish per replicate (n = 200).

Experimental feed

The feed which used in the current research was pellet-shaped. The essential amino acid which was lysine and tapioca flour were used as binders.

Experimental equipment

The equipment used in the study included 20 pieces of the aquarium in 30×30×40 cm size, aerator resun air pump LP-40, aeration hose, siphon hose, 20 pieces of the air stone, plastic bag, litmus paper to measure pH, thermometer, DO meter, ammonia test kit, digital scales, tubular fiber for water reservoirs, ruler or caliper, stationery, fecal container bottles and feed scraps, basins, buckets, and fishnet.

Experimental parameters

The current experimental study aimed to determine the effect of certain parameters on groups of fish under controlled conditions. The experimental parameters in this study included independent parameters of lysine doses in the feed as 0% (P0), 1.2% (P1), 2.2% (P2), and 3.2% (P3). The dependent parameters in this study were the content of Omega-3 and omega-6. Finally, the controlled parameters entailed experimental fish in this study in the size of 6-8 cm per head.

Measurement of Omega-6 and Omega-3 content

Omega-6 and Omega-3 content can be defined as the sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The standard technique used for fatty acid analysis and quantification has been gas chromatography (GC) with flame ionization detection (Nazari et al., 2008).

Supporting experimental parameters

The supporting parameters of the study were water quality management parameters which included pH, temperature, DO, and ammonia. Temperature and DO measurements were performed every day while ammonia was measured once every seven days.

Data analysis

The data were statistically analyzed using analysis of Variance (ANOVA 8.). ANOVA is a useful statistical model that simultaneously tests differences between means in more than two conditions (Boisgontier and Cheval, 2016). If significant results were obtained, the calculation would be continued with Duncan's Multiple Range Test with the accuracy of 5%, to provide a significance level for the difference between the mean value of each pair (Salkind, 2010).

RESULTS AND DISCUSSION

Based on the results from the current study, it was found that the Omega-3 content of catfish (*Pangasius* sp.) ranged from 1,145 to 2,076% and Linoleic Acid (LA) (Omega-6) ranged from 13.821% to 22.149%. Calculation of the content of Omega-3 and LA (Omega-6) has been presented in table 1.

The results of statistical analysis on Omega-3 content indicated a significant difference ($p < 0.05$) between treatment groups. The lowest Omega-3 content in treatment was in P3 (1.145%), while Omega-3 content in treatment P0 (1.814%), P1 (2.076%), and P2 (1.840%) presented similar results. Based on the results from Duncan's Multiple Range Test (Duncan Multiple Distance Test), there was a significant difference between the results of the Omega-3 content of Patin catfish. The results of analyzed variances by ANOVA indicated a significant difference ($P < 0.05$) between each treatment with the addition of lysine.

The results of Duncan's Multiple Range Test (Duncan Multiple Distance Test) expressed a significant difference. The P0, P1, and P2 treatment groups have the same Omega-6 content and were not significantly different, while treatment groups of P0, P1, and P2 were significantly different from P3 ($p < 0.05$). The highest Omega-6 content was found in treatment P3 (22.540%).

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Table 1. The content of Omega-3 and Linoleic Acid (Omega-6) of treated catfish (*Pangasius sp*.).

Treatment Group	Omega-3 Content ± SD	LA Content ± SD
P_0	1.814^{b} ± 0.343	14.944^{a} ± 4.176
P_1	2.076^{b} ± 0.122	14.010^{a} ± 2.013
P_2	1.840^{b} ± 0.224	14.909^{a} ± 0.848
P_3	1.145^{a} ± 0287	22.540^{b} ± 3.555

Note: Different superscripts in the same column indicate a significant difference ($p < 0.05$). LA: Linoleic acid, P0: 0% lysine, P1:1.2% lysine, P2: 2.2% lysine, P3: 3.2% lysine.

DISCUSSION

Significant changes have been obtained in the content of Omega-3 and Omega-6 in Patin catfish which were given a certain amount of fish feed containing lysine.

Omega-3 is a PUFA that has many double bonds, the first double bond is located on the third carbon atom of the methyl omega group, the next double bond lies in methyl omega, the next double bond is located on the third carbon atom from the previous double bonds. The methyl omega group is the last group of fatty acid chains. The EPA has many benefits including lowering cholesterol and anti-inflammation, and EPA is needed to help the growth and development of nerve cells to be optimal (Maulana, 2013).

The present study indicated the results of an increase in the content of Omega-3 in catfish meat after the administration of lysine in feed at a dose of 1.2% (P1) with an Omega-3 content of 2.076%. It is suspected that the administration of amino acid lysine which acts as a precursor of carnitine could be absorbed optimally by catfish so that the content of EPA and DHA could increase. Carnitine plays a role in the transfer of long-chain fatty acids into the mitochondria to be oxidized, so that the content of EPA and DHA can also increase after the oxidation process. Omega-3 has an important role in growth, immune system, lower blood cholesterol levels, enhances the metabolism of cells, essential nutrient for the eyes, and good for the central nervous system and brain in fish (Greenwood et al., 2001).

Omega-6 is included in essential fatty acids which have Linoleic acid parent compounds or called Linoleic acid (LA). Omega-6 is a PUFA that has the first double bond in the 6th position. Linoleic acid is a precursor in the synthesis of PUFA. Linoleic acid is produced from plants, it is specifically contained in seed oil. In addition, Linoleic and Linolenic acids are found in food reserves. Arachidonic Acid (AA) which is one of the Omega-6 types, is often found in cell membranes, is an important compound in interacellular communication, and becomes a precursor compound for other important compounds in the body (Belitz et al., 2009). This study indicated an increase in the content of Omega-6 in Patin catfish meat after administration of lysine amino acid to feed at a dose of 3.2% (P3) which led to Omega-6 content of 22.540%. The reason for this increase in content is the limited lysine availability and the imbalance in the composition of amino acids in the fish feed (Nunes et al., 2014). Feeds with an amino acid content of <20% are more effective than feeds which their source of protein is only raw materials (Cowey, 1994).

Omega-6 (Linoleic acid and arachidonic acid) in Patin catfish meat is influenced by the addition of Lemuru fish oil to the commercial feed of catfish (Gonçalves et al., 2012). The Omega-6 content is found in meat because most unsaturated fatty acids are stored in phospholipids in the cell membrane. The function of Omega-6 in *Pangasius* sp. is the formation of compounds such as hormones that are used as a carrier of orders from one cell to another nerve cell (Cuzon et al., 2004).

One of the supporting factors for the success of Patin catfish aquaculture is water quality. Water quality parameters observed during the present study were temperature, DO, pH, and ammonia (NH3). Patin catfish were kept in containers for 30 days with various water temperatures ranging from 27.4ºC to 28.7ºC and DO ranging from 4.47 mg/L to 7.08 mg/L. The catfish containers with different treatments and DO had similar pH of 7.0 and ammonia of 0.003 mg/L during 30 days. The level of water quality in Patin catfish containers was in accordance with the limits of water quality parameters, namely temperature ranging from 28 to 32ºC, DO levels (3-6 ppm), water pH tolerated by catfish (PH five to nine) (Khairuman and Dodi, 2002). NH3 which can still be tolerated by catfish is 1 ppm. Drastic temperature changes can cause stress in fish and stop the activity of Patin catfish in the waters. Fish need DO to perform activities such as swimming, reproduction, respiration, and growth (Buentello et al., 2000). Increased NH3 concentration is influenced by an increase in pH value and feeding amount (Effendi et al., 2007).

CONCLUSION

Based on the results from the current study, it can be concluded that the addition of lysine to commercial Patin catfish feed could increase the content of Omega-3 and Omega-6 in catfish meat. Lysine addition at the dose of 1.2% could increase the content of Omega-3 namely Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) and a 3.2% dose of lysine could increase the content of Omega-6 namely Linoleic Acid (LA) and Arachidonic Acid (AA) in catfish meat.

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DECLARATIONS

Authors' contribution

Agustono designed the study, Yaqin collected the data and Lokapimasari wrote the manuscript. Finally, all of the authors approved the final draft of the manuscript for submission.

Competing interests

The authors have declared that no competing interest exists.

Ethical considerations

All authors approved the final draft of the manuscript for submission to this journal. Ethical issues (Including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been checked by the authors.

REFERENCES

Alam MS, Teshima S, Koshio S, Ishikawa M, Uyan O, Hernandez LHH, and Michael FR (2005). Supplemental effects of coated methionine and/or lysine to soy protein isolate diet for juvenile kuruma shrimp, Marsupenaeus *japonicus*. Aquaculture, 248: 13-19. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0044848605002267

Almunadi T, Panagan YH, and Gultom JU (2001). Analasisi kualitatif dan kuantitatif asam lemak tak jenuh omega-3 dari minyak ikan patin (Pangasius pangasius) dengan metoda kromatografi gas. Jurnal Penelitian Sains, 14(4): 38-42. Available at: https://www.neliti.com/publications/168366/analisis-kualitatif-dan-kuantitatif-asam-lemak-tak-jenuh-omega-3-dari-minyak-ika

Belitz HD, Grosch W, and Schieberle P (2009). Food chemistry. Springer-Verlag, Berlin, pp. 158-247. Available at: https://www.springer.com/gp/book/9783540699330

Boisgontier MP, and Cheval B (2016). The ANOVA to mixed model transition. Neuroscience and Biobehavioral Reviews, 68: 1004-1005. DOI: https://www.doi.org/10.1016/j.neubiorev.2016.05.034

Buentello A, Gatlin DM, and Neill WH (2000). Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish (Ictalurus punctatus). Aquaculture, 182: 339-352. DOI: https://www.doi.org/10.1016/S0044-8486(99)00274-4

Cowey CB (1994). Amino acid requirements of fish: a critical appraisal of present values. Aquaculture, 124: 1-11. Available at: https://www.sciencedirect.com/science/article/abs/pii/0044848694903492

Cuzon G, Lawrence A, Gaxiola G, Rosas C, and Guillaume J (2004). Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture, 235: 513-551. DOI: https://www.doi.org/10.1016/j.aquaculture.2003.12.022

Effendi IE, Bugri HJ, and Widanarni T (2007). Effect of different rearing density on survival rate and growth of giant gouramy osphronemus gouramy Lac. fry at size of 2 cm in length. Jurnal Akuakultur Indonesia, 5(2): 127-35. DOI: https://www.doi.org/10.19027/jai.5.127-135

Gonçalves LU, Ferroli F, and Viegas EMM (2012). Effect of the inclusion of fish residue oils in diets on the fatty acid profile of muscles of males and females lambari (Astyanax altiparanae). Revista Brasileira de Zootecnia, 41(9): 1967-1974. DOI: https://www.doi.org/10.1590/S1516-35982012000900001

Greenwood RH, Titgemeyer EC, Stokka GL, Drouillard JS, and Loest CA (2001). Effects of L-carnitine on nitrogen retention and blood metabolites of growing steers and performance of finishing steers. Journal of Animal Science, 79(1): 254-60. DOI: https://www.doi.org/10.2527/2001.791254x

Khairuman SP, and Dodi S (2002). Budi daya patin secara intensif. AgroMedia Pustaka: Jakarta. pp. 52-120. Available at: https://agromedia.net/katalog/budi-daya-patin-secara-intensif/

Maulana IT (2013). Pemisahan asam elaidat (trans-9-octadecenoic acid) dan asam lemak jenuh serta peningkatan kandungan EPA dan DHA dari minyak limbah perusahaan pengolahan ikan. tesis tidak dipublikasikan. Bandung: Sekolah Farmasi, Institut Teknologi Bandung. pp. 5-70 Available at: https://digilib.itb.ac.id/index.php/gdl/view/45374

Millamena O, Bautista-Teruel M, Reyes O, and Kanazawa A (2019). Requirements of juvenile marine shrimp, Penaeus monodon (Fabricius) for lysine and arginine. Aquaculture, 164: 95-104. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0044848698001793

Nazari B, Sedigheh A, Nazila S, Saberi SA, and Noushin M (2008). Content of long chain omega-3 fatty acid composition in some Iranian canned fish. ARYA Atherosclerosis, 4: 93-97. Available at: http://arya.mui.ac.ir/index.php/arya/article/view/67

Nunes AJP, Sa MVC, Browdy CL, and Vazquez-Anon M (2014). Practical supplementation of shrimp and fish feeds with crystalline amino acids. Aquaculture, 431(20): 20-27. DOI: https://www.doi.org/10.1016/j.aquaculture.2014.04.003

Oktavianawati I, Andinata D, Isnaeni AN, Hermiastuti M, Rahmawati N, Handayani W, and Winata INA (2016). Effects of feeding diets containing Azolla pinnata and probiotics on the growth and nutritional content of patin Fish (Pangasius djambal). Agriculture and Agricultural Science Procedia, 9: 403-410. DOI: https://www.doi.org/10.1016/j.aaspro.2016.02.156

Panagan AT, Yohandini H, and Wulandari M (2012). Analisis kualitatif dan kuantitatif asam lemak tak jenuh omega-3, omega-6 dan karakterisasi minyak ikan patin (Pangasius pangasius). Jurnal Penelitian Sains, 15(3): 102-106. Available at: http://ejurnal.mipa.unsri.ac.id/index.php/jps/article/view/105/99

Salkind NJ (2010). Encyclopedia of research design. Thousand Oaks, California, pp. 45-129. DOI: https://www.dx.doi.org/10.4135/9781412961288

Tom L (1998). Nutritional and feeding of fish. Springer, USA, p. 245. Available at: https://www.springer.com/gp/book/9781475711769

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Detection of *Coxiella burnetii* (Query Fever) DNA by Nested-PCR in Beef Cattle from Ampel Slaughterhouse, Boyolali Regency, Middle Java, Indonesia

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ABSTRACT

Coxiella burnetii (*C. burnetii*) is a Gram-negative and obligate intracellular bacterium that causes Query fever (Q fever). The aim of the present study was to detect *C. burnetii* in beef cattle from Ampel slaughterhouse at Boyolali Regency, Middle Java, Indonesia. Spleen, heart, liver, lung, and kidney samples were collected from 100 cattle and used for Nested-PCR (nPCR) with four types of primers (OMP1, OMP2, OMP3, and OMP4). Five stages of pooling extraction were performed on 100 individual samples. The nPCR amplified a 437 bp DNA fragment from the fifth pool on the sampled heart, lung, and spleen. Furthermore, 10 individual samples from the fifth pool were re-tested by nPCR to find out the number of positive individual samples. Of 10 samples, the obtained result indicated the presence of *C. burnetii* DNA in 7 samples, 6 from Simmental cattle and 1 from Ongole cattle. Therefore, it can be strongly suspected that there are 7 out of 100 local breed beef cattle positive of Q fever at Boyolali Regency, Middle Java, Indonesia.

Keywords: Beef cattle, Boyolali, *Coxiella burnetii*, Nested-PCR, Query fever case.

INTRODUCTION

Coxiella burnetii (*C. burnetii*) is a Gram-negative and obligate intracellular bacterium that causes Query fever which is also known as coxiellosis. This disease is highly contagious and zoonotic (OIE, 2010; Eldin et al., 2017), and can be transmitted through aerosol and direct contact with infected animals or objects that are contaminated with the agent (Ergas et al., 2006). Flies (*Stomoxys* sp., *Musca* sp.) and ticks (Ixodidae, Argasidae) are ectoparasites that act as vectors causing the transmission of Q fever agent among animals, both from domestic to wild animals and also domestic to other domestic animals (Maurin and Raoult, 1999; Eldin et al., 2017). Livestock that can be infected are ruminants such as cattle, sheep, and goats, wild animals, pets, and even poultry.

Coxiella burnetii infection is often poorly symptomatic in animals. Symptoms that appear include decreased appetite, respiratory disorders, and reproductive disorders, such as abortion (Fournier et al., 1998). In humans, *C. burnetii* infection can be acute or chronic. The main clinical forms of acute Q fever in humans are pneumonia, hepatitis, and flu-like syndrome, while chronic Q fever causes poor conditions, including endocarditis, vascular, and chronic infections in pregnant women (Lodrigue et al., 2006; CDC, 2019). Chronic Q fever develops months or years following initial Q fever infection. Stein et al. (2005) showed that aerosol transmission of Q fever causes severe lesions in the lung.

Query fever is present in many countries around the world, such as the USA, Canada, Australia, and several countries in Africa and Asia (OIE, 2017). As a contagious disease, Q fever is the most neglected zoonosis in Indonesia. Compared to other diseases, such as rabies, anthrax, and salmonellosis, this disease has received less public attention. Considering the conducted studies on Q fever in Indonesia, Brahman Cross (BX) cattle were the main source of *C. burnetii*. In fact, Q fever cases in Indonesia were reported in BX cattle (Setiyono, 2014), Balinese cattle (Mahatmi et al., 2007), sheep, and goat (Mahatmi et al., 2007; Setiyono et al., 2008). Moreover, Q fever cases have already spreads to some regions in Indonesia, such as Bogor (Mahatmi et al., 2007; Setiyono et al., 2008), Depok (Rini et al., 2019), Jakarta (Setiyono et al., 2008), Malang (Mahatmi et al., 2007), Bali (Mahatmi et al., 2007) and Medan (Nasution et al., 2015). So far, the incidence of the disease in Boyolali Regency has not been reported and the current study was the first case of Q fever in beef cattle at Boyolali Regency, Middle Java, Indonesia. With this in mind, the present study aimed to detect *C. burnetii* in beef cattle at Boyolali Regency, Middle Java, Indonesia.

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MATERIALS AND METHODS

Sampling

The samples included heart, liver, lung, spleen, and kidney organs from 100 cattle collected by pooling technique (Rini et al., 2019). Each type of extracted organ was put in a different tube at every stage of the pooling extraction. To enhance efficiency and reduce non-specific PCR product, only the heart organ was used for the individual test which was based on pooling tests, showing the brightest band. The organ samples were collected by purposive random sampling (Rini et al., 2019) from local beef cattle, such as Simmental, Ongole, Fresian Holstein, and Brahman breeds slaughtered at the Ampel Slaughterhouse, Boyolali Regency, Middle Java, Indonesia from 19 to 21 June 2017.

Each organ was trimmed into cubes (3 cm thick), wrapped in aluminum foil, placed in a plastic container, and stored in a coolbox to maintain the cold chain and freshness of the organ. Until the last day, the collected organs were temporarily stored in the freezer (-30°C) at the Laboratory of Balai Pelayanan Kesehatan Masyarakat Veteriner (Bapel Kesmavet) Boyolali Regency. The organs were placed in a coolbox with an icepack and transported by car to be tested at the Integrated Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural University (IPB University), Bogor, Indonesia.

Nested- polymerase chain reaction

DNA extraction was carried out according to the method introduced by Ho et al. (1995) using DNA Purification Kit; Solid tissue protocol (Gentra® Puregene®, Qiagen, Germany). Frozen fresh tissue of each organ was cut into 3-5 mg pieces and grounded for the extraction. Initial screening used pooling techniques on 100 cattle samples in 5 stages. All organs (heart, liver, lung, spleen, and kidney) were extracted and placed on different tubes so there were five tubes of pooling sample organs in every stage. In the first and third pool stage, 25 samples were extracted, respectively. In the second and the fourth pool stage, every 20 samples were extracted while in the fifth pool stage as many as 10 samples were extracted. After initial screening, 10 individual samples from the fifth pool were re-tested to see which cattle were positive for Q fever.

The DNA amplification was performed by Nested-PCR (nPCR). The DNA sample was amplified on a thermal cycler machine (GeneAmp PCR systems 9600, Perkin-Elmer®, USA). The first-round PCR was programmed for 35 cycles. There were pre-denaturation (94°C, 3 minutes), denaturation (94°C, 1 minute), annealing (54°C, 1 minute), extension (72°C, 2 minutes), final extension (72°C, 4 minutes), and cooling (4°C, ∞). The first-round PCR product was 501 bp (OMP1 and OMP2, Ogawa et al., 2004). The primers used in the PCR test are shown in Table 1.

DNA amplification in nPCR was programmed for 35 cycles. It started with pre-denaturation (94ºC, 3 minutes), denaturation (94ºC, 1 minute), annealing (56ºC, 1 minute), extension (72ºC, 1 minute and 30 seconds), final extension (72ºC, 4 minutes), and cooling (4ºC, ∞). The nested-PCR product was a DNA amplicon of 437 bp (OMP3 and OMP4, Ogawa et al., 2004).

Table 1. Sequences of oligonucleotides used as primers for first-round and Nested-PCR

PCR	Primer	Sequence	Base Pair
First Round	OMP1	5‘- AGT AGA AGC ATC CCA AGC ATT- 3’	501 bp
	OMP2	5’- TGC CTG CTA GCT GTA ACG ATT- 3’	
Nested	OMP3	5‘-GAA GCG CAA CAA GAA GAA CAC-3‘	437 bp
	OMP4	5‘-TTG GAA GTT ATC ACG CAG TTG-3‘	

Reference: Ogawa et al. (2004)

Electrophoresis

After the amplification process on the PCR machine was completed, electrophoresis was carried out to see the amplification results using electrophoresis gel (Agarose Gel Electrophoresis, Takara®, Japan) (Ogawa et al., 2004). Electrophoresis reading was visualized as DNA fragments in bands. The result of visualization of electrophoresis was viewed under UV luminescence and then it was photographed.

RESULTS AND DISCUSSION

On the first-round PCR, samples did not indicate any band neither pooling (Figure 1A) nor individual sample (Figure 1B). On nPCR, there were three organ pools (Figure 2) with positive Q fever, including samples of heart, lung, and spleen from the fifth pool (Table 2). After the 10 individual samples were re-tested from the fifth pool, it was found that there were 7 out of 10 positive samples of Q fever (Figure 3). The heart organ was only used for an individual test because based on pooling tests, these organ pools showed the brightest band. As Hermansyah et al. (2018) explained, good DNA quality has a high DNA concentration and it is also characterized by the high intensity of the produced DNA bands.

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Table 2. Result of nPCR of five stages organ pool extraction

Pooling Stages	Organs				
	Heart	Spleen	Lung	Liver	Kidney
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	+	+	+	-	-

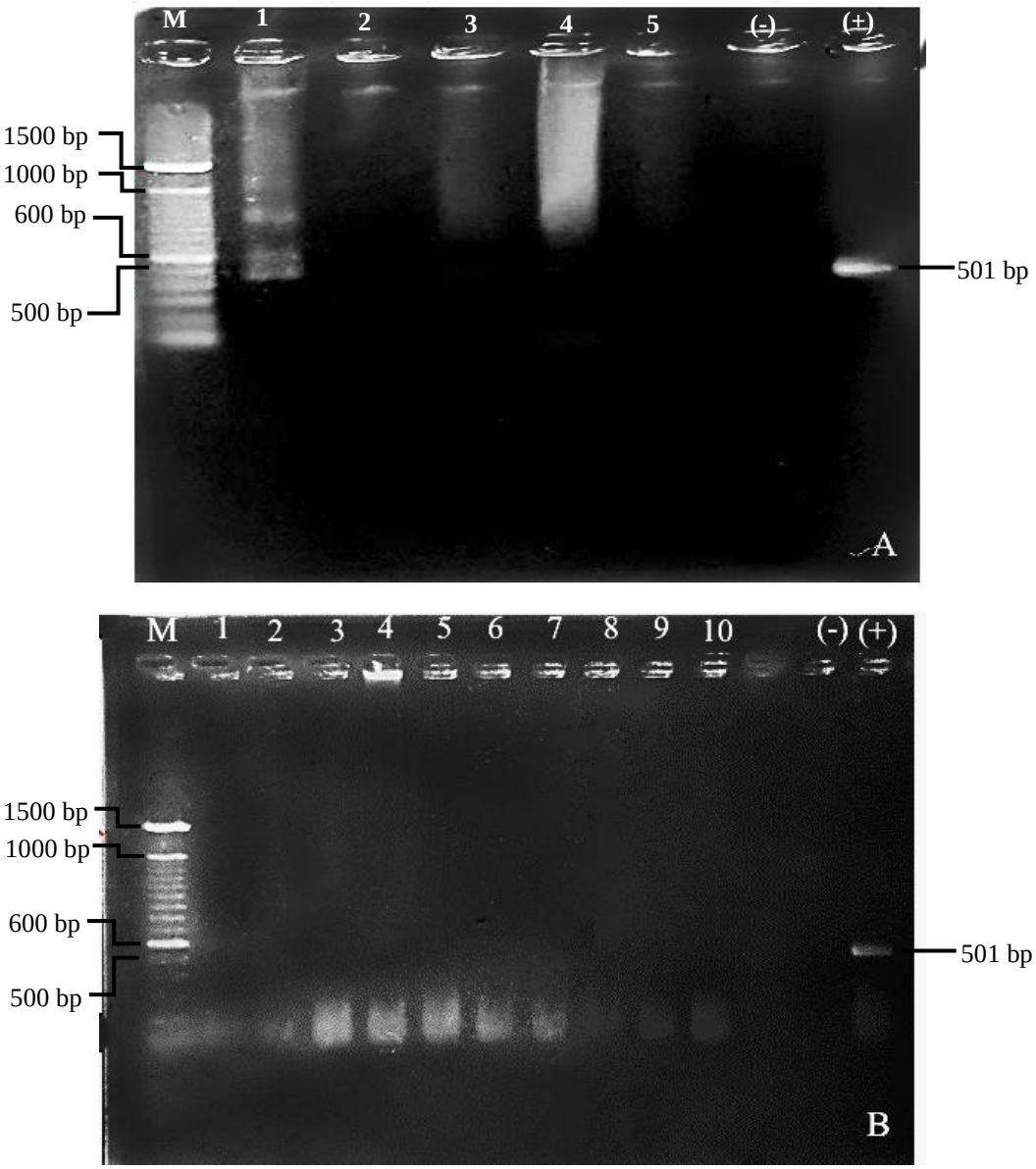

Figure 1. The result of first-round PCR result from the fifth pool (A). 1: Heart, 2: Lung, 3: Spleen, 4: Liver, 5: Kidney and individual extraction; B: 1-10: Individual sample. M: Marker, +: Positive control, *C. burnetii* strain Nine Mile, -: Negative control/ aquabidest

Both the pooling and the individual sample on the first round PCR did not show a fluorescent band at 501 bp (Figure 1). At line 1 on the pooling test (Figure 1A), the fragment wasnot formed at 501 bp (non-specific band) because PCR only used PCR products from external primers (OMP1 and OMP2) that possibly formed a non-specific band. Meanwhile, nPCR used a first-round PCR product that was re-amplified using internal primers (OMP3 and OMP4) so *C. burnetii* DNA could be detected even at low concentrations (Purnawarman et al., 2012). According to Purnawarman et al. (2012), nPCR is adequate to detect the presence of *C. burnetii* DNA with concentrations less than 300 pg, while PCR can only detect the presence of these antigens at concentrations less than 15 ng. Therefore, products from the animal origin containing *C. burnetii* DNA with concentrations less than 300 pg (nPCR) or less than 15 ng (PCR) could not be detected. The use of two pairs of primers (OMP1-OMP2 and OMP3-OMP4) is 50 times more sensitive than one pair of primers (OMP1-OMP2) (Purnawarman et al., 2012). The use of the OMP primer set (OMP1, OMP2, OMP3, and OMP4) has been used by Ho et al. (1995) and Ogawa et al. (2004) as well as other studies for highly conserved amplification of

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the outer layer protein of *C. burnetii*. Zhang et al. (1998) explained that these two pairs of oligonucleotide primers were designed to amplify a 437 bp fragment of the *com1* gene encoding a 27-kDa outer membrane protein of *C. burnetii* that highly conserved among 21 strains of *C. burnetii*.

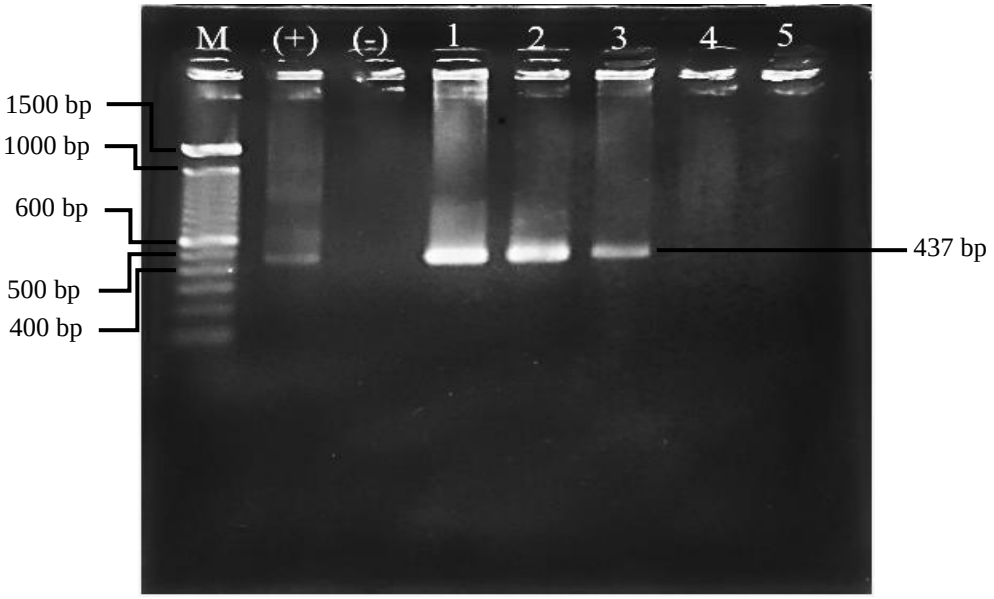

Figure 2. The result of electrophoresis of nPCR from the fifth pool. **M**: Marker; **+** : Positive control, *C. burnetii* strain Nine Mile; **-** : Negative control/ aquabidest; 1: Heart; 2: Lung; 3: Spleen; 4: Liver; 5: Kidney

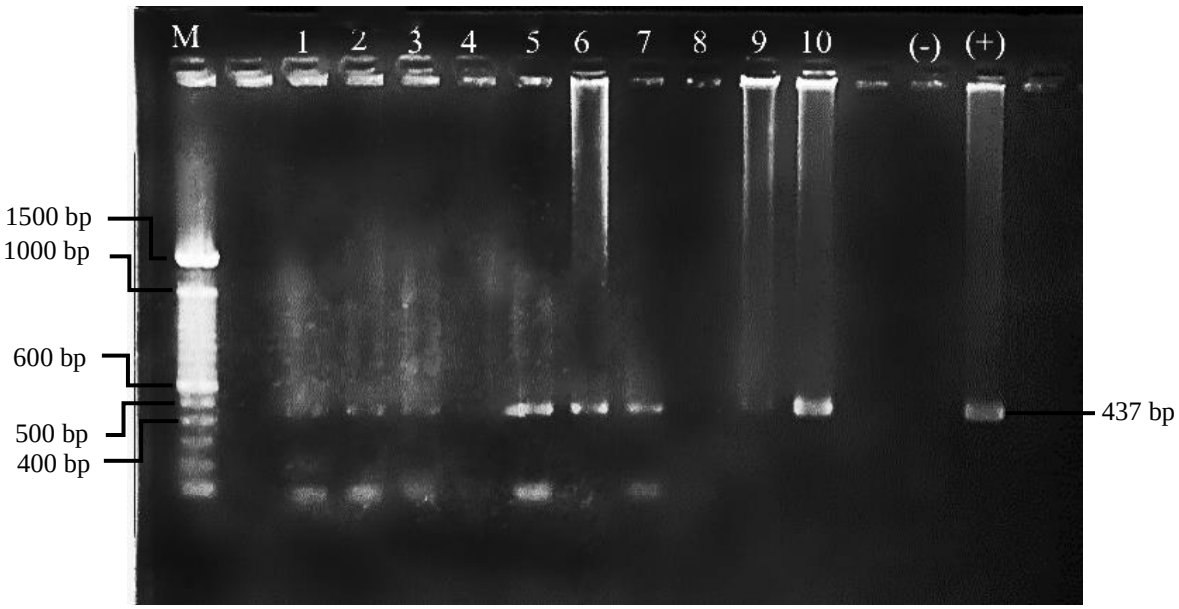

Figure 3. The result of electrophoresis of nPCR from individual extraction (heart organ). **M**: Marker; **+**: Positive control, *C. burnetii* strain Nine Mile; **-**: Negative control/ aquabidest; 1-10: Individual sample

The wide distribution of Coxiellosis around the world has lead to serious health problems both in humans and animals. Query fever, for instance, is highly contagious and can be categorized as a zoonotic emerging infectious disease (CFSPH, 2007; OIE, 2010). Query fever transmission can occur through direct contact with infected animals or contaminated dust particles, and also via vectors (as a vector-borne disease, Setiyono, 2005; CFSPH, 2007). In 2005, Indonesia imported 350,000 cattle from America and nearly 500,000 cattle from Australia (Ariningsih, 2014), where there are still instances of Q fever. Several studies in Indonesia showed that BX cattle found in Indonesia were positive for Q fever (Setiyono, 2005). Research on Balinese and BX cattle indicated 6.12% positive *C. burnetii* DNA using the nested PCR method in Bali and Bogor (Mahatmi et al., 2007). The result of immunohistochemical examination showed that 62/162 (38.3%) samples of BX cattle which were collected from slaughterhouses of Medan City and Deli Serdang Regency were immunoreactive against *C. burnetii* (Nasution et al., 2015).

A total of seven samples were found positively infected with *C. burnetii*. Six out of seven samples were from Simmental cattle and one sample was from Ongole cattle. Based on these results, local breed beef cattle at Boyolali Regency were reported positively infected with Q fever. Beef cattle slaughtered in Ampel slaughterhouse were positively infected with *C. burnetii* using PCR examination (Table 2). The results provoke more expectations about Q fever

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infection in the cattle of other regions in Indonesia. The local breed cattle dominantly raised (Simmental and Ongole) in Boyolali Regency were positive for Q fever, presumably due to the transmission from BX cattle of Bandung which were slaughtered at Ampel slaughterhouse. Contact between BX cattle and local breed cattle may occur during travel or at the shelters. Mahatmi et al. (2007) reported that there were 3 (4.29%) positive Balinese cattle that were infected with *C. burnetii* in Bali. Nugroho et al. (2021) also mentioned that beef cattle in Boyolali Regency were positive for Q fever based on immunohistochemical examinations.

Clinical signs in animals are poorly symptomatic and often characterized by decreased appetite, respiratory distress, and reproductive disorders (Lodrigue et al., 2006). Query fever in ruminants causes abortion in the last third trimester of gestation and other reproduction disorders, such as metritis (Honarmand, 2012; Eldin et al., 2017), leading to economical problems for the breeders. *Coxiella burnetii* can survive for several years in the host without any symptoms. It may be still excreted by animals with subclinical Q fever. There are still many Indonesian people who do not fully understand this disease and society often underestimates the symptoms caused by this disease. This is a particular concern to individuals who come into direct contact with livestock, such as veterinarians, breeders, or slaughterhouse workers, who are at the risk of becoming infected with this disease. The main infection route from animal to human is airborne (Maurin and Raoult, 1999). There are some signs of this disease in humans, such as flu-like symptoms, pneumonia, hepatitis, and nephritis (Maurin and Raoult, 1999; Honarmand, 2012). These signs respond differently to each individual from mild to severe. However, most acute signs are self-limiting but can develop to chronic and cause endocarditis. The mortality rate for Q fever in humans can vary from low to high (Honarmand, 2012; Eldin et al., 2017).

Infection widely spreads through almost all over the body organs, including the lung, heart, and spleen which indicates a systemic infection (Table 2). Lung infection is related to acute Q fever because this organ acts as the early site of the infection. This agent is distributed to the circulation system leading to a heart infection. The spleen is the most important organ on the lymphatic system so that systemic infection of *C. burnetii* causes infection to the spleen. Liver and kidney were negative for *C. burnetii* DNA based on the nPCR test because the infection was not in the chronic stage.

CONCLUSION

Although Query fever (Q fever) as a zoonotic disease is still neglected in Indonesia, it is of utmost importance to detect this disease in Indonesia due to its detrimental effects on both livestock and humans. The obtained results of the present study revealed that the incidence of Q fever has occurred in local beef cattle in Boyolali Regency, Middle Java, Indonesia. It can be strongly suspected that there were 7 out of 100 local breed beef cattle positive of Q fever at Boyolali Regency, Middle Java, Indonesia. Further studies are needed to obtain local isolates of *C. burnetii* so that the mapping of Q fever in Indonesia can be carried out optimally.

DECLARATION

Acknowledgment

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Ethical approval

In the current study, no ethical approval was required. The procedures were non-invasive and did not involve living animals. The samples included organs of cattle that were slaughtered at the Ampel Slaughterhouse. Slaughter was carried out by a certified butcher under the supervision of an official veterinarian.

Competing interests

All the authors declare no competing financial or personal interest

Authors' contribution

Eko Prasetyo Nugroho, Prof. Agus Setiyono, Prof. Upik Kesumawati Hadi, Dr. Wiwin Winarsih, and Dr. Dwi Astuti were the authors who have contributed to the preparation of this paper. Prof Agus Setiyono was the head of the research team who has been researching zoonotic Q fever for a long time. He was responsible for the ongoing research from the beginning until this paper can be made. Eko Prasetyo Nugroho conceptualized research ideas and implemented all laboratory activities. Prof. Upik Kesumawati Hadi, Dr. Wiwin Winarsih, and Dr. Dwi Astuti were members of the supervisory committee who provided many inputs on optimizing research methods and writing this paper. All the authors read and approved the final paper manuscript.

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 before the submission.

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REFERENCES

Centers for Disease Control and Prevention (CDC) (2019). Q fever. Georgia USA : National Center for Infectious Diseases, Division of Viral and Rickettsial Diseases, Viral and Rickettsial Zoonoses Branch, Atlanta. Available at: https://www.cdc.gov/qfever/index.html

Center for Food Security and Public Health (CFSPH) (2007). Q fever. Ames Iowa (US): Iowa State University College of Veterinary Medicine, pp. 1-6. Available at: https://www.cfsph.iastate.edu/Factsheets/pdfs/q_fever.pdf

Ariningsih E **(**2014). Performance of national beef self-sufficiency policy. Forum Penelitian Agro Ekonomi, 32(2): 137-156. DOI: http://www.doi.org/10.21082/fae.v32n2.2014.137-156

Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, Mege JL, Maurin M, and Raoult D **(**2017). From Q fever to *Coxiella burnetii* infection: A paragigm change. Clinical Microbiology Review, American Society for Microbiology, 30(1): 116-190. DOI: http://www.doi.org/10.1128/CMR.00045-16

Ergas D, Keysari A, Edelstein V, and Sthoeger ZM **(**2006). Acute Q fever in Israel: Clinical and laboratory study of 100 hospitalized patients. Israel Medical Association Journal, 8: 337-341. Available at: https://pubmed.ncbi.nlm.nih.gov/16805234/

Fournier PE, Thomas JM, and Raoult D (1998). Minireview: Diagnosis of Q fever. Journal of Clinical Microbiology, 36(7): 1823-1834. DOI: http://www.doi.org/10.1128/JCM.36.7.1823-1834.1998

Hermansyah H, Sutami N, and Miksusanti (2018). Amplifikasi PCR domain D1/D2 28S rDNA menggunakan primer ITS1 dan ITS4 sampel DNA dari *Candida tropicalis* yang diisolasi dengan metode pendinginan. Indonesian Journal of Pure and Applied Chemisty, 1(1): 1-9. DOI: http://www.doi.org/10.26418/indonesian.v1i1.26037

Ho T, Htwe KK, Yamasaki N, Zhang GQ, Ogawa M, Yamaguchi T, Fukushi H, and Hirai K **(**1995). Isolation of *Coxiella burnetii* from dairy cattle and ticks, and some characteristics of the isolates in Japan. Microbiology and Immunology, 39(9): 663-671. DOI: http://www.doi.org/10.1111/j.1348-0421.1995.tb03254.x

Honarmand H **(**2012). Q fever: An old but still a poorly understood disease. Hindawi publishing corporation, Interdisciplinary Perspectives on Infectious Disease, 4: 1-8. DOI: http://www.doi.org/10.1155/2012/131932

Lodrigue KER, Zhang GQ, McMurry DN, and Samuel JE (2006). Clinical and pathological change in a guinea pig aerosol challenge model of acute Q fever. Infection and Immunity, 6: 6085-6091. DOI: http://www.doi.org/10.1128/IAI.00763-06

Mahatmi H, Setiyono A, Damayanti RS, and Pasaribu FH (2007). Deteksi *Coxiella burnetii* penyebab Q fever pada sapi, domba dan kambing di Bogor dan Bali. Jurnal Veteriner, 8(4): 180-187. Available at: https://ojs.unud.ac.id/index.php/jvet/article/view/3213

Maurin M, and Raoult D (1999). Q fever. Clinical Microbiology Reviews, 12(4): 518-533. DOI: http://www.doi.org/10.1128/CMR.12.4.518

Nasution SS, Setiyono A, and Handharyani E (2015). Deteksi imunohistokimia antigen *Coxiella burnetii* sebagai penyebab Q fever pada sapi. Jurnal Kedokteran Hewan, 9(2): 147-151. DOI: http://www.doi.org/10.21157/j.ked.hewan.v9i2.2835

Nugroho EP, Setiyono A, Hadi UK, Winarsih W, and Astuti D (2021). Immunohistochemical detection of *Coxiella burnetii* in cattle spleen organ from Ampel Slaughterhouse, Boyolali Regency. Jurnal Medik Veteriner, 4(1): 1-8. DOI: http://www.doi.org/10.20473/jmv.vol4.iss1.2021.48-55

Office International des Epizootics (OIE) (2010). Q fever. Available at: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.16_Q_FEVER.pdf

Office International des Epizootics (OIE) (2017). Q fever. Available at: https://www.oie.int/en/disease/q-fever/

Ogawa M, Setiyono A, Sato K, Cai Y, Shiga S, and Kishimoto T (2004). Evaluation of PCR assays currently used for detection of *Coxiella burnetii* in Japan. The Southeast Asian Journal Tropical Medicine and Public Health, 35(4): 151-154. Available at: https://www.tm.mahidol.ac.th/seameo/2004_35_4/14-3322.pdf

Purnawarman T, Wibawan IWT, Pasaribu FH, Setiyono A, and Saepulloh M (2012). Sensitivitas dan Spesifisitas Nested Polymerase Chain Reaction untuk mendeteksi DNA *Coxiella burnetii*. Jurnal Veteriner, 13(1): 51-56. Available at: https://ojs.unud.ac.id/index.php/jvet/article/view/2138

Rini EP, Setiyono A, Wibawan IWT, Juniantito V, and Astuti D (2019). Detection of *Coxiella burnetii* infection in various organs from beef cattle in Depok, West Java, Indonesia. Advances in Health Sciences Research, 19: 67-70. DOI: http://www.doi.org/10.2991/isessah-19.2019.19

Setiyono A (2005). Q fever ditinjau dari aspek zoonosis (review on zoonosis of Q fever). Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor, Indonesia, 12-13 September. Available at: https://docplayer.info/40841270-Q-fever-ditinjau-dari-aspek-zoonosis.html

Setiyono A (2014). Cellular pathogenesis of Query fever in cattle. Global Veterinaria, 13(5): 668-671. DOI: http://www.doi.org/10.5829/idosi.gv.2014.13.05.85239

Setiyono A, Handaryani E, and Mahatmi H (2008). Seroprevalensi Q fever pada domba dan kambing di wilayah Jawa Barat. Jurnal Ilmu Ternak dan Veteriner, 13(1): 61-66. Available at: http://download.garuda.ristekdikti.go.id/article.php?article=460541&val=7168&title=Seroprevalence%20of%20Q%20fever%20in%20sheep%20and%20goat%20in%20West%20Java%20area

Stein A, Louveau C, Lepidi H, Ricci F, Baylac PB, Davoust B, and Raoult D (2005). Q fever pneumonia: Virulence of *Coxiella burnetii* pathovars in a murine model aerosol infection. Infection and Immunity, 73(4): 2469-2477. DOI: http://www.doi.org/10.1128/IAI.73.4.2469-2477.2005

Zhang GQ, Nguyen SV, To H, Ogawa M, Hotta A, Yamaguchi T, Kim HJ, Fukushi H, and Hirai K (1998). Clinical evaluation of a new PCR assay for detection of *Coxiella burnetii* in human serum samples. Journal of Clinical Microbiology, 36(1): 77-80. DOI: http://www.doi.org/10.1128/JCM.36.1.77-80.1998

To cite this paper: Nugroho EP, Setiyono A, Hadi UK, Winarsih W, and Astuti D (2021). Detection of *Coxiella burnetii* (query fever) DNA by nested-PCR in beef cattle from Ampel slaughterhouse, Boyolali Regency, middle java, Indonesia. *World Vet. J.*, 11 (2): 267-272.

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The Effect of Lipopolysaccharide Subunit Vaccine of *Brucella abortus* on Montanide ISA 70 Adjuvant on Sheep

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ABSTRACT

Brucellosis is one of the most important zoonotic diseases in the entire world. This disease results in serious economic loss and public health problems. The disease is caused by gram-negative bacteria of the genus *Brucella*. There is a need to perform control programs, such as conducting a vaccination program on livestock. One of the vaccine components that can be used is *B. abortus* lipopolysaccharide. The present study aimed to find out the effect of *B. abortus* lipopolysaccharide subunit vaccine in adjuvant Montanide ISA 70 against antibody titer and interferon-gamma (IFN- γ) level by administrating different doses and different post-vaccination sampling times. *B. abortus* lipopolysaccharide was used in the current study as an antigen and Montanide ISA 70 as an adjuvant. The samples were divided into three groups, each containing six sheep. In the control group (P0), the samples received no treatment. In the first treatment group (P1), the samples were subjected to the injection of *B. abortus* lipopolysaccharide subunit in Montanide ISA 70 adjuvant of 50 mg/ml. Regarding the second treatment group (P2), the samples had an injection of *B. abortus* lipopolysaccharide subunit vaccine in 100 mg/ml. The results showed that the administration of *B. abortus* lipopolysaccharide subunit vaccine in the Montanide ISA 70 adjuvant could influence the formation of antibodies and IFN-γ secretion on sheep. The administration of a dose of 100 µg/ml indicated a greater antibody titer, compared to the dose of 50 µg/ml. The administration of the vaccine at a dose of 50 µg/ml revealed a greater IFN-γ level value in comparison with the dose of 100 µg/ml. The result of the study on IFN-γ level indicated the control group had a greater IFN-γ level rather than the treatment group. In Conclusion, The administration of *B. abortus* lipopolysaccharide subunit vaccine in Montanide ISA 70 adjuvant could influence the formation of IFN-γ antibody and secretion on sheep.

Keywords: *B. abortus*, Lipopolysaccharide, Montanide ISA 70 Adjuvant, Vaccine

INTRODUCTION

Brucellosis is known as one of the most significant zoonotic diseases in the entire world since it leads to critical economic and public health problems. Brucellosis which is caused by gram-negative bacteria of the genus *Brucella* can cause chronic infection both in animals and humans (Xavier et al., 2014). Brucellosis in Indonesia is considered an infectious reproductive disease in livestock. Animals infected with *Brucella* may experience abortion, retained placenta, orchitis, epididymitis which may excrete germs into the uterus and milk. The economic loss caused by this disease reaches 138.5 billion rupiah per year due to miscarriage rates, infertility, premature death, weak calves, and decreased milk production (Sari et al., 2014).

Although Brucellosis has no effective treatment, vaccination of young cows can be an efficient way to control the disease. The three most common vaccines include the attenuated strain 19 vaccines (S19), the adjuvant vaccine 45/20, and the RB51 vaccine (Davis and Elzer, 2002). In Indonesia, the government approved the *B. abortus* RB51 vaccine to control brucellosis in cows. Nowadays, the RB51 vaccine is used instead of the S19 vaccine, since it may lead to some problems, including latent infection and prolonged antibodies. These issues can interfere with the serological diagnosis of Brucellosis. The protection caused by the RB51 vaccine is the same as the S19 vaccine leading to infection since it is an active vaccine. Therefore, there is an urgent need to develop an effective and safe vaccine to control the disease.

The lipopolysaccharide (LPS) subunit is a fragment used in developing the *B. abortus* vaccine. Lipopolysaccharide is the largest surface antigen found in gram-negative bacteria. Therefore it is the basis for the serological classification of various bacterial species and can be useful for the diagnosis of the disease. Moreover, lipopolysaccharides not only play an important role in stabilizing the outer membrane and the biological activity of the most outer membrane protein but also function as receptors for various bacteriophages (Ratnasari et al., 2018). Gram-negative bacterial lipopolysaccharide is the potential antigen that can directly induce the humoral-specific immune response, namely B lymphocyte cells so that they stimulate antibody formation more quickly. An antigen is a substance that can be recognized and bound

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properly by the immune system. The part of the antigen that binds directly to the molecular receptor is known as the antigen determinant or an epitope (Rantam, 2003).

The adjuvant selection also greatly influences the immune response caused during the vaccine. One of the adjuvants that can be used is Montanide ISA 70. Montanide ISA 70 Adjuvant is a water-in-oil adjuvant consisted of oil and a very fine emulgator from the mannide monooleate group. The employed formulation for Montanide induces a long-term and strong immunity, which makes it more stable and easier for injection, compared with the traditional oil emulsion. Furthermore, Montanide has a high immunopotentiation and low side effects (Tehrani et al., 2014) which are the significant factors in the preparation of effective and safe vaccines to manage the disease in livestock. The immunization of animals with a high-quality vaccine is the main control suggestion for different animal diseases. There are some national reports showing that vaccine administration could successfully control and eradicate the disease. The use of Montanide ISA 70 is an alternative choice (Tehrani et al., 2014). This study was aimed to determine the effect of the lipopolysaccharide subunit vaccine in Montanide ISA 70 adjuvant to the antibody titers and interferon-gamma (IFN-γ) level of the sheep.

MATERIALS AND METHODS

Ethical approval

All process of the present experiment was conducted in Department of Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia and the process was ethically approved by the committee of Universitas Airlangga in terms of animal welfare and ethics.

The present study was an experimental laboratory study in which the effects of adjuvant Montanide ISA 70 and LPS of *B. abortus* were investigated. Montanide ISA 70 Adjuvant could affect the efficiency of *B. abortus* LPS as an antigen. The current study was conducted at a sheep farm owned by PT. Agro Great Indoberkah Probolinggo, and at the Virology Laboratory Department of Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

The samples in the current study were divided into three treatment groups, namely treatment group (P1), treatment group (P2), and finally the control group (P0). Before starting the treatment, the sheep were divided and put randomly into different cages, each containing six male sheep approved by the veterinary assistant for healthy status before the study. The P0 was the control group that received no treatment entailed six sheep aged two years. P1 referred to the group involved six sheep aged two years treated by giving *B. abortus* LPS injection in Montanide ISA 70 adjuvant at a dose of 50 µg/ml subcutaneously. Finally, P2 was for the group with six sheep aged two years treated by giving *B. abortus* LPS injection in Montanide ISA 70 adjuvant at a dose of 100 µg/ml subcutaneously.

Subunit vaccine formulation

In the present study, the LPS *B. abortus* subunit vaccine was administered in the Montanide ISA 70 adjuvant with the dosages of 50 µg/ml and 100 µg/ml. The vaccine was made by adding Montanide ISA 70 adjuvant into LPS *B. abortus* with a proportion of 3:7 meaning that each ml of the subunit vaccine contained 0.3 ml of LPS *Brucella abortus* and 0.7 ml of Montanide ISA 70 adjuvant (Tehrani et al., 2014).

Raising experimental animals

The samples of 18 male sheep (Ovis aries) used in this study were divided into three treatment groups, i.e. P1, P2, and P0 (as control). To begin with, the sheep were divided into three cages where each cage entailed six sheep. In treatment 1, the samples were subjected to the injection of LPS *B. abortus* with Montanide ISA 70 Adjuvant with a dose of 50µl/ml subcutaneously. On the other hand, in treatment 2 (Sari et al., 2014), the samples were injected with LPS *B. abortus* in Montanide ISA 70 adjuvant as much as 100µl/ml subcutaneously. The sheep in the control group received no treatment. It should be noted that the sheep were kept in wooden stage cages. Feeding and drinking followed the norms of "Preferences for sheep or goats in Indonesia" (Budisatria et al., 2010).

Sampling technique

Sampling was carried out in two turns; the first turn was performed on August 29, 2016, and the second turn was run on September 12, 2016. A total of 18 samples were selected for blood sampling by taking blood from the jugular vein using a 3-5 ml syringe.

Serum separation

To procure the blood serum, the collected blood was either put into a tube and centrifuged at a speed of 3000 rpm for 20 minutes or was left aside so that the blood serum became separated and came to the surface of the tube.

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Antibody titer examination with indirect ELISA technique

The present study aimed to find out whether there was an antibody formed by the LPS *B. abortus* vaccine employing a serological test using the Indirect-ELISA test technique. The principle of the Indirect-ELISA test was antigen + antibody + antibody labeled enzyme. The Indirect-ELISA involved placing 100 µl of LPS antigen from *B. abortus* weighing 1.25 µg/ml into a microplate and incubating at 37°C for 18 hours. Each plate hole was washed three times with a washing buffer of 200µl per plate. In the next step, 200 µl of 4% buffer blocking was added to each plate hole, and then incubated at 37˚C for 1 hour, washed again by placing the washing buffer of 200 µl into each plate three times. To each standard antibody, the antibodies were added (1: 100 diluted), and 100 µl was placed into the microplate hole and incubated at 37˚C for 2 hours followed by the addition of 100 µl anti-sheep antibodies labeled with the Alkaline Phosphatase enzyme in each plate hole, and incubated at 37˚C for 1 hour. In the next step, the p-PNP substrate was added and incubated in a dark place at room temperature for 15-30 minutes, then 50 µl of NaOH 3n stopper solution was added and a microplate reading was performed on the ELISA reader with a wavelength of 405 nm (Pangestika and Ernawati, 2018).

IFN-γ level examination with direct sandwich-ELISA

In the current study, a serological test was employed using the Direct Sandwich-ELISA technique in order to find out the presence of IFN-γ expression caused by LPS *B. abortus* (Prescott et al., 2002). The examination was carried out using the ELISA kit (Legend maxTM). With regard to the principle of the Direct Sandwich-ELISA Test, the first specific antibody was placed in the solid phase, then the added antigen was examined, and followed by the addition of the second antibody labeled as the enzyme. The Direct Sandwich-ELISA Test prepared a 96 well microplate that has been plotted with a monoclonal antibody against IFN-γ. Then, it was washed with a 400µl washing buffer twice. After that, 100µl of IFN-γ standard solution was added to each plate hole, so that the concentration of each was 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml, 15 6 pg/ml, and 7.8 pg/ml. Similarly, each plate hole was filled with 50µl of standard serum, and 50µl of Biotin-Conjugate were put in microwell and incubated for 2 hours at a temperature of 18-25˚C. After washing the microwell with washing buffer and drying it, 100µl of Sterptividin-HRP was added to all microwells and incubated for an hour at a temperature of 18-25˚C. Again after washing with washing buffer three times and adding 100µl of TMB substrate (3,3′,5,5′-Tetramethylbenzidine) to all plate holes, they were incubated at a temperature of 18-25˚C for 10 minutes. Then, a 100µl of stopper solution was added to each plate hole, and the performance was read by ELISA reader with a wavelength of 450nm.

Data analysis

Data of antibody titer and IFN-γ level were statistically analyzed using the Analysis of Variance (ANOVA) test. P-value less than 0.05 ($p < 0.05$) was considered statistically significant. Honest Significant Difference test (Tukey) was run using SPSS software, ver.21 (Statistical Product and Service Solution, 21).

RESULTS

Examination and analysis of antibody titer data of the sheep by indirect ELISA test

As mentioned earlier, the obtained data related to the sheep's antibody titer were statistically analyzed using ANOVA. In case of any significant difference ($P < 0.05$), the Honest Significant Difference (Tukey) test was employed using SPSS software, version 21. Based on the result of the examination using the indirect ELISA technique, the data of antibody titer were listed in Table 1, while the difference in antibody titer in the second-week post-vaccination and the fourth-week post-vaccination could be seen in Figure 1.

The result of the ANOVA test showed that there were significant differences in each treatment ($p < 0.05$). Therefore, data was analyzed again using the Honestly Significant Difference test (HSD test). Based on p-value ($p < 0.05$), the treatment group P0 (nothing is given) was significantly different from the P1 treatment group (50 µg/ml given) and P2 (100µg/ml given), but there was no significant variance between P1 treatment group (50 µg/ml) and P2 treatment group (100 µg/ml). The comparison test between the antibody titer in the second-week post-vaccination and the antibody titer in the fourth-week post-vaccination showed no substantial difference ($p = 0.304$).

The average antibody titer in the P0 group in the second and fourth weeks neither increased nor decreased. However, the average antibody titer was increased in the P1 treatment group (50 µg/ml) and P2 (100 µg/ml) in the fourth week, compared to the antibody titer in the second week.

Examination and analysis of IFN-γ level data with ELISA direct sandwich test

The obtained data on the IFN-γ level of sheep were analyzed statistically using ANOVA. P value less than 0.05 was considered statistically significant. Honestly Significant Difference test was also employed using SPSS software, version 21. The examination of the Interferon-gamma level was aimed to find out the role of IFN-γ in inducing the

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cellular immune system. Based on the results of the ELISA reader, the data listed in Table 2 were obtained, while the post-vaccination differences in the second week and the fourth week could be seen in Figure 2.

The average level of the IFN-γ was determined in each treatment group, and it showed a significant difference among treatments ($p < 0.05$). There was a noteworthy difference among treatment groups followed by the HST test as a multiple comparison test. The comparison test between P0 and P1 showed a significant difference ($p = 0.034$). In addition, there was a substantial difference in the comparison test between P0 and P2 ($p = 0.005$). However, the comparison test of P1 with P2 did not reveal any significant difference ($p = 0.716$). The comparison between IFN-γ level in the second-week post-vaccination and IFN-γ level in the fourth-week post-vaccination was not prominently different ($p = 0.220$).

The average of IFN-γ level in the control group (P0) in the second and the fourth weeks post-vaccination showed a stable trend. In the P1 (50 µg/ml) and P2 (100 µg/ml) groups, the sampling of IFN-γ level in the fourth-week post-vaccination experienced a decrease, compared to the sampling of IFN-γ level in the second-week post-vaccination.

Table 1. Average and standard deviation of sheep antibody titer for the not-given-treatment group (P0) and the given-treatment groups (P1, P2) in the second and fourth weeks of post-vaccination.

Treatments	Antibody Titer (X±SD)	
	2nd Week	4th Week
P0	0,000[a] ± 0,000*	0,000[a] ± 0,000*
P1	0,350[b] ± 0,437*	0,883[b] ± 1,183*
P2	0,800[b] ± 1,232*	0,966[b] ± 1,148*
Average	0,383[a] ± 0,785**	0,616[a] ± 1,001**

* different superscripts in the same column, showing significant differences ($p < 0.05$); ** different superscripts on the same row, showing significant differences. P0: The control group which received no treatment entailed six sheep aged two years, P1: The group involved six sheep aged two years treated by giving B. abortus LPS injection in Montanide ISA 70 adjuvant at a dose of 50 µg/ml subcutaneously, P2: The group included six sheep aged two years treated by giving B. abortus LPS injection in Montanide ISA 70 adjuvant at a dose of 100 µg/ml subcutaneously.

Table 2. Average and standard deviation of IFN-γ level of sheep of a not-given-treatment group (P0) and given-treatment groups (P1, P2) in the second and fourth weeks of post-vaccination.

Treatments	Titer antibody (X±SD)	
	2nd Week	4th Week
P0	5.267[a] ± 0.911*	5.392 [a]± 0.094*
P1	4.998[b] ± 0.776*	4.468 [b]± 0.313*
P2	4.701[b] ± 0.490*	4.410[b] ± 0.257*
Average	4.988 ± 0.741**	4.872 ± 0.639**

* different superscripts in the same column, showing significant differences ($p < 0.05$); ** different superscripts on the same row, showing significant differences ($p < 0.05$); P0: The control group which received no treatment entailed six sheep aged two years, P1: The group involved six sheep aged two years treated by giving B. abortus LPS injection in Montanide ISA 70 adjuvant at a dose of 50 µg/ml subcutaneously, P2: The group included six sheep aged two years treated by giving B. abortus LPS injection in Montanide ISA 70 adjuvant at a dose of 100 µg/ml subcutaneously.

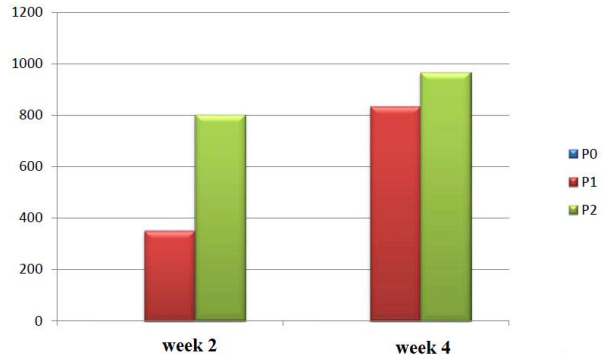

Figure 1. Average graph of sheep antibody titer for a not-given-treatment group (P0) and given-treatment groups (P1, P2) at the second and fourth weeks of post-vaccination

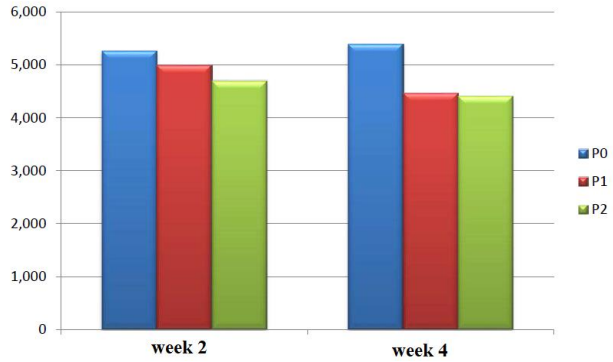

Figure 2. Average graph of IFN-γ level of sheep of the not-given-treatment group (P0) and given-treatment groups (P1, P2) in the second and fourth weeks of post-vaccination

DISCUSSION

The administration of *B. abortus* lipopolysaccharide subunit in the Montanide ISA 70 adjuvant at doses of 50 µg/ml and 100 µg/ml in the post-vaccination sampling at the second week showed an increase in titer values of 0.350 and 0.833, respectively, compared to the control group's titer. Animal antibodies provided by *B. abortus* vaccine were detected in the second-week post-vaccination, and it peaked in the sixth-week post-vaccination (Sudibyo, 1995). After the sixth week, the formed antibodies started to decrease. Other studies stated that no antibody responses during the fourth week after the inoculation, and there was only a sharp increase at the sixth week after the inoculation (Praja et al., 2017). The intensity of the humoral immune response could be demonstrated by the serum antibody level (Pooley et al., 2019).

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When animals or humans are exposed to an antigen, a premier immune response characterized by the appearance of IgM occurs a few days after the exposure, leading to an increase in the serum antibody level. The IgM level peaks on the seventh day (Sathe and Cusick, 2021). After the sixth and seventh days, IgG is detected in the serum, whereas IgM begins to decrease before IgG level peaked 10-14 days after the exposure (Pajuaba et al., 2010) The antibody level continues to decrease, and only is slightly detected after four to five weeks.

The administration of *B. abortus* lipopolysaccharide subunit in Montanide ISA 70 adjuvant at the doses of 50 µg/ml and 100 µg/ml in post-vaccination sampling at the second week showed a reduction in IFN-γ level of 4.998 and 4.701, respectively, compared to 5.267 value of IFN-γ for the control group. Moreover, IFN-γ level value of animals that did not receive any treatment showed lower values than those animals that received treatments (Segura et al., 2007). Many factors that affected the antibody response were produced in vivo, including the composition of conjugation, carrier protein, immunization route, adjuvant, animal species, and detection method (Davar et al., 2015). At high doses, interferon could reduce cellular and humoral immune responses. At low doses, interferon-stimulated the immune system by increasing the activity of Natural Killing cells (NK cells), macrophage, T cells, and by regulating antibody production (Baratawidjaja, 2006). Regarding time, the value of IFN-γ level in the sampling of the second-week post-vaccination was higher than the value of IFN-γ level in the sampling of the fourth-week post-vaccination. The result showed that IFN-γ level reached the optimal level in the second-week post-vaccination. Based on the statistical test, the obtained result was indicative of 0.220 ($P > 0.05$). Consequently, it could be inferred that the time factor did not have any significant effect on the IFN-γ level of the sheep which received the *Brucella abortus* lipopolysaccharide subunit vaccine.

Davar et al. (2015) mentioned that IFN-γ level peaked seven days after the vaccination, the IFN-γ level continued to decrease after seven days, and very little IFN-γ was detected 180 days after the vaccination. The IFN-γ was a major cytokine for macrophage activating cytokine, and played a major role in the non-specific and cellular-specific immunity. IFN-γ is believed to be a cytokine that activated macrophages to kill phagocytes. IFN-γ stimulates the expression of the Major Histocompatibility Complex (MHC-I), Major Histocompatibility Complex Class 2 (MHC-II), and Antigen-Presenting Cells (APC) costimulator. IFN-γ increases CD4 cell differentiation to the Thiamine biosynthetic bifunctional enzyme (Th1) subcells, and prevents Th2 cell proliferation. IFN-γ acts against B cells in the transfer of IgG subclasses that bound Fcγ-R to phagocytes. and activates the complement. Both of these processes increase phagocytosis of the microbes operationalized (Baratawidjaja, 2006).

The adaptive or specific immune response was slower, because it needed antigen sensitivity, but has better protection against the same antigen. This immune system was run by B lymphocytes and T lymphocytes derived from lymphoid progenitor cells. T lymphocyte cells were responsible for the production of cytokines and cytotoxicity (cellular immunity), and B lymphocytes produced antibodies (humoral immunity; Skendros and Boura, 2013). The IFN-γ could divert Ig that participated in the microbial elimination. IFN-γ activated neutrophils and stimulated the cytolytic effect of NK cells (Baratawidjaja, 2006).

Adjuvant has an important role in the success of vaccination helping to form antibody responses. The use of adjuvant should be adapted to several criteria, namely target species, antigen, type of immune response, inoculation route, and immunity duration (Aucouturier et al., 2001). Montanide was a water-in-oil adjuvant consisting of oil and a very fine emulgator from the mannide monooleate group. Formulation using Montanide adjuvant-induced a long-term and strong immunity. Compared to the traditional oil emulsion, Montanide was more stable and easier to be injected. In addition, Montanide also has a higher immunopotentiation and fewer side effects (Tehrani et al., 2014). The addition of an adjuvant to the vaccine could increase the immunogenicity of the vaccine. Adjuvant was an ingredient mixed into a vaccine to enhance the immune response, both humoral and cellular immunity (Murphy et al., 1999).

CONCLUSION

The administration of *B. abortus* lipopolysaccharide subunit in the Montanide ISA 70 adjuvant can influence the formation of antibodies and IFN-γ secretion on the sheep. The difference in dose given during the vaccination influenced the formation of antibodies and IFN-γ secretion. Administration of 100 µg/ml produced antibody titer value greater than the dose of 50 µg/ml and injecting the dose of 50 µg/ml produced an IFN-γ level greater than the dose of 100 µg/ml. There were significant differences regarding antibody titer and IFN-γ level in the second-and fourth-week post-vaccination.

DECLARATION

Author's contribution

All authors have similar roles in conducting, writing, and editing the manuscript.

Conflict of Interests

The authors have not declared any conflict of interest in this work.

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REFERENCES

Aucouturier J, Dupuis L, and Ganne V (2001). Adjuvants designed for veterinary and human vaccines. Vaccine, 19: 2666-2672. DOI: https://www.doi.org/10.1016/s0264-410x(00)00498-9

Baratawidjaja K (2006). Imunologi Dasar Yogyakarta: Balai Penerbit Fakultas Kedokteran Universitas Airlangga, Ailangaa, pp. 30-47. Available at: http://lib.ui.ac.id/detail?id=20417471

Budisatria I, Udo H, Eilers C, Baliarti E, and Zijpp A (2010). Preferences for sheep or goats in Indonesia. Small Ruminant Research, 88: 16-22. DOI: https://www.doi.org/10.1016/J.SMALLRUMRES.2009.11.002

Davar S, Vaziri F, and Eftekhary M (2015). Preparation and evaluation of a new lipopolysaccharide-based conjugate as a vaccine candidate for brucellosis. Osong Public Health and Research Perspectives, 6(1): 9-13. DOI: http://www.dx.doi.org/10.1016/j.phrp.2014.10.012

Davis DS, and Elzer PH (2002). *Brucella* vaccines in wildlife. Veterinary Microbiology, 90: 533-544. DOI: https://www.doi.org/10.1016/s0378-1135(02)00233-x

Murphy F, Gibbs E, Horzinek M, and Michael S (1999). Veterinary virology third edition. Elsevier Academic Press, United States of America. pp. 170-181. Available at: https://www.elsevier.com/books/veterinary-virology/murphy/978-0-12-511340-3

Pajuaba AC, Silva DA, and Mineo JR (2010). Evaluation of indirect enzyme-linked immunosorbent assays and IgG avidity assays using a protein A-peroxidase conjugate for the serological distinction between *Brucella abortus* S19-vaccinated and -infected cows. Clinical and Vaccine Immunology, 17(4): 588-595. DOI: https://www.doi.org/10.1128/CVI.00444-09

Pangestika R, and Ernawati R (2018). Antiviral activity effect of silver nanoparticles (agnps) solution against the growth of infectious bursal disease virus on embryonated chicken eggs with Elisa Test. KnE Life Sciences, 3(6): 536-548. DOI: http://www.dx.doi.org/10.18502/kls.v3i6.1181

Pooley HB, Begg DJ, Plain KM, Whittington RJ, Purdie AC, and de Silva K (2019). The humoral immune response is essential for successful vaccine protection against paratuberculosis in sheep. BMC Veterinary Research, 15(1): Article number 223. DOI: https://www.doi.org/10.1186/s12917-019-1972-z

Praja RN, Handijatno D, Koesdarto S, Yudhana A, Mikrobiologi D, and Helmintologi L (2017). Characterization of virb4 protein from local isolate of *Brucella abortus* using sodium dodecyl sulfate polyacrylamide gel electrophoresis technique. Jurnal Kedokteran Hewan, 18(36): 416-421. DOI: https://www.doi.org/10.21157/j.ked.hewan.v12i1.8091

Prescott JF, Menzies PI, and Hwang YT (2002). An interferon-gamma assay for diagnosis of Corynebacterium pseudotuberculosis infection in adult sheep from a research flock. Veterinary Microbiology, 88(3): 287-297. DOI: https://www.doi.org/10.1016/s0378-1135(02)00121-9

Rantam FA (2003). Metode imunologi. Surabaya: Airlangga University Press, Airlangaa.

Ratnasari R, Handijatno D, Suwarno, and Rantam FA (2018). Antigenic derterminants omp2a gene of B. abortus local isolate. Acta Veterinaria Indonesia, 2(1): 17-25. Available at: https://journal.ipb.ac.id/index.php/actavetindones/article/download/8784/6858/

Sari RR, Oktorina R, Rofiqul A, and Yuniarti LS (2014). Validasi metode uji mikroskopis dan real time polymerase chain reaction pada bahan baku pakan ternak. pros konf ilm vet nas ke-13, Palembang, Indonesia. (23-26 November 2014). Prosiding Konferensi Ilmiah Veteriner Nasional, 13: 23-26. Available at: http://journal.ipb.ac.id/index.php/hemera/article/download/18650/13157

Sathe A, and Cusick JK (2021). Biochemistry, Immunoglobulin M. StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK555995/

Segura S, Gamazo C, Irache JM, and Espuelas S (2007). Gamma interferon loaded onto albumin nanoparticles : *In vitro* and *in vivo* activities against *Brucella abortus*. Antimicrobial Agents and Chemotherapy, 51(4): 1310-1314. DOI: https://www.doi.org/10.1128/AAC.00890-06

Skendros P, and Boura P (2013). Immunity to brucellosis. Revue Scientifique Et Technique, 32(1): 137-147. DOI: https://www.doi.org/10.20506/rst.32.1.2190

Sudibyo A (1995). The difference of serological responses between naturally infected, experimentally infected, and vaccinated cattle with *Brucella abortus* strain 19 vaccine. Jurnal Ilmu Ternak dan Veteriner, 1(2): 117-122. DOI: http://www.dx.doi.org/10.14334/jitv.v1i2.21

Tehrani NK, Mahdavi M, Imani Fooladi AA, and Tabatabaie F (2014). Survey protein vaccine formulated with montanide ISA 70 effects following immunization and after challenge with Leishmania major. Biosciences Biotechnology Research Asia, 11(1): 53-60. Available at: http://www.biotech-asia.org/vol11no1/survey-protein-vaccine-formulated-with-montanide-isa-70-effects-following-immunization-and-after-challenge-with-leishmania-major/

Xavier NM, Den Hartigh A, Santos LR, Paixao AT, and Tsolis MR (2014). Pathogenesis of *Brucella* spp. The Open Veterinary Science Journal, 4(1): 109-118. DOI: http://www.dx.doi.org/10.2174/1874318801004010109

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$(GTG)_5$-PCR Mediated Molecular Typing of Zoonotic Bacteria

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ABSTRACT

The present review aimed to reveal the role of $(GTG)_5$-PCR microbial typing in indicating the routes and source of infections, investigate the outbreaks and genotypes of clinical strains, as well as finding virulent strains and epidemiology of bacterial isolates. All available and published data in Google scholar, PubMed, ResearchGate, and Science Direct during the past two decades that used the $(GTG)_5$-PCR method for genotyping the bacterial isolates were included in the current study. The findings have indicated that $(GTG)_5$-PCR can be recommended as a possible, cost-effective, fast, and easy tool for molecular typing of bacterial isolates.

Keywords: Zoonotic bacteria, $(GTG)_5$-PCR, Molecular typing

INTRODUCTION

It is of utmost importance to understand clonal relatedness between the microbial strains since it can help to identify the source and routes of infections, examine the outbreaks, detect cross-transmission of healthcare-associated pathogens, distinguish particularly virulent strains and assess the efficacy of control measures (Tenover et al., 1997; MacCannell, 2013; Rafei et al., 2014). Bacterial typing has also helped in the improvement of monitoring programs and provided important data for public health prevention strategies.

The traditional typing methods were based on phenotypic typing while modern molecular methods are based on genome components of bacteria (Bradford, 2018). Antibiogram, biotyping, serotyping, and phage typing are examples of traditional epidemiological typing methods that have sometimes been effective in understanding the epidemiology of infectious diseases. Serotyping, phage typing, and antibiotic resistance patterns could provide useful information for short-term epidemiological studies in pathogens worldwide (Tenover et al., 1997; MacCannell, 2013). However, conventional methods are of various types, which are labor-intensive and time-consuming in epidemiological investigations (MacCannell, 2013). As a result, DNA-based typing methods play a significant role in the analysis of most microbial pathogens' epidemiology.

Numerous epidemiological typing systems have been used, with a diverse armamentarium ranging from non-molecular techniques to more advanced molecular typing technologies. The most widely utilized molecular typing methods include the DNA-based methods, particularly restriction endonuclease analysis of genomic and plasmid DNA, southern hybridization analysis with the use of specific DNA probes, plasmid profiling, chromosomal DNA profiling using either pulse-field gel electrophoresis or polymerase chain reaction (PCR)-based methods (Tenover et al., 1997; MacCannell, 2013). Molecular genetic techniques, such as PCR, nucleic acid fingerprinting, DNA sequence analysis of bacterial chromosomes are important techniques in identifying the prevalence of nosocomial infections, food contamination reservoirs or the release of pathogenic plant strains into the environment, and isolation of specific genotypes in conjugation with a particular bacterium (Ranjbar et al., 2008).

However, the aforementioned methods also have been recognized with some disadvantages, such as the need for special tools, considerable cost, and time-consuming procedure. Regarding this background, the present study aimed to review the importance of using $(GTG)_5$-PCR as a simple and low-cost method for subtyping bacterial isolates.

Sources

All available and published data in Google scholar, PubMed, ResearchGate, and ScienceDirect that used the $(GTG)_5$-PCR method for genotypes of *A. baumannii* isolates were included in the current study.

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Subtyping of bacterial isolates

Genetic variation in the genome of bacteria causes a molecular typing method to determine bacterial strains. For microbial typing of bacteria, many approaches are available; however, each of them has its own merits and demerits that make it useful in some studies and restrictive in others. High discriminatory power, good reproducibility, the simplicity of the method, simple interpretation of the results, and low costs of the method are important issues to choose an approach as a typing method (Li et al., 2009).

$(GTG)_5$-PCR

This method is effective in screening a large number of bacterial strains and it is beneficial for intraspecies differentiation and identification of bacterial genomes. Indeed, $(GTG)_5$-PCR is a type of repetitive extragenic palindromic (rep)-PCR that amplifies the $(GTG)_5$ repetitive element which lays throughout the bacterial genomes (Gevers et al., 2001). This PCR-based method can be used for a broad range of Gram-negative bacteria and a narrow range of Gram-positive bacteria (Gevers et al., 2001; Kathleen et al., 2014).

Acinetobacter baumannii

There are a few studies on molecular typing of *A. baumannii* using the $(GTG)_5$-PCR. Huys et al. (2009) presented that $(GTG)_5$-PCR was a reliable and cost-efficient tool to differentiate members of pan-European multi-resistant *A. baumannii* (MAB) clone III from different MAB clones isolated from the University Hospital of Ghent, Belgium (Huys et al., 2009). In another study, the $(GTG)_5$-PCR-based typing method was used to differentiate *A. baumannii* strains recovered from hospitals of Tehran, Iran. The findings of this research highlighted the high power of typing since more than 95% of the isolates were typable and $(GTG)_5$-PCR differentiated the strains into 13 $(GTG)_5$ clusters (Ranjbar and Babazadeh, 2021).

***Staphylococcus* species**

The highly resistant coagulase-negative staphylococci are important factors of nosocomial diseases and veterinary infections. The identification of staphylococcal species using traditional biochemical tests is not efficient due to the increase in the number of known subspecies. There are many reports for genotypic and phenotypic detection of staphylococci (Braem et al., 2011). A study conducted on 35 type strains and 253 field isolates of *Staphylococcus* species indicated $(GTG)_5$-PCR fingerprinting is a useful method to determine the identity of bovine *Staphylococcus* species. $(GTG)_5$-PCR fingerprinting achieved a typeability of 94.7% and an accuracy of 94.3%, compared to identifications based on gene sequencing in this study (Stepán et al., 2004). Nováková et al. (2010) By using rep-PCR using the (GTG)5 primer could differentiate *Staphylococcus microti* DSM 22147 from viscera of common voles.

***Lactobacilli* species**

Svec et al. (2011) used the $(GTG)_5$-PCR to characterize vaginal *lactobacilli*. It is declared that $(GTG)_5$-PCR is a simple, beneficial, and rapid method for the identification of many vaginal *lactobacilli* isolates and also suggested that this method can be more reliable in combination with other techniques (Svec et al., 2008; Svec et al., 2011).

***Salmonella* species**

Salmonella enterica Serotype 4,[5],12:i:- is an emerging serotype with worldwide distribution and a significant infection rate of humans and domestic animals (Yang et al., 2015). The methods of BOX-A1R-based (BOX)-PCR, repetitive extragenic palindromic (REP)-PCR, enterobacterial repetitive intergenic consensus (ERIC)-PCR, and GTG5-PCR were compared in a study. The findings indicated that both the high average number of amplicons bands and high value of discrimination index suggested BOX-PCR and GTG5-PCR as better molecular typing methods than REP-PCR and ERIC-PCR in their capability to distinguish among closely genetically related *S.* 4,[5], 12:i:- isolates in hospitalized patients and minced pork samples. Also, BOX-PCR and GTG5- PCR generated more clusters for each serotype from the same and different sources (Pooncharoen et al., 2019).

***Klebsiella* species**

Klebsiella pneumoniae is a Gram-negative nosocomial pathogen detected in many infectious diseases, such as bacteremia, pneumonia, urinary tract infections, and in patients with underlying diseases (Shakib et al., 2012; Mancini et al., 2018). The pathogenicity of this organism is notable because of its ability to produce some important enzymes which destroy an extended spectrum of antimicrobial agents (Shakib et al., 2012). In a study, 88 *K. pneumoniae* isolates were subjected to $(GTG)_5$–PCR assay, and the strains were differentiated into 9 clusters which showed the ability of this method for molecular typing of *K. pneumoniae* strains (Ranjbar and Afshar, 2019).

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***Streptococcus* species**

Svec et al. (2008) evaluated the $(GTG)_5$-PCR for the fast screening of bacterial strains isolated from dental plaque of children with early childhood caries. It is proposed that the $(GTG)_5$-PCR fingerprinting is a fast and valid method for the identification of *Streptococcus Mutans*, compared to other used molecular typing methods (Svec et al., 2008).

***Campylobacter* species**

Campylobacteriosis is one of the most common zoonotic diseases derived from the consumption of contaminated poultry meat (Poonchareon et al., 2019). There are few studies that assay the *Campylobacter* species via (GTG) 5–PCR. In one study conducted in Cape Town for eight years, DNA fingerprinting using $(GTG)_5$ was performed to type 100 isolates of *Campylobacter concisus* strains. The findings of a study conducted by Matsheka et al. (2006) provided that homologous lineages of *C. concisus* may belong to a heterogeneous species complex. The mentioned study also confirmed the discriminability and simplicity of $(GTG)_5$-PCR (Matsheka et al., 2006).

Escherichia coli

Extraintestinal *Escherichia coli*, the main cause of colibacillosis in chickens, also cause different infections in humans, such as neonatal meningitis, urinary tract infections, and sepsis (Mellata, 2013). Knowing the abundance of this bacterium in its natural source is very strategical for controlling the occurrence of infectious agents in humans and animals. A study on the feces of many poultry and free-living birds revealed that from 95% to 70.7% of fecal *E. coli* isolates were classified into the correct host source (Mohapatra et al., 2008).

***Enterococcus* species**

Many phenotypic and molecular methods have been described for the identification of enterococci (Domig et al., 2003). The rep-PCR with $(GTG)_5$ primer has also been presented as a reliable method for species identification of all enterococci strains which grouped clearly into well-separated clusters and representing single species (Švec et al., 2005).

***Yersinia* species**

Huang et al. (2013) analyzed the variation of molecular characteristics of *Yersinia ruckeri* isolates collected in northwest Germany and reported that this method was one of the best methods of molecular typing, compared to the methods which could obtain four patterns of *Yersinia ruckeri* isolates. In order to compare the obtained molecular profiles of *Yersinia* enterocolitica, rep-PCR method was suggested using the primer (GTG) 5 (Versalovic et al., 1994). Regarding rep-PCR, the comparison revealed that the contaminated pigs with *Yersinia* enterocolitica could carry the microorganism to different points in the abattoir environment (*Moreira* et al., 2019).

***Vibrio* species**

Ben-Haim et al. (2003) studied the *Vibrio coralliilyticus* species and found that the inner AFLP and GTG5-PCR pattern similarities were higher than 64 %. Balcázar et al. (2010) suggested that rep-PCR fingerprinting technique using (GTG)5-PCR allowed differentiating *V. alginolyticus* and *V. splendidus* in epidemiological analyses, particularly in large studies and critical situations.

Other bacterial species

Kathleen et al. (2014) reported (GTG)5 PCR is useful in the differentiation of unknown bacterial isolates. De Vuyst et al. (2008) found some new *Acetobacter* species and reported (GTG)5-PCR DNA fingerprinting was useful for the identification and classification of acetic acid bacteria to the species level. Acetic acid bacteria Gram-negative, ellipsoidal to rod-shaped, obligate aerobic bacteria that currently classified into 10 genera and 44 species, such as *Acetobacter* and, *Gluconacetobacter* species. It is reported Rep-PCR using $(GTG)_5$ could provide better differentiation of the isolates of *Listeria monocytogenes* than did RAPD PCR and resulted in discrimination of the isolates into a larger number of unique profiles (Hadjilouka et al., 2014).

CONCLUSION

The current study supports the idea that $(GTG)_5$-PCR is a possible, cost-effective, and easy technique for molecular typing of bacterial isolates; however, these data can be compared to more known typing techniques.

DECLARATION

Author`s contribution

Daryoush Babazadeh reviewed the articles and wrote the draft of the manuscript. Reza Ranjbar revised the draft of the manuscript and prepared it for submission. All authors check and confirmed the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Consent to publish

Not applicable.

REFERENCES

Balcázar JL, Gallo-Bueno A, Planas M and Pintado J *(*2010*).* Isolation of *Vibrio alginolyticus* and *Vibrio splendidus* from captive-bred seahorses with disease symptoms. Antonie van Leeuwenhoek, 97: article number 207. DOI: https://doi.org/10.1007/s10482-009-9398-4

Ben-Haim Y, Thompson FL, Thompson CC, Cnockaert MC, Hoste B, Swings J, and Rosenberg E (2003). *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. International Journal of Systematic and Evolutionary Microbiology, 53: 309-315. DOI: https://doi.org/10.1099/ijs.0.02402-0

Bradford PA (2018). Epidemiology of Bacterial Resistance. In, Fong I., Shlaes D., Drlica K. (Eds) Antimicrobial Resistance in the 21st Century. Emerging Infectious Diseases of the 21st Century. Springer, Cham. Available at: https://www.springer.com/gp/book/9783319785370

Braem G, De Vliegher S, Supré K, Haesebrouck F, Leroy F, and De Vuyst L (2011). (GTG) 5-PCR fingerprinting for the classification and identification of coagulase-negative Staphylococcus species from bovine milk and teat apices: a comparison of type strains and field isolates. Veterinary Microbiology, 147: 67-74. DOI: https://www.doi.org/10.1016/j.vetmic.2010.05.044

De Vuyst L, Camu N, De Winter T, Vandemeulebroecke K, Van de Perre V, Vancanneyt M, De Vos P, and Cleenwerck I (2007). Validation of the (GTG) (5)-rep-PCR fingerprinting technique for rapid classification and identification of acetic acid bacteria, with a focus on isolates from Ghanaian fermented cocoa beans. International Journal of Food Microbiology, 125(1):79-90. DOI: https://doi.org/10.1016/j.ijfoodmicro.2007.02.030

Domig KJ, Mayer HK, and Kneifel W (2003). Methods used for the isolation, enumeration, characterization and identification of Enterococcus spp. 2. Pheno- and genotypic criteria. International Journal of Food Microbiology, 88: 165-188. DOI: https://www.doi.org/ 10.1016/s0168-1605(03)00178-8 .

Gevers D, Huys G, and Swings J (2001). Applicability of rep-PCR fingerprinting for identification of Lactobacillus species. FEMS Microbiology Letters, 205: 31-36. DOI: https://www.doi.org/10.1111/j.1574-6968.2001.tb10921.x

Hadjilouka A, Andritsos ND, Paramithiotis S, Mataragas M, and Drosinos EH (2014). Listeria monocytogenes serotype prevalence and biodiversity in diverse food products. Journal of Food Protection, 77(12): 2115-2120. DOI: https://doi.org/10.4315/0362-028X.JFP-14-072

Huang Y, Runge M, Michael GB, Schwarz S, Jung, A and Steinhagen D (2013). Biochemical and molecular heterogeneity among isolates of *Yersinia ruckeri* from rainbow trout (Oncorhynchus mykiss, Walbaum) in north west Germany. BMC Veterinary Research, 9: Article number 215. DOI: https://doi.org/10.1186/1746-6148-9-215

Huys G, Cnockaert M, Nemec A, Dijkshoorn L, Brisse S, Vaneechoutte M, and Swings J (2005). Repetitive-DNA-element PCR fingerprinting and antibiotic resistance of pan-European multi-resistant A. baumannii clone III strains. Journal of Medical Microbiology, 54: 851-856. DOI: https://www.doi.org/10.1099/jmm.0.45986-0

Kathleen MM, Samuel L, Felecia C, Ng KH, Lesley MB, and Kasing A (2014). (GTG) 5-PCR analysis and 16S rRNA sequencing of bacteria from Sarawak aquaculture environment. International Food Research Journal, 21(3): 915-920. Available at http://www.ifrj.upm.edu.my/21%20(03)%202014/10%20IFRJ%2021%20(03)%202014%20Kahleen%20723.pdf

Kathleen MM, Samuel L, Felecia C, Ng KH, Lesley MB, and Kasing A (2014). (GTG)5-PCR analysis and 16S rRNA sequencing of bacteria from Sarawak aquaculture environment. International Food Research Journal, 21(3): 915-920.

Li W, Raoult D, and Fournier PE (2009). Bacterial strain typing in the genomic era. FEMS Microbiol Review, 33: 892-916. DOI: https://www.doi.org/10.1111/j.1574-6976.2009.00182.x

MacCannell D (2013). Bacterial strain typing. Clinics In Laboratory Medicine, 33(3): 629-650. DOI: https://www.doi.org/10.1016/j.cll.2013.03.005

Mancini S, Poirel L, Corthesy M, Greub G, and Nordmann P (2018). Klebsiella pneumoniae co-producing KPC and RmtG, finally targeting Switzerland. Diagnosis of Microbiology and Infectious Diseases, 90(2): 151-152. DOI: https://www.doi.org/10.1016/j.diagmicrobio.2017.10.019

Matsheka MI, Lastovica AJ, Zappe H, and Elisha BG (2006). The use of (GTG) 5 oligonucleotide as an RAPD primer to type Campylobacter concisus. Letters in Applied Microbiology, 42: 600-605. DOI: https://www.doi.org/10.1111/j.1472-765X.2006.01900.x

Mellata M (2013). Human and avian extraintestinal pathogenic Escherichia coli: infections, zoonotic risks, and antibiotic resistance trends. Foodborne Pathogenes and Disease, 10: 916-932. DOI: https://www.doi.org/10.1089/fpd.2013.1533

Mohapatra BR, Broersma K, and Mazumder A (2008). Differentiation of fecal Escherichia coli from poultry and free-living birds by (GTG) 5-PCR genomic fingerprinting. International Journal of Medical Microbiology, 298: 245-252. DOI: https://www.doi.org/10.1016/j.ijmm.2007.03.019

Moreira LM, Milan C, Gonçalves TG, Ebersol CN, de Lima HG, and Timm CD (2019). Contamination of pigs by Yersinia enterocolitica in the abattoir flowchart and its relation to the farm. Ciencia Rural, 49 (8). DOI: https://doi.org/10.1590/0103-8478cr20181040

Nováková D, Pantůček R, Hubálek Z, Falsen E, Busse HJ, Schumann P, and Sedláček I (2010). Staphylococcus microti sp. nov., isolated from the common vole (Microtus arvalis). International Journal of Systematic and Evolutionary Microbiology, 60(Pt 3): 566-573. DOI: www.doi.org/10.1099/ijs.0.011429-0

Pooncharеon K, Pulsrikarn C, Nuanmuang N, and Khamai P (2019). Effectiveness of BOX-PCR in differentiating genetic relatedness among Salmonella enterica serotype 4,[5], 12: i:-isolates from hospitalized patients and minced pork samples in northern Thailand. International Journal of Microbiology, Article ID: 5086240. DOI: https://www.doi.org/10.1155/2019/5086240

Rafei R, Dabboussi F, Hamze M, Eveillard M, Lemarié C, Gaultier MP, Mallat H, Moghnieh R, Husni-Samaha R, Joly-Guillou ML et al. (2014). Molecular analysis of Acinetobacter baumannii strains isolated in Lebanon using four different typing methods. PLoS One, 9(12): e115969. Available at: https://dx.plos.org/10.1371/journal.pone.0115969

Ranjbar R, Pourshafie M, Sadeghifard N, Parzadeh M, Izadi M, Jonaidi N, and Sedaghat M (2008). Molecular characterization of epidemic isolates of Vibrio cholerae O1 by Arbitrarily Primed PCR (AP-PCR). Iranian Journal of Public Health, 37: 83-87. DOI: https://www.doi.org10.5812/jjm.8(5)2015.18119

Ranjbar R, and Babazadeh D (2018). DNA fingerprinting of clinical strains of *Acinetobacter baumannii* isolated from some hospitals of Tehran, Iran by method of $(GTG)_5$-PCR, thesis. 23-32. Available at: http://research2.bmsu.ac.ir/homepage/homePage.action

Ranjbar R, and Afshar D (2019). Evaluation of (GTG) 5-PCR for Genotyping of Klebsiella pneumonia Strains Isolated from Patients with Urinary Tract Infections. Iranian Journal of Public Health, 48(10): 1879-1884. DOI: https://doi.org/10.18502/ijph.v48i10.3496

Shakib P, Ghafourian S, Zolfaghary MR, Hushmandfar R, Ranjbar R, and Sadeghifard N (2012). Prevalence of OmpK35 and OmpK36 porin expression in beta-lactamase and non-betalactamase-producing Klebsiella pneumoniae. Biologics, 6: 1-4. DOI: https://www.doi.org/10.2147/BTT.S27582

Stepán J, Pantucek R, and Doskar J (2004). Molecular diagnostics of clinically important staphylococci. Folia Microbiologica, 49: 353-386. Available at: https://link.springer.com/article/10.1007/BF03354664

Svec P, Nováková D, Zácková L, Kukletová M, and Sedlácek I (2008). Evaluation of (GTG) 5-PCR for rapid identification of Streptococcus mutans. Antonie Van Leeuwenhoek, 94: 573-579. DOI: https://www.doi.org/10.1007/s10482-008-9275-6

Svec P, Sedláček I, Chrápavá M, and Vandamme P (2011). (GTG) (5) -PCR fingerprinting of lactobacilli isolated from cervix of healthy women. Folia Microbiologica (Prague), 56: 80-83. DOI: https://www.doi.org/10.1007/s12223-011-0006-4

Švec P, Vancanneyt M, Seman M, Snauwaert C, Lefebvre K, Sedláček I, and Swings J (2005). Evaluation of (GTG) 5-PCR for identification of Enterococcus spp. FEMS Microbiology Letters, 247(1): 59-63. DOI: https://www.doi.org/10.1016/j.femsle.2005.04.030

Tenover FC, Arbeit RD, and Goering RV (1997). How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections a review for healthcare epidemiologists. Infection Control and Hospital Epidemiology, 18(6): 426-439. Available at: http://cel.webofknowledge.com/InboundService.do?customersID=atyponcel&smartRedirect=yes&mode=FullRecord&IsProductCode=Yes&product=CEL&Init=Yes&Func=Frame&action=retrieve&SrcApp=literatum&SrcAuth=atyponcel&SID=F39sOMH5iQtkkKv1Sb6&UT=WOS%3AA1997XC21200008

Versalovic J, Schneider M, de Bruijn FJ, and Lupski JR (1994). Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods in Molecular and Cellular Biology, 5: 25–40. Available at: https://www.scienceopen.com/document?vid=7a2acf61-8c89-4d19-89e1-f2987fdfa0f1

Yang X, Wu Q, Zhang J, Huang J, Guo W, and Cai S (2015). Prevalence and characterization of monophasic Salmonella serovar 1,4, [5], 12: i: -of food origin in China. PLoS One, 10(9): e0137967. Available at: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0137967

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Effect of Lysine Supplementation in Commercial Feed on Energy Retention and Feed Conversion Ratio of Carp (*Osphronemus gouramy*)

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ABSTRACT

The long period of raising carp *(Osphronemus gouramy)* causes the need for excessive feed. One way that can accelerate the growth of this fish in order to shorten the maintenance period is by the addition of essential amino acids, such as lysine. However, this certainly gives its own influence on energy retention. Therefore, the aim of this study was to determine the influences of addition of lysine in feed on energy retention and feed conversion ratio of carp. The research method used an experimental method with a completely randomized design consisting of five treatments and four replications. The treatments used were the addition of Lysine 0%, 1%, 1.5%, 2%, and 2.5% to the feed. The present experiment was conducted for a year. The results showed that the addition of lysine as much as 2% in commercial feed can increase the energy retention of carp *(Osphronemus gouramy).* Moreover, the addition of lysine by giving up to 2.5% cannot reduce the feed conversion ratio in carp *(Osphronemus gourami)* rearing. It can be concluded that the use of lysine has different effects related to the increase in retention and decrease conversion ratio in carp.

Keywords: Carp, Conversion ratio, Energy retention, Lysine

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INTRODUCTION

Carp (*Osphronemus gouramy*) in Indonesia experiences an increase in production every year. As in 2011, the market demand for carp was 9,322 tons and in 2012 the demand increased to 10,303 tons (Zakaria, 2008).

The growth of carp is slow to reach an average weight of 250 grams/head in male species and 200 grams/head in female takes 10-12 months (Handajani, 2007). The long period of raising carp causes the need for feed consumption (Muzdalipah and Yulianto, 2018). One way that can accelerate the growth of this fish in order to shorten the maintenance period is by the addition of essential amino acids, such as lysine (Simanjuntak et al., 2016; Nguyen et al., 2019). Essential amino acids are amino acids that cannot be synthesized by animals or plants and they trigger growth rate (Lovell, 1998). The requirement of lysine for carp is greater than that of other essential amino acids which is 5.7% (Viola et al., 1992). Lysine may improve the balance of utilization of amino acids so that it can increase the growth rate (Alam et al., 2005).

Amino acids in proteins are used as forming new proteins during growth and reproduction or replacing damaged proteins during the maintenance and growth period (Hidayat, 2016). The lysine supplementation as an essential amino acid made up of protein is expected to increase the energy retention of carp (Güroy et al., 2017; Nguyen et al., 2019).

On the other hand, the benefits of lysine as a supplemented ingredient to accelerate the growth of carp can be observed from the value of the FCR (Gan et al., 2013). FCR is the ratio between the dry weights of feed consumed and fish weight gain (Afrianto and Liviawaty, 1992). The addition of lysine is expected to reduce the Food Conversion Ratio (FCR), so that it can accelerate the growth of carp and shorten the maintenance time (rearing period) to the commercial size of consumption. For this reason, the present study was conducted with the aim of analyzing the addition of lysine in commercial feed to energy retention and conversion ratio of carp feed.

MATERIALS AND METHODS

This research was conducted from April 2016 to May 2016 in the Laboratory of the Faculty of Fisheries and Maritime Affairs, Universitas Airlangga. Proximate analysis of carp feed and the meat was conducted at the Feed Laboratory of Universitas Airlangga. Through this, several tools were used, namely 40x25 × 25 cm3 aquariums of 20 pieces, small aerators, aerated hoses, aeration stones, *seser* (fishes trap), plastic bags, pH meters, thermometers, DO meters, refractometers, analytical scales, *sipon* (suction device).

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The animals used for testing in this study were carp (*Osphronemus gouramy*). The carps used are 8-10 cm in size and each aquarium contains 10 fishes. Five treatments and four replications were arranged, with 20 aquariums and 200 fishes. The maintenance media used in this study are fresh water with a volume of 15 liters per aquarium. The diet was included commercial feed in the form of pellets and lysine (CAS Number 56-87-1, L-Lysine ≥98% (TLC), Sigma-Aldrich).

Along with conducting the research, carps were selected before being stocked in an aquarium. In this case, the selection is based on uniformity in size and completeness of the body's organs so that it can be ascertained that the carp is healthy and homogeneous. Before stocking, carps are acclimatized for 30 minutes. Stocking of carp was done in the morning or evening to avoid stress.

The study compared the effect between commercial feed which was not given lysine and commercial feed including lysine at different doses in each treatment (P_0, P_1, P_2, P_3, P_4) on energy retention and FCR in carp (*Osphronemus gouramy*). Analyses of variance were performed using the GLM procedure of SAS Institute Inc. (2005) as a completely randomized design consisting of five treatments with four replications.

Water Quality

Water quality can be defined as the suitability of water for fish survival and growth which is generally determined by several water quality parameters (Mahasri et al., 2009). The range of water quality during this study has a dissolved oxygen content of 4 mg/ l. The content of dissolved oxygen in this study was in the physiological range. The temperature of maintenance media during the study was between 28^{o}C - 31^{o}C (Indonesia, 2000). The pH value during the study ranged from 7.5 to 8.0. This is according to the optimal water pH ranges from 7.5 to 8.5 (Mahasri et al., 2009). The optimal ammonia concentration in aquaculture, in this case, is not more than 1 ppm (Indonesia, 2000). Ammonia concentration was not more than 1 ppm. Ammonia concentration values range from 0 to 0.09 (mg/l). Nevertheless, the water quality for 34 days of carp maintenance based on the data above shows that the optimal gourami breeding media and does not cause toxic results in death, this is evidenced by the survival rate of carp as many as 100% (Mahasri et al., 2009).

RESULT AND DISCUSSION

Energy Retention

The results showed the energy retention value of carp ranged from 23.36 to 37.42%. Data on average energy retention can be seen in Table 1.

The results of statistical analysis according to table 1 showed findings that were not significantly different on carp's energy retention. There was a significant difference between treatments, the highest energy retention was P_3 (37.42%) and the lowest energy retention was P_0 (23.36%). P_3 is not significantly different from P_4 but significantly different from P_2, P_1, and P_0. Furthermore, it is known that energy is obtained from an overhaul of chemical bonds through the process of oxidation reactions to feed components namely proteins, fats, and carbohydrates into simpler compounds (amino acids, fatty acids, and glucose) in order to be absorbed and to be used or stored by the body (Afrianto and Liviawaty, 1992). Energy retention is the amount of feed energy that can be stored in the body of a fish (Chusminah et al., 2018). Meanwhile, ANOVA statistical test results showed that the administration of amino acid lysine in commercial feed showed no significant difference in the energy retention of carp. Based on Duncan's Multiple Range Test, there was a difference between treatments with the highest supplemented amount at P_3 and the lowest amount at P_0. This shows that the supplementation of lysine in commercial feed can increase energy retention in comparison with feed without the addition of lysine.

Increased energy retention can occur due to lysine; because it's one of the essential amino acids that cannot be synthesized by the animal body. So it must be included through feed, with proper feeding and the appropriate dosage then an increase in energy retention can occur because the main source of energy in fish is a protein (Zhang et al., 2009). The dietary addition of lysine which is a monomer from protein causes absorption will be faster because it occurs directly in the intestine (Zhang et al., 2009). It is well known that protein is an important component of energy compilers in fish (Lovell, 1998). Therefore, amino acid metabolism can occur through two stages, namely transamination, and deamination (Buwono, 2000). Through these events, amino acids can be converted into acetyl Co-A which then generates to the Krebs cycle to produce energy (Buwono, 2000).

The mean value of energy retention ranged from 23.36 to 37.42% with the highest energy retention in P_3 with the addition of lysine as much as 2%. This can be interpreted that out of every 3149.8179 Kcal/kg of feed energy consumed, it can be utilized for daily growth and metabolism in carp (0.3742 x 3149.8179 Kcal /kg) or 1,178.66 Kcal/kg. On the other hand, the lowest energy retention calculation results are at P_0 as many as 23.36%. Such conditions indicate that energy that can be utilized for the growth and metabolism of carp is (0.2336 x 3044.2726 Kcal/kg) or 711.14 Kcal/kg.

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Through this matter, it is known that energy retention is related to protein feed levels because they also contain protein in addition to carbohydrates and fats (Saravanan et al., 2012). Analysis of feed protein for P_0, P_1, P_2, P_3, and P_4 are 25.15; 27.82; 28.94; 30.61; 32.06. Moreover, energy analysis levels of P_0, P_1, P_2, P_3, and P_4 are 3138.47; 3317.91; 3323.779; 3238.94; 3360.34. The highest energy retention is P_3 with the addition of lysine as many as 2%, the protein content of the feed is 30.61%, and the energy content of 3238.94 Kcal/kg. The protein-energy level is the optimal ratio for carp because the protein content of the feed approaches the minimum requirement for its protein feed in the growth phase of 32%.

The addition of 2.5% lysine possibly produced a protein content of feed in accordance with the protein requirements of carp feed is 32.06% in P_4 but the resulting energy retention was lower than P_3. This is due to an imbalance between protein-energy requirements in feed, so excess protein will cause extra energy demands to do the deamination process so that the energy that should be used for growth will be reduced (NRC, 1989). Therefore, without the right energy-protein balance will not have an impact on growth, the level of protein-energy in the feed also affects feed consumption, if the protein-energy level exceeds the need, it will reduce consumption so that the uptake of other nutrients including protein will decrease (Lemos et al., 2014). Comparison between protein retention and energy must be optimal in order to stimulate growth. In fact, high energy intake can reduce protein consumption as an energy source (Souto et al., 2013).

Feed Conversion Rate

The results showed the FCR of carp ranged from 3.39 to 5.05. Data on average FCR can be seen in Table 2.

The results of the statistical analysis of lysine amino acids in commercial feed, in Table 2, showed results that were not significantly different ($p > 0.05$) to the conversion ratio of carp feed. Based on Duncan's Multiple Range Test, it was found that there were no differences between treatments. Therefore, it is known that the FCR is the ratio between the dry weights of the food consumed and fish weight gain (Afrianto and Liviawaty, 1992) The FCR is used as one of the benchmarks of success both technically and financially. On the other hand, the value of the FCR is inversely proportional to the growth in fish weight. The higher FCR shows that the feed given is increasingly ineffective in the growth of carp.

Meanwhile, ANOVA statistical results and Duncan's Multiple Range Test showed that the supplementation of lysine in commercial feed did not affect the FCR value in carp ($p > 0.05$). Based on the calculation of feed consumed, the amount of feed consumed P_0 and P_3 has a small difference but the addition of P_3 fish weight is greater than that of P_0. Consequently, there is a tendency to decrease the value of feed conversion to occur at P_3. Feed consumption data can be seen in Table 2. The value of the P_3 FCR is 3.39, which means that 3.39 grams of feed give 1 gram of carp weight gain. P_0 FCR value is 5.05, which means to add 1 gram of carp weight, feed consumption of 5.05 grams is required.

On the other hand, the ANOVA statistical results of giving lysine in commercial feed to FCR were not significantly different, it possibly could be due to the content of lysine used in this study. In addition to achieving optimal growth, feed energy must meet the needs for daily activities, metabolism, and maintenance needs. Energy in feed is still used for the daily needs of fish, namely for metabolic processes so that maximum growth has not yet occurred. These conditions are in accordance with circumstances where not all the incoming energy can be digested and utilized for growth (Handajani and Widodo, 2010; Zuidhof, 2019). In this case, the dietary energy is physiologically used for maintenance and metabolism; if there is residual it will be deposited as body tissue in the growth process (Zuidhof, 2019).

Table 1. Average energy retention in carp

Treatment	Energy Retention ± SD (%)	Energy Retention √y±SD
P_0	23.36^{b} ± 9.84	4.76 ± 1,01
P_1	23.90^{b} ± 4.53	4.87 ± 0.46
P_2	24.73^{b}± 4.58	4.95 ± 0.46
P_3	37.42^{a} ± 8.61	6.09 ± 0.73
P_4	26.03^{ab} ± 5.16	5.08 ± 0.49

Notes: P_0: Lysine 0%, P_1: Lysine 1%, P_2: Lysine 1.5%, P_3: Lysine 2%, P_4: Lysine 2.5 %, and SD: standard deviation. Different superscripts in the same column show significant differences in Duncan's Multiple Range Test (*Duncan's Multiple Range Test*).

Table 2. FCR in different treatment in carp feed conversion ratio

Treatment	Feed Conversion Rate ± SD	Feed Conversion Rate √y+0.5±SD
P_0	5.05 ± 1.55	2.33 ± 0.32
P_1	4.50 ± 0.91	2.23 ± 0.20
P_2	4.15 ± 0.85	2.15 ± 0.19
P_3	3.39 ± 1.00	1.96 ± 0.25
P_4	3.82 ± 0.75	2.07 ± 0.18

Notes: P_0: Lysine 0%, P_1: Lysine 1%, P_2: Lysine 1.5%, P_3: Lysine 2%, P_4: Lysine 2.5 %, and SD: Standard Deviation

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CONCLUSION

Based on present findings, it can be concluded, the supplementation of lysine as much as 2% in commercial feed can increase the energy retention of carp *(Osphronemus gouramy*). As well as, the dietary supplementation of lysine to commercial feed by giving up to 2.5% may not reduce the feed conversion ratio in carp (*Osphronemus gouramy*) breeding.

DECLARATIONS

Authors' contribution

Authors (Ataina Thaiin, Agustono, and Mohammad Anam Al Arif) had equal roles in conducting, writing, and editing manuscript as teamwork.

Competing interests

The authors have declared that no competing interest exists.

Ethical considerations

All authors approved the final draft of the manuscript for submission to this journal. Ethical issues (Including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been checked by the authors.

REFERENCES

Afrianto E, and Liviawaty E (1992). Pengendalian Hama dan Penyakit Ikan. Kanisius. Yogyakarta, p. 89. Available at: https://opac.perpusnas.go.id/DetailOpac.aspx?id=211610

Alam MS, Teshima S, Koshio S, Ishikawa M, UyanL O, Hernandez LHH, and Michael FR (2005). Supplemental effects of coated methionine and/or lysine to soy protein isolate diet for juvenile kuruma shrimp, Marsupenaeus japonicas. Aquaculture, 248: 13-19. DOI: https://www.doi.org/10.1016/j.aquaculture.2005.04.015

Buwono ID (2000). Kebutuhan asam amino esensial dalam ransum ikan ibnu dwi buwono, Kanisius, p. 668. Available at: http://opac.lib.ugm.ac.id/index.php?mod=book_detail&sub=BookDetail&act=view&typ=htmlext&buku_id=646123&obyek_id=1

Chusminah C, Haryati RA, and Kristiani D (2018). Efektifitas implementasi e-tilang kendaraan bermotor dalam rangka tertib berlalu lintas pada korps lalu lintas polri. Widya Cipta: Jurnal Sekretari dan Manajemen, 2(2): 217-224. Available at: https://ejournal.bsi.ac.id/ejurnal/index.php/widyacipta/article/view/4318

Gan L, Liu YJ, Tian LX, Yue YR, Yang HJ, Liu FJ, Chen YJ, and Liang GY (2013). Effects of dissolved oxygen and dietary lysine levels on growth performance, feed conversion ratio and body composition of grass carp, Ctenopharyngodon idella. Aquaculture Nutrition, 19(6): 860-869. DOI: https://www.doi.org/10.1111/anu.12030

Güroy D, Karadal O, Güroy B, Mantoğlu S, Çelebi K, Şimşek O, Eroldoğan OT, Genç MA, and Genç E (2017). The effects of dietary protein levels with amino acid supplementation on the growth performance, haematological profile and histology of meagre (Argyrosomus regius) in two different size classes. Aquaculture Research, 48(12): 5751-5764. DOI: https://www.doi.org/10.1111/are.13398

Handajani H (2007). Perendaman larva gurami (Osphronemus gouramy) dengan umur yang berbeda pada hormon metiltestoteron terhadap keberhasilan pembentukan monosex jantan. jurusan Perikanan Universitas Muhammadiyah Malang. Hal. Available at: https://onesearch.id/Record/IOS1.INLIS000000000242746

Handajani H, and Widodo W (2010). Nutrisi Ikan. Universitas Muhammadiyah Malang Press. Malang, p. 271. Available at: https://ummpress.umm.ac.id/katalog/detail/nutrisiikan.html

Hidayat T (2016). Quality deteoration gourami fish *(Osphronemus gouramy*) during storage. International Journal of Materials Chemistry and Physics, 2(1): 40-44. Available at: https://docplayer.net/47678606-Quality-deteoration-gourami-fish-osphronemus-gouramy-during-storage.html

Lemos MV, Antunes de A, Thiago Q, Souto CN, Martins GP, Janaína Gomes A, and Gomes GI (2014). Effects of digestible protein to energy ratios on growth and carcass chemical composition of siamese fighting fish (*Betta splendens*). Ciência e Agrotecnologia, 38(1): 76-84. DOI: https://www.doi.org/10.1590/S1413-70542014000100009

Lovell T (1998). Fish nutrition and feeding experiments. Nutrition and Feeding of Fish, pp. 123-134. Available at: https://link.springer.com/chapter/10.1007/978-1-4615-4909-37

Mahasri G, Mubarak AS, Alamsjah MA, and Manan A (2009). Bahan ajar: Manajemen kualitas air. fakultas perikanan dan kelautan. Universitas Airlangga hal. Available at: https://sinta.ristekbrin.go.id/journals/detail?page=4&id=3997

Muzdalipah I, and Yulianto E (2018). Ethnomathematics study: The technique of counting fish seeds (Osphronemus gouramy) of sundanese style. Journal of Medives, 2(1): 25-40. DOI: https://www.doi.org/10.31331/medives.v2i1.555

National Research Council (NRC) (1989). Diet and health: Implications for Reducing Chronic Disease Risk, p. 768. Available at: http://www.nap.edu/catalog/1222.html

Nguyen MV, Espe M, Conceição L, Le HM, Yúfera M, Engrola S, Jordal AO, and Rønnestad I (2019). The role of dietary methionine concentrations on growth, metabolism and N-retention in cobia (*Rachycentron canadum*) at elevated water temperatures. Aquaculture Nutrition, 25(2): 495-507. DOI: https://www.doi.org/10.1111/anu.12875

Saravanan S, Schrama JW, Figueiredo-Silva AC, Kaushik SJ, Verreth JA, and Geurden I (2012). Constraints on energy intake in fish: the link between diet composition, energy metabolism, and energy intake in rainbow trout. PloS One, 7(4): e34743. DOI: https://www.doi.org/10.1371/journal.pone.0034743

Simanjuntak SBI, Wibowo ES, and Indarmawan I (2016). Stimulation of deprivation cycles with spirulina platensis feed supplementation on osphronemus gouramy physiological responses. Biosaintifika Journal of Biology and Biology Education, 8(3): 377-384. DOI: https://www.doi.org/10.15294/biosaintifika.v8i3.7274

Souto CN, Lemos MVA, Martins GP, Araújo JG, Lopes KLAM, and Guimarães IG (2013). Protein to energy ratios in goldfish (*Carassius auratus*) diets. Ciência Agrotecnologia, 37(6): 550-558. DOI: http://www.dx.doi.org/10.1590/S1413-70542013000600008

Viola S, Lahav E, and Angconi H (1992). Reduction of feed protein levels and of nitrogenous N-excretions by lysine supplementation in intensive carp culture. Aquatic Living Resources, 5(4): 277-285. DOI: https://www.doi.org/10.1051/alr:1992026

Zakaria R (2008). Kemunduran mutu ikan gurami (*Osphronemus gouramy*) pasca panen pada penyimpanan suhu chilling. Program Studi Teknologi Hasil Perikanan, IPB, Bogor, pp. 1-90. Available at: https://docplayer.info/38896934-Kemunduran-mutu-ikan-gurami-osphronemus-gouramy-pasca-panen-pada-penyimpanan-suhu-chilling-rijan-zakaria.html

Zhang Q, Ames JM, Smith RD, Baynes JW, and Metz TO (2009). A Perspective on the maillard reaction and the analysis of protein glycation by mass spectrometry: Probing the pathogenesis of chronic disease. Journal of Proteome Research, 8(2): 754-769. DOI: https://www.doi.org/10.1021/pr800858h

Zuidhof MJ (2019). A Review of dietary metabolizable and net energy: Uncoupling heat production and retained energy. Journal of Applied Poultry Research, 28(2): 231-241. DOI: https://www.doi.org/10.3382/japr/pfx062

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Clinical and Laboratory Findings in Dogs Undergoing Adjuvant Chemotherapy with Gemcitabine/Carboplatin Combination for Mammary Neoplasia

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ABSTRACT

Adjuvant chemotherapy might be indicated in some canine mammary cancer cases due to metastatic potential. In this regard, studies to determine adverse events following chemotherapy protocols are valuable. The purpose of this prospective clinical trial was to evaluate the safety and tolerability of gemcitabine and carboplatin combination in dogs with malignant mammary tumors. For this prospective clinical trial, 21 female dogs mastectomized due to malignant mammary neoplasia underwent adjuvant chemotherapy with gemcitabine (3 mg/kg, 60-minute IV infusion) and carboplatin (10 mg/kg, 20-minute IV infusion) based protocol every 21 days for three cycles. They were monitored periodically for treatment-related adverse events by clinical and laboratory evaluations. A total of 17 (80.9%) dogs developed leukopenia, 10 (47.6%) neutropenia, and 15 (71.4%) thrombocytopenia at least once along with the three chemotherapy cycles. All these hematologic toxicities were grade 1, 2, or 3. Two (9.5%) animals had evidence of gastrointestinal toxicity; however, clinical signs were mild to moderate (grades 1 and 2). No dog had life-threatening adverse events (grade 4) or even died (grade 5) of treatment-related complications. The adjuvant chemotherapy protocol with gemcitabine and carboplatin was well-tolerated and safe in female dogs for mammary cancer treatment with self-limiting hematological and gastrointestinal adverse events.

Keywords: Adverse event, Canine, Mastectomy, Toxicity, Tumor

INTRODUCTION

Mammary tumors are the most common neoplasms in female dogs. They have genetic, anatomopathological, and clinical similarities with human breast cancer. The recommended treatment for canine mammary cancer is surgical excision (Cassali et al., 2020; Sorenmo et al., 2020). Adjuvant chemotherapy is indicated in some cases of malignant neoplasms due to metastatic potential (Lavalle et al., 2012) although it might lead to several side effects, mainly myelosuppression and gastrointestinal (GI) disorders (Rodaski et al., 2008). The toxicity occurs since chemotherapeutic agents do not target exclusively neoplastic cells, but high mitotic rate cells, including the regular ones (Rodaski et al., 2008; Gustafson and Bailey, 2020).

Chemotherapy protocols in companion animals may be linked to a good quality of life and extended long-term survival. However, the potential for adverse events that impact the overall quality of life should be taken into consideration (Vail, 2009). This emphasizes the importance of detailed studies on the drug doses and administration frequencies in veterinary cancer patients in order to increase success rates with as few side effects as possible (Medeiros, 2017). Gemcitabine is an antineoplastic drug that requires cell absorption and intracellular phosphorylation to act (Garnett et al., 2016). Doses ranging from 350 to 1,000 mg/m^2 (IV) are known to cause the least side effects (Lana et al., 2007; Dominguez et al., 2009) related to hematologic, dermatological, GI, and renal disturbances (Rodaski et al., 2008). Gemcitabine administration has also been found to sensitize the neoplastic cell to a better carboplatin action (Silva et al., 2018). Carboplatin belongs to the second generation of platinum compounds. The drug acts through covalent binding to DNA through displacement reactions resulting in bifunctional lesions and intrastrand crosslinks (Fichtinger-Schepman et al., 1985). Carboplatin has less toxicity than other anticancer agents from the same group (Wagstaff et al., 1989). Side effects observed are nausea and vomiting, nephrotoxicity as well as myelosuppression with neutropenia and thrombocytopenia (Selmic et al., 2014). Lavalle et al. (2012) demonstrated that dogs with malignant mammary tumors treated with carboplatin after surgical excision had a statistically significant longer overall survival when compared with animals submitted to surgical treatment alone.

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An *in vitro* study showed that gemcitabine and carboplatin decreased cell proliferation, increased apoptosis, and induced cell cycle disruption. Cell cycle interruption and apoptosis were higher when both drugs were administered in sequence (Anjos et al., 2012). The true utility of gemcitabine might act as a potentiator of carboplatin cytotoxicity (Dominguez et al., 2009). The success of combination chemotherapy over single-agent treatment is providing maximal cell kill within the range of tolerable toxicity, accomplishing more interaction between the chemotherapeutic agents and tumor cell population, and slowing the development of cellular drug resistance (Gustafson and Bailey, 2020). Gemcitabine and carboplatin in combination therapy have been evaluated for the treatment of canine osteosarcoma (McMahon et al., 2011) and varied carcinomas (Dominguez et al., 2009). In addition, it is one of the regimens recommended for mammary cancer in dogs (Cassali et al., 2020) although there are no previous studies on toxicity and safety of gemcitabine and carboplatin doublet therapy for canine malignant mammary tumors as far as one can tell from the literature.

In veterinary medicine, there is a lack of studies on the toxicity of chemotherapy regimens. In this context, the aim of this prospective clinical trial was to assess the adverse events, safety, and tolerability of adjuvant chemotherapy with gemcitabine and carboplatin combination for mammary cancer in female dogs through clinical and laboratory evaluations.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Ethics Committee on the Use of Animals of Universidade Federal Fluminense with the verdict number 279/12. In addition, written informed consent was obtained from owners to authorize the study.

Patients

The present study was conducted at the Professor Firmino Másrico Filho Veterinary Teaching Hospital, Universidade Federal Fluminense, Brazil from March 2012 to December 2013.

Dogs were enrolled in this prospective study according to the following criteria: (1) at least one histopathologically confirmed malignant mammary neoplasia; (2) surgical treatment of disease by unilateral or bilateral mastectomy in cases of nodules in one or both mammary chains, respectively; (3) no previous history of cancer; and (4) disease stage I to IV by modified TNM staging system based on WHO classification (Owen, 1980), which means the absence of distant metastasis suggestion by 3-view radiographic examination of the thorax and abdominal ultrasonography.

After the mastectomy, mammary chain resected were fixed in 10% buffered formalin solution for 48 hours, embedded in paraffin, and cut in 5 µm sections. Tumor sections were stained by hematoxylin and eosin, then classified according to Cassali's histopathological criteria (Cassali et al., 2020). Tumor histological type and grade, regional lymph node status, and resection margin status were obtained. These data associated with cancer disease staging and owner willingness to offer an antineoplastic treatment determined whether or not to start adjuvant chemotherapy. In cases of multiple types of mammary tumors in the same dog, the clinical decision-making for adjuvant chemotherapy treatment was based on the most aggressive tumor type and grade.

Chemotherapy administration

The chemotherapy protocol consisted of three cycles. The interval between cycles was 21 days. Dogs received gemcitabine at a dose of 3 mg/kg (Rodaski and De Nardi, 2008) IV over 60 minutes. The carboplatin was administered 5 minutes after gemcitabine through the same catheter at a dose of 10 mg/kg (Rodaski and De Nardi, 2008; Dominguez et al., 2009) IV for 20 minutes. Before, between, and after chemotherapy administrations, 25 ml/kg/hour of 0.9% saline solution was administered as recommended for platinum-derived protocols (Repetti and Daleck, 2007). As chemotherapy-induced toxicity prophylaxis, ondansetron hydrochloride (0.5 mg/kg PO every 12 hours) was given for 6 days and starting 3 days before each chemotherapy session for all dogs. In addition, immediately before anticancer agents infusion, ondansetron (0.5 mg/kg IV), ranitidine hydrochloride (1.0 mg/kg SC) and promethazine (1.0 mg/kg IV) were administered (Viana, 2007).

Adverse events evaluation

Adverse events were graded according to the Veterinary Cooperative Oncology Group - Common Terminology Criteria for Adverse Events following investigational therapy in dogs and cats (LeBlanc et al., 2021) based upon clinical and laboratory evaluations (E). About 20 days after mastectomy and before the first cycle of adjuvant chemotherapy, baseline clinical and laboratory evaluations (E0) were performed. Table 1 shows the schedule of chemotherapy cycles as well as clinical and laboratory evaluations. Dogs were evaluated on days 0, 15, 20, 36, 41, 57 and 62, identified as E0, E1, E2, E3, E4, E5 and E6, respectively, for this 63-day study. The female dogs were subjected to blood count tests from

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E0 to E6 and serum biochemistry and urinalysis with urine protein-creatinine ratio exams in E0, E2, E4, and E6. These findings were accounted for as laboratory changes. Clinical evaluation and complete blood counts (including manual white blood cell differential) were also performed on E1, E2, and E5. Clinical evaluation from E0 to E6 included physical examination, abdominal palpation, cardiopulmonary auscultation, oral, and ocular mucosa examination, as well as monitoring of weight, temperature, and hydration status. Besides, clinical history and anamnesis were carried out with the dog's owner with regard to appetite, vomiting, diarrhea, lethargy, and other non-ordinary events related to antineoplastic drugs side effects.

Table 1. Schedule of clinical and laboratory evaluations (E0 to E6) and the three cycles of gemcitabine and carboplatin combination chemotherapy performed on female dogs with mammary cancer

Days	Evaluation					Chemotherapy	
	Period	Clinical	Laboratory				
			Blood count	Serum biochemistry	Urinalysis	Session	Cycle
0	E0	x	x	x	x		
1						x	First
15	E1	x	x				
20	E2	x	x	x	x		
22						x	Second
36	E3	x	x				
41	E4	x	x	x	x		
43						x	Third
57	E5	x	x				
62	E6	x	x	x	x		

E0: Baseline evaluation, E1: First evaluation, E2: Second evaluation, E3: third evaluation, E4: Fourth evaluation, E5: Fifth evaluation, E6: Sixth evaluation, x: Marks the evaluated parameter.

Adverse events managing

Toxicity management varied according to tumor type and grade from chemotherapy cycle delay to symptomatic or prophylactic therapy prescription.

Statistical analysis

In the clinical trial, one-way analysis of variance (Dunn, 1964; Scheffé, 1999) and the paired Student's t-test considered GI toxicosis (emesis, diarrhea, hyporexia, and weight loss) and other clinical toxicity (such as lethargy, fever, hypothermia). The laboratory findings were hematological (complete blood count, global leukometry, neutrophil count, platelet count, red blood cells, haemoglobin, and hematocrit) and metabolic toxicoses (increase in creatinine, urea, alanine aminotransferase [ALT] and alkaline phosphatase [ALP], decrease in serum albumin, and increase in urine protein-creatinine ratio). The abnormal parameters taken were classified into five degrees (LeBlanc et al., 2021).

RESULTS

Patients' characteristics

A total of 98 female dogs met the inclusion criteria. From these, 77 were excluded from the current study for the following reasons: (1) not having chemotherapy indication, since they had stage I disease and low aggressive tumors; (2) their owners declining the adjuvant chemotherapy recommendation; or (3) were dogs undergoing a different chemotherapy protocol. Finally, a total of 21 female dogs of varying ages and breeds, including mixed breed dogs, with mammary cancer met inclusion criteria and were enrolled for this prospective clinical trial. Seven (33.3%) dogs were spayed and 14 (66.7%) dogs were not. The mean age was 10.4 years (range 7-14 years). There were eight (38.09%) Poodles Toy, four (19.04%) mixed breed dogs, two (9.52%) Daschunds, one (4.76%) Bichon Frise, one (4.76%) Border Collie, one (4.76%) Bull Terrier, one (4.76%) Rottweiler, one (4.76%) English Cocker Spaniel, one (4.76%) Brazilian Terrier and one (4.76%) Pinscher. The mean weight was 11.5 kg (ranged from 3.1 to 37.5 kg) wherein 16 dogs were above 15.0 kg. The time interval between surgery and initiation of chemotherapy was approximately 21 days (range 16–44 days). No significant correlation between toxicity and age, breed, or even weight was found.

Staging, tumor type, and grade

With regards to disease staging, 47.6% (n = 10/21), 28.6% (n = 6/21), 19.1% (n = 4/21) and 4.7% (n = 1/21) of the animals were stage I, II, III and IV, respectively. The most common mammary tumor type was carcinoma in a mixed

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tumor grade II (66.6%, n = 14/21), followed by tubular carcinoma grade II (19.0%, n = 4/21), papillar carcinoma grade II, solid carcinoma grade III, and carcinosarcoma grade II (4.8%, n = 1/21 each). No significant correlation between toxicity and cancer stage, tumor type, or grade was found. Ten animals received adjuvant chemotherapy due to tumor size over 3 cm (six dogs stage II plus four dogs stage III) and one because of positive lymph node metastasis (stage IV). Among these animals, two dogs stage III had more aggressive tumor types such as carcinosarcoma grade II and solid carcinoma grade III. All female dogs with stage I cancer had mammary tumor grade II and underwent chemotherapy by owners' voluntary choice. The clients were aware that no scientific evidence of adjuvant chemotherapy benefits for grade II malignant mammary tumors is available, but they were concerned about metastatic disease potential and therefore opted for adjuvant treatment after surgery.

Laboratory findings

The main hematological changes during all three adjuvant chemotherapy cycles were leukopenia (43 events in 126 blood count exams), neutropenia (24 events in 126 exams), and thrombocytopenia (38 events in 126 exams). About 80.9% (n = 17/21) of the female dogs had leukopenia at least once from E1 to E6 (43 events in 17 dogs). A higher incidence of grades 1 (global leukometry 5,499-6,000/µl) and 2 leukopenia (global leukometry 3,499-5,500/µl) was noticed in 39.5% (n = 17/43 each), followed by grade 3 leukopenia (global leukometry 1,499-3,500/µl) (Figure 1) that summed up 21.0% (n = 9/43) of events. The most severe episodes of leukopenia happened in E1 (blood count 14 days after the first chemotherapy session) and E4 (blood count before the third session). However, only leukopenia in E1 was statistically significant ($p < 0.05$) when compared to leukopenia in E2, E3, E4, E5, and E6.

With regards to neutropenia, 47.6% (n = 10/21) of animals had it at least once from E1 to E6. To sum up all 24 episodes of neutropenia (24 events in 10 dogs), 66.7% (n = 16/24) were grade 1 (Absolute Neutrophil Count [ANC] 1,500-3,000/µl), 20.8% (n = 5/24) were grade 2 (ANC 1,000-1,499/µl) and 12.5% (n = 3/24) were grade 3 (ANC 500-999/µl, Figure 2). Two animals (a 19.7 kg Mixed Breed dog and a 25.0 kg Border Collie) had grade 3 neutropenia and one of them (Border Collie) showed afebrile grade 3 neutropenia again later (Neutrophil count = 925/µl). Neutropenia had a higher frequency and was statistically significant ($p < 0.05$) on blood count after the first chemotherapy cycle (E1). No animal had febrile neutropenia.

Around 38.1% of female dogs (n = 8/21) had a delayed or second nadir about 19 days after chemotherapy administration, totalizing 12 neutropenia events in E2, E4, and E6. About 71.4% (n = 15/21) of the female dogs had thrombocytopenia at least once from E1 to E6 (38 events in 15 dogs). Thrombocytopenia was more frequently detected in the blood count after the second (E3) and third (E5) chemotherapy sessions (Figure 3). It was mild / grade 1 (platelet count 100,000-200,000/µl) in most events (68.4%, n = 26/38). Grade 2 (platelet count 50,000-99,000/µl) and grade 3 (platelet count 25,000-49,000/µl) thrombocytopenia were observed in 18.4% (n = 7/38) and 13.2% (n = 5/38) of cases, respectively. The decrease in platelet count was highly significant ($p < 0.0001$) in E3, considerably significant ($p < 0.001$) in E1 and E5, and significant ($p < 0.05$) in E2, E4, and E6. The most severe thrombocytopenia cases occurred in E3 and E5 blood counts (both platelet count = 40,000/µl), performed 14 days after the second and the third chemotherapy sessions, respectively.

No animal had anemia during this clinical trial. Finally, descriptive analyzes of the different events (leukopenia, neutropenia, and thrombocytopenia), related to toxicity degree and chemotherapy cycle, showed a higher hematological adverse event in E1, E3, and E5 control blood counts with E1 being the most severe. It is noteworthy that one 12-year-old Poodle breed dog (4.7%, n = 1/21) did not present myelosuppression at any time from E0 to E6. No dog developed elevations in hepatic or renal values during treatment. Nonetheless, one animal already had a pre-existing grade 4 elevation in ALT (1,440U/l) and grade 1 in ALP (246U/l) at the beginning of the study and before hepatopathy treatment. No other female dog had abnormalities noted on pretreatment clinical and laboratory evaluations (E0).

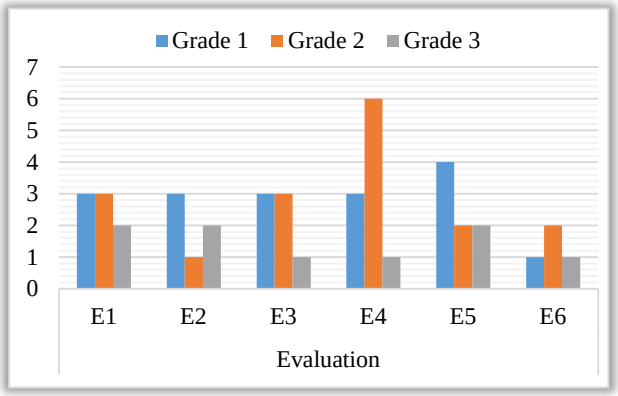

Figure 1. Number of leukopenia events (n = 43) distributed in grades from E1 to E6 in 21 female dogs who underwent adjuvant chemotherapy with gemcitabine and carboplatin combination. E1: 14 days (nadir) after first chemotherapy cycle; E2: 2 days before the second chemotherapy cycle; E3: 14 days (nadir) after second chemotherapy cycle; E4: 2 days before the third chemotherapy cycle; E5 and E6: 14 (nadir) and 19 days after third chemotherapy cycle, respectively.

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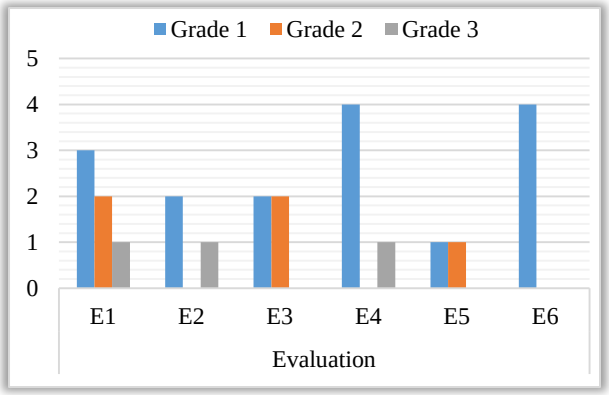

Figure 2. Number of neutropenia events (n = 24) distributed in grades from E1 to E6 in 21 female dogs who underwent adjuvant chemotherapy with gemcitabine and carboplatin chemotherapy combination. E1: 14 days (nadir) after first chemotherapy cycle; E2: 2 days before the second chemotherapy cycle; E3: 14 days (nadir) after second chemotherapy cycle; E4: 2 days before the third chemotherapy cycle; E5 and E6: 14 (nadir) and 19 days after third chemotherapy cycle, respectively.

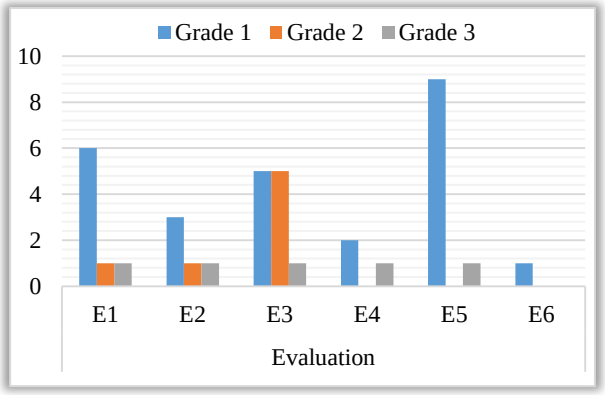

Figure 3. Number of thrombocytopenia events (n = 38) distributed in grades from E1 to E6 in 21 female dogs underwent adjuvant chemotherapy with gemcitabine and carboplatin chemotherapy combination. E1: 14 days (nadir) after first chemotherapy cycle; E2: 2 days before the second chemotherapy cycle; E3: 14 days (nadir) after second chemotherapy cycle; E4: 2 days before the third chemotherapy cycle; E5 and E6: 14 (nadir) and 19 days after third chemotherapy cycle, respectively.

Clinical findings

Clinical signs of GI toxicity were based on episodes of emesis, diarrhea, hyporexia, and weight loss. Only two dogs (9.5%, n = 2/21) had vomiting and diarrhea both a few days after the first chemotherapy session. One animal, a nine-year-old Bull Terrier, was classified with GI toxicity grade 1, and the other, a seven-year-old Border Collie, grade 2. The constitutional clinical sign of lethargy was reported by owners in three female dogs (14.3%, n = 3/21) and occurred only after the first chemotherapy cycle, classified as grade 1. Regarding other clinical adverse events, no animal had another GI sign or even other constitutional clinical (fever, hypothermia and weight loss), allergic, cardiac, dermatologic, ear and labyrinth, hemorrhagic, musculoskeletal/soft tissue, neurological, ocular, respiratory nor genitourinary disorders.

Adverse events managing

Neutropenia, thrombocytopenia, and GI signs lead to adverse events management protocols, which varied according to toxicity type and grade. Neutropenia grades 2 and 3, as well as grade 1 when ANC was under 2,500/µl, led to treatment delay of 2 to 7 days considering the severity and individual recovery time. Therefore, around 11.2% (n = 7/63) of chemotherapy cycles were postponed due to neutropenia events in E2 (n = 2/7) and E4 (n = 5/7) in 5 dogs. The two animals that showed neutropenia under cutoff value in E2, presented neutopenia again in E4. Hemogram was performed every 48 or 72 hours for neutropenic dogs and the chemotherapy session delayed was retaken when ANC reached 2,500/µl.

Prophylactic broad-spectrum antibiotic (enrofloxacin 5 mg/kg PO every 12 hours for 7 days) was prescribed for two dogs with afebrile grade 3 neutropenia with ANC under 750/µl (both in the nadir of first chemotherapy cycle, one in E1, and other in E2). One of those dogs presented afebrile grade 3 neutropenia once again in E4 (nadir of second chemotherapy cycle) nevertheless antibiotic was not prescribed since ANC was 925/µl, which is over 750/µl (Bisson et al., 2020).

Thrombocytopenia grades 3 and 2 when platelets were under 75,000/µl led to chemotherapy administration delay in 2 to 7 days considering the severity and individual recovery time. Thus, 4.8% (n = 3/63) chemotherapy cycles were postponed due to thrombocytopenia events in E2 (n = 2/3) and E4 (n = 1/3) given that one dog in E2 also had concurrent grade 3 neutropenia (ANC = 640/µl) contributing to session suspension. Hemogram was performed every 48 or 72 hours for thrombocytopenic dogs and the delayed chemotherapy session was retaken when platelets accomplished the cutoff value of 75,000/µl.

It is should be noted that neutropenia and thrombocytopenia under cutoff values occasioned chemotherapy delay only in E2 and E4 since those were the evaluations before chemotherapy sessions. Concerning GI adverse events (vomiting plus diarrhea) presented by two animals, diet adjustments, ondansetron (1.0 mg/kg PO every 12 hours), and omeprazole (1.0 mg/kg PO every 24 hours) were prescribed. No antineoplastic drug dosage has been reduced even for animals that previously showed adverse events. No dog had a life-threatening adverse event (grade 4) or even died (grade 5) of chemotherapy treatment-related complications in this clinical trial. No animal was hospitalized due to

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chemotherapy-related adverse events.

Clinical outcomes

Regarding treatment response, no dog had local recurrence nor metastasis (by 3-view radiographic examination of the thorax and abdominal ultrasonography 3 months after surgery) during the 63-day study period. After chemotherapy treatment, owners were recommended to bring their dogs for follow-up every three months to evaluate disease recurrence and metastasis. Nevertheless, 19 (90.5%) dogs lost follow-up along the long-term period of 60 months. Therefore, only two (9.5%) patients were enrolled in the overall survival time (OST) analysis (from surgical treatment to death). The first was a thirteen-year-old Daschund with carcinoma in a mixed tumor grade 2 and stage I. The latter was an eight-year-old Poodle with carcinoma in a mixed tumor grade 2 and stage III. The OST was 1,125 and 1,797 days, respectively. The cause of both deaths was unknown (information through pet owners' phone contact).

DISCUSSION

The efficacy of systemic therapy for mammary gland tumors has not been confirmed according to the highest evidence-based standards, even though chemotherapy is routinely recommended for dogs with large tumors (tumor size > 3 cm diameter), lymph node metastasis, and aggressive tumor histology after surgery (Sorenmo et al., 2020) since these features are related to recurrence / distant metastasis and shorter survival time (Rasotto et al., 2017).

Although Coffee et al. (2020) found carboplatin-induced neutropenia and thrombocytopenia especially in dogs under 15.0 kg, the findings of the current study indicated no correlation between myelosuppression and body weight. However, it must be considered that the authors studied a canine population much larger than the current study (101 versus 21) which leads to higher statistical reliability. Interestingly, the dog that had the worst neutropenic events (two episodes of grade 2 neutropenia in E3 and E6 plus two episodes of grade 3 neutropenia in E2 and E4) was a seven-year-old 25kg Border Collie. In addition, the same canine had 3 thrombocytopenic events (one grade 1 episode in E5 and two grade 2 events in E2 and E3) and was one out of the two dogs that had vomiting (grade 2) and diarrhea (grade 1) after the first chemotherapy session. Border Collie breed dogs may have polymorphisms (Alves et al., 2011) and even nt230 (del4) *ABCB1* gene mutation (Dekel et al., 2017; Marelli et al., 2020), interfering with glycoprotein P substrates metabolism. Several chemotherapeutic agents have already been identified as substrates of the glycoprotein P (Mealey, 2004; Linardi and Natalini, 2006; Mealey et al., 2017), but gemcitabine and carboplatin were not so far. Thus, no PCR test for *ABCB1* mutation was requested for the Border Collie dog that showed exacerbated toxicity compared to other animals studied.

Bone marrow suppression is a frequently encountered chemotherapy toxicity. The bone marrow cells divide rapidly, so it is a prime target since the activity of most anticancer agents is greatest in tissues with a high growth rate. The clinical result of myelosuppression is varying degrees of peripheral blood cytopenias (MacDonald, 2009). Indeed leukopenia, neutropenia, and thrombocytopenia were the most common hematological findings along 126 blood count exams (from E1 to E6) in 21 canines during gemcitabine and carboplatin doublet treatment. However, these blood adverse events were considered acceptable since most were grade 1 and no grade 4 or 5 were found. As with other authors (Dominguez et al., 2009; McMahon et al., 2011), the hematological toxicosis observed in dogs was minimal to moderate. The most frequent side effects in humans were hematological, mainly thrombocytopenia and neutropenia (Maisano et al., 2011). In the current study, leukopenia was also considered statistically significant in dogs in addition to thrombocytopenia and neutropenia. However, it should be considered that only neutropenia and thrombocytopenia are considered for clinical judgment and decision making.

In humans with lung cancer undergoing gemcitabine and carboplatin chemotherapy, a quarter of patients developed myelosuppression grades 3 and 4, as another quarter showed no sign of myelosuppression (Gréen et al., 2016). In the current study, about one-third of the animals (28.6%, n = 6/21) had grade 3 leukopenia at some point of the treatment while one female dog (4.7%, n = 1/21) did not present myelosuppression at any time. The results of the current study are similar to another research in dogs (Dominguez et al., 2009) that found grades 3 and 4 neutropenia in 32% of canines (n = 12/37) and to a pilot study in cats (Martinez-Ruzafa et al., 2009) in which two animals (14.3%, n = 2/14) developed grade 3 or 4 neutropenia. However, grades 3 and 4 neutropenia were substantially more frequent (65%) with the same antineoplastic drugs in humans (Usami et al., 2010). Regarding this discrepancy in toxicity, the doses employed for humans are different from dogs and cats, and so is the incidence of adverse events.

Neutropenia is usually the most serious and dose-limiting cytopenia associated with chemotherapeutic drug administration. Animals with neutropenia that are afebrile and asymptomatic should have chemotherapy delayed for a few days to one week based on the severity (MacDonald, 2009), as it was done in the current study in 11.2% of chemotherapy cycles (n = 7/63). Even though neutropenia was detected in 47.6% (n = 10/21) of dogs in the current study, febrile neutropenia was observed in none of the cases as expected since this event is more common with other

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anticancer agents use, such as vincristine, doxorubicin (Britton et al., 2014) and lomustine (Cunha et al., 2017). Pyrexia accompanying neutropenia is a more clinical concern than if a patient is afebrile and clinically well, as it indicates the probable presence of infection (Bisson et al., 2018). Since all neutropenia reported in this clinical trial were afebrile and dogs did not show alterations at clinical screening or the owner's questionnaire, antimicrobial prophylaxis was not prescribed for animals with grades 1 and 2 neutropenia. Two animals had grade 3 neutropenia, both clinically well, but due to ANC under 750/µl, antibiotic was prescribed according to Bisson et al. (2020) and Fournier et al. (2017) recommendation for ANC cutoff for antimicrobial prophylaxis. The enrofloxacin was chosen for antimicrobial prophylaxis due to other authors' guidance (Thamm and Vail, 2007; Vail, 2009; Boudreaux, 2014; Gustafson and Bailey, 2020). One of those two dogs showed afebrile grade 3 neutropenia (ANC = 925/µl) again, but antibiotic was not prescribed this time since ANC was over 750/µl (Bisson et al., 2018; Bisson et al., 2020) and the animal did not have risk factors, such as hematological malignancies, concomitant disease, or weight less than 14kg - was a 25kg Border Collie (Bisson et al., 2018).

Chemotherapy cycles were delayed in 23.8% (n = 5/21) of animals, while McMahon et al. (2011) deferred treatment in 12% (n = 6/50) and Dominguez et al. (2009) in 18.9% (n = 7/37) of dogs mainly due to neutropenia severity. On the other hand, in a study with cats (Martinez-Ruzafa et al., 2009) receiving gemcitabine (2mg/kg IV) on days 1 and 8 and carboplatin (10mg/kg IV) on day 1, the findings indicated that 6 out of 14 cats (42.9%) experienced treatment delays. If instead of 2,500/µl we had opted for a lower ANC cutoff value, such as 1,500/µl, as recommended by other authors (MacDonald, 2009), we probably would have reported less delayed sessions.

Dose reductions are deleterious to the optimum delivery of chemotherapy (Gustafson and Bailey, 2020). For that reason, no antineoplastic drugs dosage has been reduced for animals that previously showed adverse events in the current trial inasmuch as no grade 4 toxicity has occurred as recommended by MacDonald (2009) and different from Dominguez et al. (2009) who decided for gemcitabine and carboplatin dose reduction of 25% as a result of grade 3 neutropenia in one dog. McMahon et al. (2011) administrated the combination of carboplatin and gemcitabine in 50 dogs every 21 days. They reduced carboplatin dosage 15 times in 6 dogs (12%) during treatment cycles 2 and 3 due to grade 3 and 4 neutropenia, grade 3 thrombocytopenia, grade 2 lethargy, and grade 2 anorexia. Guidelines for dose adjustments of anticancer agents are not standardized, and the administrations are done empirically (10% to 25% dose reduction) whenever severe or unacceptable hematologic or GI toxicosis has happened (Gustafson and Bailey, 2020).

The nadir varies with individual drugs, however, it commonly occurs 5-10 days after chemotherapy administration (MacDonald, 2009). Around 38.1% of female dogs (n = 8/21) had a delayed or a second nadir about 19 days (E2, E4, and E6) after the chemotherapy session probably due to carboplatin use (Chun et al., 2007). Thrombocytopenia cutoff value of 75,000/µl followed MacDonald (2009) recommendation. Given the fact that anemia is a rare treatment-related event (MacDonald, 2009), indeed it was expected no anemia report in the current study. Considering that our study lasted 63 days, even if chemotherapeutic agents affected red cells development in bone marrow, we would not have enough time to evaluate since the canine erythrocyte lifespan is about 110 days (Lindena et al., 1986).

A study in dogs receiving gemcitabine 2mg/kg by slow intravenous infusion, followed by carboplatin 10mg/kg on day 1, gemcitabine alone on day 8, and then repeating the cycle on day 21, reported minimal to moderate hematological toxicity (Dominguez et al., 2009). Another research on dogs with osteosarcoma using gemcitabine 2mg/kg followed by carboplatin 300mg/m^2 both by intravenous infusion and repeating the cycle on day 22 concluded that the hematological toxicity of this protocol was acceptable (McMahon et al., 2011). In the present study, hematological adverse events were also considered mild to moderate. Besides, gemcitabine was infused first followed by carboplatin to avoid antagonistic effects. Further, this infusion order is considered more effective since provides synergism between chemotherapeutic agents (Wang et al., 2010).

Even though there are reports of renal function changes related to these drugs (Calvert et al., 1989; Barabas et al., 2008), no renal toxicity degree was observed. It is known that platinum derivatives tend to predispose to renal toxicity. However, when compared to cisplatin, carboplatin has been shown to be significantly less toxic (Dominguez et al., 2009). Moreover, one dog already had increased ALT (grade 4) and ALP (grade 1) activities in E0 (before the first chemotherapy session). Nevertheless, due to hepatopathy treatment (Silimarin 20mg/kg every 12 hours PO), those parameters were normalized along with the clinical trial period even with chemotherapy maintenance. The animal presented grade 2 ALT and grade 1 ALP when started the first chemotherapy cycle. Given the dog did not have marked enzyme activities increasing along with the treatment (E1 to E6), hepatotoxicity was not considered to be an important adverse effect of gemcitabine and carboplatin combined protocol. In this context, no degree of treatment-related hepatic or renal toxicity was observed in the current study. Liver toxicity due to gemcitabine has not been observed in humans either (Toschi et al., 2005; Saif, 2010).

In 20 human patients treated with gemcitabine and carboplatin, nausea and vomiting were not commonly observed (Usami et al., 2010) similar to the current study in which only two female dogs (9.5%, n = 2/21) had vomiting and diarrhea after the first chemotherapy session and no longer in the subsequent cycles even with the maintenance of antineoplastic agents dosages for following sessions. Martinez-Ruzafa et al. (2009) and Dominguez et al. (2009) studied

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tolerability of gemcitabine (2mg/kg IV) on days 1 and 8 and carboplatin (10mg/kg IV) on day 1 in cats and dogs, respectively, with carcinomas from different origins. The former found GI toxicity in 4 out of 14 cats (28.6%) while the latter observed mild to moderate severity and self-limiting GI signs in 27 out of 37 dogs (73.0%). Although both studies reported higher rates of GI adverse events, compared to the findings of the current study, they administrated gemcitabine twice totalizing 4mg/kg for cycle whereas 3mg/kg was used once for each chemotherapy cycle in the present research. Using carboplatin and gemcitabine combination every 21 days, McMahon et al. (2011) observed 17 episodes of grade 1 or 2 GI toxicity in 26% of dogs (n = 13/50), including anorexia, nausea, diarrhea, and vomiting. The GI toxicity signs management with diet adjustments, antiemetic (ondansetron), and proton pump inhibitor (omeprazole) was performed according to Gustafson and Bailey (2020) recommendations.

Given the prophylactic medication, the vomiting low rate might be due to ondansetron prescription. It means that the incidence and degree of GI toxicosis may be underestimated in the current study. Nevertheless, Dominguez et al. (2009) also used concurrent metoclopramide as prophylactic antiemetic medication in 22 of 37 dogs and found GI toxicosis in 27 animals (73%, n = 27/37), which is considerably higher than the results of the present study. This could be related to gemcitabine only once administered in the present protocol or even to ondansetron superiority over metoclopramide as an antiemetic for dogs receiving chemotherapy. Metoclopramide had no effect on either vomiting or nausea for dogs treated with cisplatin while 5-HT3 receptor antagonist ondansetron demonstrates greater antiemetic and anti-nausea efficacy over metoclopramide and maropitant (Kenward et al., 2017).

Less frequent adverse events have been reported in humans by some authors, such as acute myocardial infarction, skin rash, thrombosis, or pulmonary embolism (Fidias et al., 2008; Lee et al., 2009), pulmonary toxicity (Baig et al., 2019), neurotoxicity (Joerger et al., 2002; McWhinney et al., 2009), skin fibrosis (Hashimoto et al., 2011), and skin necrosis (Holstein et al., 2010), even though these effects were not observed in the current study.

Most veterinary patients enjoy a good quality of life while on chemotherapy treatment (MacDonald, 2009) as seen in the present study. Therefore, anticipating and managing adverse events is essential to allow chemotherapeutic administration modifications and improve the quality of life of dogs during treatment (Gustafson and Bailey, 2020).

Chemotherapy may fail due to unacceptable toxicity (Gustafson and Bailey, 2020). The knowledge of gemcitabine and carboplatin doublet therapy main side effects in canine cancer patients, especially in a homogeneous group, including same tumor (mammary neoplasm) and treatment objective (adjuvant therapy following mastectomy), is essential on veterinary oncologist practicing. Understanding drug activity and chemotherapy toxicity, can help the physician in treatment decision making and ensuring dog owners are appropriately educated as to the type and likelihood of adverse events and planning for appropriate preventive and therapeutic protocols to manage them (Vail, 2009; Gustafson and Bailey, 2020).

The lack of commitment of the owners with their animals' follow-up led to insufficient information collection about disease-free intervals and overall survival time. Since we obtained the OST data of only two female canines (OST = 1,125 and 1,797 days) and no further information, it is not possible to evaluate the efficacy of gemcitabine and carboplatin doublet chemotherapy protocol in reducing disease recurrence and metastasis or increasing survival time.

The current prospective clinical trial had some limitations, including the small number of female dogs, evaluations lasting until the third chemotherapy cycle without further monitoring of most dogs, administration of only one schedule and dosage, and GI toxicity probably underestimated due to prophylactic ondansetron therapy. Therefore, further long-term follow-up studies aiming at survival time, therapeutic gain, maximum tolerated dose, biologically effective dose, and dosing schedule to make these drugs combination use more effective and less toxic as possible are required.

Even though novel drugs are a great promise for cancer treatment in animals, optimizing the use of currently already available agents might bring new benefits (Thamm and Gustafson, 2020). In this regard, both gemcitabine and carboplatin are medications known for decades, but there is still a lack of information about these chemotherapeutic agents association for use in dogs. The current study represented the first assessment of toxicity and safety of gemcitabine and carboplatin given in combination for mammary cancer adjuvant treatment in female dogs.

CONCLUSION

The adjuvant chemotherapy protocol with gemcitabine and carboplatin combination was well-tolerated and safe in female dogs with mammary cancer. Most hematological and GI adverse events were considered mild to moderate, but all self-limiting.

ECLARATIONS

Authors' contribution

All authors have made substantial contributions to all parts of the study. CBI and MLGF designed the project.

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CBI and TM performed animals' selection, surgery, chemotherapy, clinical evaluations, sampling, analysis, and interpretation of data and literature review. AMRF carried out the histopathological analysis. CBI executed data collection. CBI and TM prepared the draft manuscript. TM and MLGF critically reviewed and edited writing. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

REFERENCES

Alves L, Hulsmeyer V, Jaggy A, Fischer A, Leeb T, and Drogemullee M (2011). Polymorphisms in the ABCB1 Gene in Phenobarbital Responsive and Resistant Idiopathic Epileptic Border Collies. Journal of Veterinary Internal Medicine, 25(3): 484-489. DOI: https://www.doi.org/10.1111/j.1939-1676.2011.0718.x

Anjos CPG, Vasconcelos AC, Crosara PFTB, Anjos GC, Beckere CG, and Guimarães RES (2012). Poptosis in eosinophilic nasal polyps treated in vitro with mitomycin C. Brazilian Journal of Otorhinolaryngology, 78(3): 32-37. Available at: http://www.bjorl.org.br/en-apoptosis-in-eosinophilic-nasal-polyps-articulo-S180886941530241X

Baig J, Shokouh-Amiri M, Chan J, Chowdhery R, Danthurthy S, and Venepalli NK (2019). The spectrum of pulmonary toxicity in pancreatic cancer patients receiving gemcitabine combination chemotherapy. Case Reports in Oncology, 12(2): 506-512. Available at: https://www.karger.com/Article/FullText/500242

Barabas K, Milner R, Lurie D, and Adin C (2008). Cisplatin: A review of toxicities and therapeutic applications. Veterinary and Comparative Oncology, 6: 1-18. DOI: https://www.doi.org/10.1111/j.1476-5829.2007.00142.x

Bisson JL, Argyle DJ, and Argyle SA (2018). Antibiotic prophylaxis in veterinary cancer chemotherapy: A review and recommendations. Veterinary and Comparative Oncology, 16(3): 301-310. DOI: https://www.doi.org/10.1111/vco.12406

Bisson JL, Fournier Q, Johnston E, Handel I, and Bavcar S (2020). Evaluation of a 0.75 × 109/L (=750 /uL) absolute neutrophil count cut-off for antimicrobial prophylaxis in canine cancer chemotherapy patients. Veterinary and Comparative Oncology, 18(3): 258-268. DOI: https://www.doi.org/10.1111/vco.12544

Boudreaux B (2014). Antimicrobial use in the veterinary cancer patient. Veterinary Clinics of North America: Small Animal Practice, 44(5): 883-891. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0195561614000825?via%3Dihub

Britton BM, Kelleher ME, Gregor TP, and Sorenmo KU (2014). Evaluation of factors associated with prolonged hospital stay and outcome of febrile neutropenic patients receiving chemotherapy: 70 cases (1997–2010). Veterinary and Comparative Oncology, 12(4): 266-276. DOI: https://www.doi.org/10.1111/vco.12001

Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, Siddik ZH, Judson IR, Gore ME, and Wiltshawet E (1989). Carboplatin dosage: prospective evaluation of a simple formula based on renal function. Journal of Clinical Oncology, 7(11): 1748-1756. Available at: https://ascopubs.org/doi/abs/10.1200/JCO.1989.7.11.1748

Cassali GD, Jark PC, Gamba C, Damasceno KA, EstrelaLima A, De Nardi AB, Ferreira E, Horta RS, Firmo BF, Sueiro FAR et al. (2020). Consensus Regarding the Diagnosis, Prognosis and Treatment of Canine and Feline Mammary Tumors - 2019. Brazilian Journal of Veterinary Pathology, 13(3): 555-574. Available at: https://bjvp.org.br/banner/2020/11/v-13-n-3-1/

Chun R, Garret L and Vail D (2007). Cancer chemotherapy. In: Withrow SJ, Vail DM (Editors), Withrow& MacEwen's Small Animal Clinical Oncology. 4th Ed. Saunders Elsevier, St. Louis, Missouri, USA, pp. 163-192.

Coffee C, Roush JK, and Higginbotham ML (2020). Carboplatin-induced myelosuppression as related to body weight in dogs. Veterinary and Comparative Oncology, 18(4): 804-810. DOI: https://www.doi.org/10.1111/vco.12622

Cunha SCS, Silva FBF, Corgozinho KB, Silva KVGC, and Ferreira AMR (2017). Adverse Effects of Chemotherapy in Dogs. World's Veterinary Journal, 7(3): 74-82. Available at: http://wvj.science-line.com/vol-7-no-3-sep-2017.html

Dekel Y, Machluf Y, Stoler A, Aderet A, Baumel D, Kellerman E, Plotsky Y, Partouche ON, Elhalal G, Ben-Shlomo I et al. (2017). Frequency of canine nt230(del4) MDR1 mutation in prone pure breeds, their crosses and mongrels in Israel – insights from a worldwide comparative perspective. BMC Veterinary Research, 13: 333-341. Available at: https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-017-1251-9

Dominguez PA, Dervisis NG, Cadile CD, Sarbu L, and Kitchell BE (2009). Combined gemcitabine and carboplatin therapy for carcinomas in dogs. Journal of Veterinary Internal Medicine, 23: 130-137. DOI: https://www.doi.org/10.1111/j.1939-1676.2008.0248.x

Dunn OJ (1964). Multiple comparisons using rank sums. Technometrics, 6(3): 241-252. Available at: https://amstat.tandfonline.com/doi/abs/10.1080/00401706.1964.10490181

Fichtinger-Schepman AM, van der Veer JL, den Hartog JH, Lohman PHM, and Reedijk J (1985). Adducts of the antitumor drug cis-diamminedichloroplatinum (II) with DNA: formation, identification, and quantitation. Biochemistry, 24(3): 707-713. Available at: https://pubs.acs.org/doi/pdf/10.1021/bi00324a025

Fidias PM, Dakhil SR and Lyss AP (2008). Phase III study of immediate compared with delayed docetaxel after front-line therapy eith gemcitabine plus carboplatin in advanced nonsmall-cell lung cancer. Journal of Clinical Oncology, 27(4): 591-598. Available at: https://ascopubs.org/doi/pdfdirect/10.1200/jco.2008.17.1405

Fournier Q, Serra JC, Handel I, and Lawrence J (2017). Impact of pretreatment neutrophil count on chemotherapy administration and toxicity in dogs with lymphoma treated with CHOP chemotherapy. Journal of Veterinary Internal Medicine, 32: 384-393. DOI: https://www.doi.org/10.1111/jvim.14895

Garnett CL, Guerrero TA, and Rodriguez Jr CO (2016). Fixed dose-rate administration of gemcitabine in cancer-bearing cats: A pilot study. The Canadian Veterinary Journal, 57(11): 1149-1155. Available at: http://europepmc.org/article/med/27807377

Gréen H, Hasmats J, Kupershmidt I, Edsgard D, Petris L, Lewensohn R, Blackhall F, Vikingsson S, Besse B, Lindgren A et al. (2016). Using Whole-Exome Sequencing to Identify Genetic Markers for Carboplatin and Gemcitabine-Induced Toxicities. Clinical Cancer Research, 22(2): 366-373.

Available at: https://clincancerres.aacrjournals.org/content/clincanres/22/2/366.full.pdf

Gustafson DL, and Bailey DB (2020). Cancer Chemotherapy. In: Vail DM, Thamm DH, Liptak JM (Editors), Withrow & MacEwen's Small Animal Clinical Oncology. 6th Ed. Saunders Elsevier, St. Louis, Missouri, USA, pp. 182-208.

Hashimoto A, Yamamoto N, Hata Y, Takahashi M, and Shimokawa R (2011). Dermal fibrosis related to combination chemotherapy with carboplatin and gemcitabine. European Journal of Dermatology, 21(6): 999-1001. Available at: https://www.jle.com/fr/revues/ejd/e-docs/dermal_fibrosis_related_to_combination_chemotherapy_with_carboplatin_and_gemcitabine_291038/article.phtml

Holstein A, Batge R, and Egberts EH (2010). Gemcitabine induced digital ischaemia and necrosis. European Journal of Cancer Care, 19(3): 408-409. DOI: https://www.doi.org/10.1111/j.1365-2354.2008.01057.x

Joerger M, Gunz A, Speich R, and Pestalozzi BC (2002). Gemcitabine-related pulmonary toxicity. Swiss Medical Weekly, 132(1-2): 17-20. Available at: https://pubmed.ncbi.nlm.nih.gov/11901447/

Kenward H, Elliott J, Lee T, and Pelligand L (2017). Anti-nausea effects and pharmacokinetics of ondansetron, maropitant and metoclopramide in a low-dose cisplatin model of nausea and vomiting in the dog: a blinded crossover study. BMC Veterinary Research, 13(244): 1-12. Available at: https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-017-1156-7

Lana SE, Rutteman GR, and Withrow SJ (2007). Tumors of the mammary gland. In: Withrow SJ, Vail DM (Editors), Withrow& MacEwen's Small Animal Clinical Oncology. 4th Ed. Saunders Elsevier, St. Louis, Missouri, USA, pp. 619-638.

Lavalle GE, De Campos CB, Bertagnolli AC, and Cassali GD (2012). Canine malignant mammary gland neoplasms with advanced clinical staging treated with carboplatin and cyclooxygenase inhibitors. In Vivo, 26(3): 375-379. Available at: https://pubmed.ncbi.nlm.nih.gov/22523289/

Leblanc AK, Atherton M, Bentley RT, Boudreau CE, Burton JH, Curran KM, Dow S, Giuffrida MA, Kellihan HB, Mason NJ et al. (2021). Veterinary Cooperative Oncology Group - Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) following investigational therapy in dogs and cats. Veterinary and Comparative Oncology, pp. 1-42. DOI: https://www.doi.org/10.1111/vco.12677

Lee SM, Rudd R, and Woll PJ (2009). Randomized double-blind placebo-controlled trial of thalidomide in combination with gemcitabine and carboplatin in advanced non-small lung cancer. Journal of Clinical Oncology, 27(31): 5248-5254. Available at: https://ascopubs.org/doi/10.1200/JCO.2009.21.9733

Linardi RL, and Natalini CC (2006). Multi-drug resistance (MDR1) gene and P-glycoprotein influence on pharmacokinetic and pharmacodymanic of therapeutic drugs. Ciência Rural, 36: 336-341. Available at: https://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782006000100056

Lindena J, Wittenberg H, Diederichs F, and Trautschold I (1986). The Decline of Catalytic Enzyme Activity Concentration of In Vivo Ageing Erythrocytes of the Man, the Dog and the Rat. Journal of clinical chemistry and clinical biochemistry, 24(1): 49-59. Available at: https://edoc.hu-berlin.de/bitstream/handle/18452/13008/cclm.1986.24.1.49.pdf?sequence=1

MacDonald V (2009). Chemotherapy: Managing side effects and safe handling. The Canadian Veterinary Journal, 50(6): 665-668. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2684058/

Maisano R, Zavettieri M, Azzarello D, Raffaele M, Maisano M, Bottari M, and Nardi M (2011). Carboplatin and Gemcitabine Combination in Metastatic Triple-negative Anthracycline and Taxate- Pretreated Breast Cancer Patient: a Phase II study. Journal of Chemotherapy, 23: 40-43. Available at: https://www.tandfonline.com/doi/abs/10.1179/joc.2011.23.1.40

Marelli SP, Polli M, Frattini S, Cortellari M, Rizzi R, and Crepaldi P (2020). Genotypic and allelic frequencies of MDR1 gene in dogs in Italy. Veterinary Record Open, 7(1): 1-5. Available at: https://europepmc.org/article/med/32617164

Martinez-Ruzafa I, Dominguez PA, Dervisis NG, Sarbu L, Newman RG, Cadile CD, and Kitchell BE (2009). Tolerability of gemcitabine and carboplatin doublet therapy in cats with carcinomas. Journal of Veterinary Internal Medicine, 23(3): 570-577. DOI: https://www.doi.org/10.1111/j.1939-1676.2009.0279.x

McMahon M, Mathie T, Stingle N, Romansik E, Vail D, and London C (2011). Adjuvant Carboplatin and Gemcitabine Combination Chemotherapy Postamputation in canine Appendicular osterosarcoma. Journal of Veterinary Internal Medicine, 25: 511-517. DOI: https://www.doi.org/10.1111/j.1939-1676.2011.0697.x

McWhinney SR, Goldberg RM, and McLeod HL (2009). Platinum neurotoxicity pharmacogenetics. Molecular Cancer Therapeutics, 8: 10-16. Available at: https://mct.aacrjournals.org/content/molcanther/8/1/10.full.pdf

Mealey KL (2004). Therapeutic implications of the MDR-1 gene. Journal of Veterinary Pharmacology and Therapeutics, 27(5): 257-264. DOI: https://www.doi.org/10.1111/j.1365-2885.2004.00607.x

Mealey KL, Dassanayake S, and Burke NS (2017). Establishment of a cell line for assessing drugs as canine P-glycoprotein substrates: proof of principle. Journal of Veterinary Pharmacology and Therapeutics, 40(5): 545-551. DOI: https://www.doi.org/10.1111/jvp.12390

Medeiros VB (2017). Câncer da mama na cadela. Journal of Surgical and Clinical Research, 8: 118-129. DOI: https://www.doi.org/10.20398/jscr.v8i1.13039

Owen LN (1980). Mammary Glands. In: Owen LN (Editor), TNM Classification of tumours in Domestic Animals. 1st Ed. World Health Organization, Geneva, Switzerland, pp. 16-20.

Rasotto R, Berlato D, Goldschmidt MH and Zappulli V (2017). Prognostic significance of canine mammary tumor histologic subtypes: an observational cohort study of 229 cases. Veterinary Pathology, 54(4): 571-578. Available at: https://journals.sagepub.com/doi/10.1177/0300985817698208

Repetti CFS, Daleck CR (2007). Associação do methimazole e do ondansetron à quimioterapia com cisplatina em cães submetidos a quatro diferentes protocolos de fluidoterapia. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 59 (3): 665-672. Available at: https://www.scielo.br/scielo.php?script=sci_abstract&pid=S0102-09352007000300018&lng=en&nrm=iso&tlng=en

Rodaski S, and De Nardi (2008). Quimioterapia Antineoplásica em cães e gatos. 3rd Ed. MedVet, São Paulo, SP, Brasil, pp. 305.

Rodaski S, De Nardi AB, and Piekarz CH (2008). Quimioterapia Antineoplásica. In: Daleck CR, De Nardi AB, Rodaski S (Editors), Oncologia em cães e gatos. 1st Ed. Roca, São Paulo, SP, Brasil, pp. 161-177.

Saif M (2010). Pulmonary toxicity associated with gemcitabine. Journal of the Pancreas, 11(2): 189-190. Available at: http://www.serena.unina.it/index.php/jop/article/view/3859

Scheffé H (1999). The Analysis of Variance. 1st Ed. Wiley, New York, USA, p. 53.

Selmic LE, Burton JH, Thamm DH, Withrow SJ, and Lana SE (2014). Comparison of carboplatin and doxorubicin-based chemotherapy protofols in 470 dogs after amputation for treatment of appendicular osteosarcoma. Journal of Veterinary Internal Medicine, 28(2): 554-563. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4857984/

Silva AA, Carlotto J, and Rotta I (2018). Padronização da ordem de infusão de medicamentos antineoplásicos utilizados no tratamento dos cânceres de mama e colorretal. Einstein, 16(2): 1-9. Available at: https://www.scielo.br/pdf/eins/v16n2/pt_1679-4508-eins-16-02-eRW4074.pdf

Sorenmo KU, Worley DR, and Zappulli V (2020). Tumors of the Mammary Gland. In: Vail DM, Thamm DH, Liptak JM (Editors), Withrow & MacEwen's Small Animal Clinical Oncology. 6th Ed. Saunders Elsevier, St. Louis, Missouri, USA, pp. 604-625.

Thamm DH, and Gustafson DL (2020). Drug dose and drug choice: Optimizing medical therapy for veterinary cancer. Veterinary and Comparative

Oncology, 18(2): 143-151. DOI: https://www.doi.org/10.1111/vco.12537

Thamm DH, and Vail DM (2007). Aftershocks of cancer chemotherapy: managing adverse effects. The American Animal Hospital Association, 43: 1-7. DOI: https://www.doi.org/10.5326/0430001

Toschi L, Finochiaro G, Bartolini S, Gioia V, and Cappuzzo F (2005). Role of gemcitabine in cancer therapy. Future Oncology, 1: 7-17. Available at: https://www.futuremedicine.com/doi/full/10.1517/14796694.1.1.7

Usami N, Yokoi K, Hasegawa Y, Taniguchi H, Shindo J, Yamamoto M, Suzuki R, Imaizumi K, Kondo M, and Shimokata K (2010). Central Japan Lung Study Group: phase II study of carboplatin and gemcitabine as adjuvant chemotherapy in patients with completely resected non-small cell lung cancer: a report from the Central Japan Lung Study Group. International Journal of Clinical Oncology, 15: 583-587. Available at: https://link.springer.com/article/10.1007/s10147-010-0118-x

Vail DM (2009). Supporting the Veterinary Cancer Patient on Chemotherapy: Neutropenia and Gastrointestinal Toxicity. Topics in Companion Animal Medicine, 24(3): 122-129. Available at: https://www.sciencedirect.com/science/article/abs/pii/S1938973609000154

Viana FAB (2007). Guia terapêutico veterinário. 2nd Ed. Cem, Lagoa Santa, MG, Brasil, pp. 463. Available at: https://www.magazineluiza.com.br/guia-terapeutico-veterinario-4-edicao-editora-cem/p/eja6c90hc4/li/ldvt/

Wagstaff AJ, Ward A, Benfield P, and Heel RC (1989). Carboplatin. A Preliminary Review of its Pharmacodynamic and Pharmacokinetic Properties and Therapeutic Efficacy in the Treatment of Cancer. Drugs, 37: 162-190. Available at: https://link.springer.com/article/10.2165/00003495-198937020-00005

Wang S, Zhang H, Cheng L, Evans C, and Pan CX (2010). Analysis of the cytotoxic activity of carboplatin and gemcitabine combination. Anticancer Ressearch, 30(11): 4573-4578. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4562399/pdf/nihms720106.pdf

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Pharmacokinetics of the Slow-release Drug in the Form of Moxidectin-based Solution for Dogs and Cats

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ABSTRACT

The pharmacokinetic characteristics of the moxidectin-based drugs have been studied in the blood serum of animals after a single oral administration of the drug at the therapeutic dose in form of syrup. The drug is intended to control parasitic diseases of cats and dogs. The present studies on cats and dogs (drug administration and blood sampling) were conducted in the experimental farm of Kurilovo, Russia, for three months. The study involved six dogs and six cats, half breed, aged one to four years. The samples included six dogs (four male and two female) and six cats (three male and three female), and groups were formed according to the principle of analog groups. The drug, moxidectin, was orally administered once at the dose of 1.5 mg per one kg of animal's weight. The active substance of the drug was identified in the blood serum of animals by High-Performance Liquid Chromatography (HPLC) with fluorescence detection. The result of the current study showed that based on the pharmacokinetics of moxidectin, the concentration of the active substance in the blood serum after three hours reached 134.80-498.09 ng/ml in cats and 479.07-1459.40 ng/ml in dogs. The obtained results indicated that a single administration of the drug at the recommended therapeutic dose could ensure the maintenance of therapeutic concentrations of moxidectin in the blood, and accordingly, the protection of animals from parasites for up to 90 days.

Keywords: Cats, Dogs, Moxidectin, Pharmacokinetics, Solution

INTRODUCTION

Numerous studies have been performed on a comparative assessment of the pharmacokinetics of various macrocyclic lactones. Macrocyclic lactones are natural avermectins produced by soil actinomycetes of the species *Streptomyces avermitilis*, and they are structurally similar to milbemycins (produced by *Streptomyces hygroscopicus*) that possess a wide spectrum of both nematicidal and insectoacaricidal activities. The most widespread macrocyclic lactones are kindred; natural products include avermectin B1a and B1b, aversect-1, doramectin, milbemectin, as well as milbemycins A3 and A4, and semi-synthetics are also used in products, such as ivermectin, eprinomectin, selamectin, lepimectin, and moxidectin which are a nemadectin derivative (Platonova and Avsevieva, 2018; Jafarov et al., 2019).

The study on the effect of their pharmacokinetics on fat deposition in pigs showed an increased resistance of moxidectin in the plasma of pigs having a normal diet, compared to those having a diet with an increased linoleic acid content, while there were no differences in the pharmacokinetic of ivermectin among animals receiving maintenance or normal ration (Craven et al., 2002). The decreased rate of fat deposition affected the pharmacokinetic location of highly lipophilic drugs like moxidectin, but did not affect the pharmacokinetic location of the less lipophilic drugs, such as ivermectin. Given the pharmacokinetic parameters for the parent molecules, the persistence of doramectin and moxidectin is significantly longer than that of ivermectin, which may positively influence their efficacy after subcutaneous injection, associated with an interval among doses (Oukessou et al., 1999; Sallovitz et al., 2003; McCall, 2005; Al-Azzam et al., 2007; Gokbulut et al., 2010).

The pharmacokinetics of moxidectin was proportional to the doses used in previous studies (3 to 36 mg per person) and a long half-life was noted on average as 20-35 days. Thus, the results have shown that moxidectin is safe and well-tolerated by the body at doses ranging from 0.05 to 0.6 mg/kg. It is noted that moxidectin in liquid dosage form reaches its maximum concentration in blood plasma faster than tablets by 0.9 hours (Cotreau et al., 2003, Korth-Bradley et al., 2012). Regarding a recent study on the pharmacokinetics of moxidectin in Gelmintal Syrup on cats and dogs at single administered at a dose of 0.3 mg/kg, it was found that the maximum moxidectin concentration in blood of cats reached 9.3 ng/ml after 3 hours of drug administration, dropped to 3.0 ng/ml by 24 hours, and remained at 1-3 ng/ml up to 720 hours (Arisov et al., 2016a). In another study addressing the effect of the drug on dogs, a similar procedure was observed. The maximum concentration was reached three to six hours after the administration (once at a dose of 0.3 mg/kg) (75.1 ng/ml), and by 24 hours, it decreased to 12-16 ng/ml and remained in the range of two to five ng/ml

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throughout the study (Arisov et al., 2016b). Although many studies on the pharmacokinetics of moxidectin have been conducted worldwide, the presence of this substance in therapeutic concentrations in the serum of domestic carnivores for such a long time as 90 days has not been studied. The present study aimed to investigate the pharmacokinetics of moxidectin in the blood serum of dogs and cats after a single application of the drug "Neoterica Protecto syrup" at the recommended therapeutic dose of 1.5 mg/kg for 90 days.

MATERIALS AND METHODS

Ethical approval

When the experiment was being designed, the involved researchers were guided by the principles of humane treatment of experimental animals according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123). Animal handling was controlled according to directive 2010/63/EU of the European Parliament and the European Union Council dated 22 September 2010 on the protection of animals used for scientific purposes.

Experimental groups

The experiment involved six mongrel dogs (four males and two females) aged one to five years weighing 9.3-14.7 kg, and six mongrel cats (four males and two females) aged one to five years weighing 1.5-4.5 kg. The animals were kept in nursery conditions, and had not received any chemotherapeutic drugs (30 days before the study), and were clinically healthy (Guidelines for the examination of medicines, 2013).

Sample collection

To study the pharmacokinetics of moxidectin in the body of cats and dogs after the administration, the drug was individually administered orally in a single dose of 1.5 mg per one kg of animal's weight. Blood samples were collected from the internal femoral vein or the anterior saphenous vein of the forearm with sterile needles directly into special tubes. Two ml of blood samples from the cats and 2-5 ml from the dogs were taken before the drug administration, and this procedure was repeated after 3, 6, 12, and 24 hours as well as 3, 6, 10, 20, 45, 60, 75, and 90 days. At each study period, blood samples were taken from six cats and six dogs (Guidelines for the examination of medicines, 2013).

Blood samples were collected in cipher-marked polymeric disposable tubes without a coagulation activator. At least 1 ml of the serum samples was separated and taken into cipher-marked Eppendorf tubes, and the serum samples were frozen. All samples were stored in a freezer at a temperature of -30°C until the initiation of the study. The main parameter that was identified during the study was the moxidectin content in the blood serum. The pharmacokinetic parameters of the active substance in the body of cats and dogs were calculated (using the PKSolver - a program that includes a formula for calculating) based on the obtained results (Alvinerie M. et al., 1995, Zhang Y. et al., 2010).

Methodology of experiment

The concentrations of moxidectin in the serum samples of cats and dogs were measured using a validated technique. The stability and correctness of the measurements were monitored by adding them to the analytical run of calibration standards for comparison and blank samples of bioactive matrices, and control samples were prepared based on blood serum free of any analyte. The analyte and internal standards were identified by comparing the peak retention times of these components. To determine the retention times, the obtained extracts were processed according to the procedure for preparing blood serum samples containing specified concentrations of moxidectin and internal standards.

When obtaining calibration graphs for moxidectin, linear interpolation with an intercept was used (y: kx+b) with the balance 1/x depending on moxidectin concentration (C_{Mox}) in blood. To calculate the concentrations in the studied blood serum samples, the equations obtained for the trend line of the calibration graphs were utilized by extracts of the blood serum sample model:

$$C_{Mox}: \frac{\frac{S_{Mox}}{S_{IS}} - b}{k},$$

Where, *k* is a slope coefficient of the calibration function and *b* denotes an intercept of the calibration function.

Equipment

The utilized pieces of equipment in the current study included Shimadzu AUW220D laboratory balance (Shimadzu, Japan), Shimadzu LC-20 Prominence chromatographic system (Shimadzu, Japan), Kromasil 100-3.5-C8 chromatographic column 3.0 x 150 mm (Nouryon, Netherlands), Kromasil 100-3.5-C8 pre-column 2.1x10 mm (Nouryon, Netherlands), Biosan Vortex V-1Plus Vortex (BioSan, Latvia), SNOL 58/350 low-temperature electric oven (drying oven) (SNOL-THERM, Russia), SNOL 7.2/900 laboratory electric furnace (muffle furnace) (SNOL-THERM, Russia), Sartorius mechanical dispensers (Sartorius AG, Germany), Eppendorf 5418 centrifuge (Eppendorf, Germany).

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Statistical analysis

The results were statistically processed using Microsoft Excel, 2013. The Descriptive statistics of the obtained data included finding the mean values, and relative standard deviations from the mean, and standard errors in Microsoft Excel. The pharmacokinetic parameters were calculated using the PKSolver program (add-in for Microsoft Excel) (Zhang et al., 2010). Figures were made using MS Excel and Shimadzu LabSolutions programs.

RESULTS

The results of studying the pharmacokinetics of moxidectin in the blood serum of cats and dogs have shown that moxidectin is rapidly absorbed from the gastrointestinal tract, and its concentration reaches its maximum values by three hours in both cats and dogs. Maximum moxidectin concentrations in the serum ranged from 136.211 to 467.116 ng/ml in cats and 491.861 to 1370.217 ng/ml in dogs. Then, the active substance concentration in the blood serum of animals decreased and was determined by 90 days after the administration in the range of 1.310-2.603 ng/ml and 1.268-2.821 ng/ml in cats and dogs, respectively. Moxidectin concentrations were identified in the blood serum of all animals on day 90 and were above the lower limit of quantitative determination (1 ng/ml).

The pharmacokinetic parameters of moxidectin were calculated using the PKSolver (Zhang et al., 2010), and are presented in tables 1 and 2. Changes in the moxidectin concentration in the cats involved in the experiment are shown in Figure 1. The figure shows the mean values of observed concentrations and interpolation lines obtained as a result of applying the model hypothesis of the moxidectin distribution using an approximant in the approximation of a two-compartment model of the active substance distribution. In addition, marked confidence intervals (CI = 0.95) characterized the variability of individual concentrations.

The volume of distribution is a hypothetical volume of body fluid required to evenly distribute the entire administered dose at a concentration similar to that in blood plasma. High volume of distribution indicated that a drug actively penetrates into biological fluids and tissues. If a drug is actively bound, for example, by adipose tissue, its concentration in the blood can almost instantly become very low, and the volume of distribution will reach several hundred liters, and exceed the actual amount of body fluids. In this regard, it is also called as apparent Volume of Distribution (Vss). In the present study, Vss averaged 0.0972 ng/ml in cats, and 0.019428 ng/ml in dogs. Total body clearance is the amount of plasma or blood that is completely cleared of a drug in a time unit. Due to the fact that the main excretion routes are the kidneys and the liver, and the total body clearance is the sum of renal and hepatic clearance. The main physiological factors that determine clearance are functional state of the main physiological systems of the body, blood supply volume, and blood velocity in the organ. As a result of the studies, CL averaged 0.000093 (ng/ml)/h in cats, and 0.000084 (ng/ml)/h in dogs. A half-life is the time required to reduce the drug concentration in plasma by 50%. Fifty percent of a drug is excreted from the body in almost one half-life period, 75% in two periods, and 87% in three periods, etc. In the current study, the half-life ($t_{1/2}$) averaged 935 hours (39 days) in cats, and 1096 hours (45 days) in dogs.

Table 1. Pharmacokinetic parameters of moxidectin in the blood serum samples of cats after a single oral administration of the drug at a dose of 1.5 mg/kg within 90 days

Parameter	Cat number						Mean	RSD (%)
	1	2	3	4	5	6		
$t_{1/2}$, h	1548.241	737.2329	1113.7695	645.8868	416.9375	1148.7031	935.128	43.95
Tmax, 1/h	3	3	3	3	3	3	3	0
Cmax, ng/ml	136.211	289.563	467.116	405.359	376.221	168.924	307.232	43.33
C_0, ng/ml	0	0.018	596.61265	0.065	0.054	0.040	0.0378	64.49
Clast /Cmax	0.01082	0.006613	0.0028515	0.006421	0.003817	0.007754	0.00637	44.71
AUC $_{0\text{-}t}$, ng/ml *h	8608.841	12904.37	20845.367	20882.20	20797.35	8964.15	15351.2	38.38
$AUC_{0\text{-}inf}$, ng/ml *h	11901.22	14941.17	22985.664	23307.73	21661.13	11135.119	17506.2	31.34
AUC $_{0\text{-}t}$/0-inf	0.72335	0.863678	0.9068855	0.895935	0.960123	0.805034	0.0835	9.72
AUMC 0-inf, ng/ml *h^2	1941475	1334016	15238669	1680769	1017160	13441464	1473572	21.65
MRT, h	1631.323	892.8460	662.96405	721.1209	469.5784	1207.1235	935.3	44.93
Vz,(ng/ml)	0.281522	0.106779	0.1048586	0.059968	0.041654	0.223243	0.1370	69.25
CL, (ng/ml)/h	0.000126	0.000100	0.000065	0.000064	0.000069	0.000134	0.000093	33.34
Vss, (ng/ml)	0.205607	0.089636	0.0432637	0.046408	0.032517	0.162610	0.0972	73.25

RSD: Relative standard deviation; $t_{1/2}$: Drug elimination time from the body by biotransformation and excretion of 1/2 of the administered or received and absorbed dose; T_{max}: Time to reach the maximum concentration of the active substance; C_{max}: Active ingredient maximum concentration; Clast: last measured concentration of a substance; $AUC_{0\text{-}t}$: Area under the curve "active substance concentration-time" in the time frame from 0 to the moment (t) of the last biomaterial sampling; $AUC_{0\text{-}inf}$: Area under the curve "active substance concentration-time" in the timeframe from 0 to ∞; $AUMC_{0\text{-}inf}$: Area under the curve "product of time and drug concentration"; MRT: The average substance retention time in the systemic circulation; Vz: Distribution volume - the ratio of the total content of a substance in the body to its serum concentration; CL: Clearance or extraction coefficient - an indicator of the of a substance excretion rate from the body; V_{ss}: apparent volume of distribution at equilibrium.

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Table 2. Pharmacokinetic parameters of moxidectin in the blood serum samples of dogs after a single oral administration of the drug at a dose of 1.5 mg/kg within 90 days

Parameters	Dog number						Mean	RSD (%)
	1	2	3	4	5	6		
$t_{1/2}$ (h)	957.773	1703.632	1508.1140	1343.670	462.827	599.555	1095.928	45.93
Tmax (1/h)	3	3	3	3	3	3	3.0	0
Cmax (ng/ml)	887.536	1058.026	1129.64	1370.217	521.31	491.861	909.765	38.34
Clast /Cmax	0.001428	0.001901	0.0022219	0.001577	0.004120	0.005735	0.002830	60.92
AUC $_{0\text{-}t}$ (ng/ml*h)	28585.27	34217.60	42851.047	38523.5	33635.9	44936.0	37124.88	16.57
$AUC_{0\text{-}inf}$ (ng/ml*h)	30337.36	39162.73	48312.176	42712.64	35070.16	47376.10	40495.19	17.38
AUC $_{0\text{-}t}$ /0-inf	0.942246	0.873728	0.8869616	0.901923	0.959103	0.948495	0.91874	3.88
AUMC 0-inf (ng/ml*h^2)	13025019	31274176.1	34442217	26415797.8	19667710.9	27823896.3	25441469	30.90
MRT (h)	429.3392	798.5696	712.90966	618.4538	560.8104	587.2981	617.896	20.65
Vz, (ng/ml)	0.068320	0.094138	0.0675528	0.068077	0.028559	0.027386	0.059005	44.21
CL (ng/ml)/h	0.000049	0.0000383	0.000310	0.0000351	0.0000427	0.0000316	0.000084	130.9
Vss (ng/ml)	0.021228	0.030586	0.0004596	0.021719	0.023986	0.018594	0.019428	52.19

RSD: Relative standard deviation; $t_{1/2}$: Drug elimination time from the body by biotransformation and excretion of 1/2 of the administered or received and absorbed dose; T_{max}: Time to reach the maximum concentration of the active substance; C_{max}: Active ingredient maximum concentration; Clast: last measured concentration of a substance; $AUC_{0\text{-}t}$: Area under the curve "active substance concentration-time" in the time frame from 0 to the moment (t) of the last biomaterial sampling; $AUC_{0\text{-}inf}$: Area under the curve "active substance concentration-time" in the timeframe from 0 to ∞; $AUMC_{0\text{-}inf}$: Area under the curve "product of time and drug concentration"; MRT: The average substance retention time in the systemic circulation; Vz: Distribution volume - the ratio of the total content of a substance in the body to its serum concentration; CL: Clearance or extraction coefficient - an indicator of the of a substance excretion rate from the body; V_{ss}: apparent volume of distribution at equilibrium.

Bioavailability is the part of a drug dose that reaches systemic blood after its extravascular injection. The bioavailability can be absolute and relative, and is defined as the ratio of Area Under Curve (AUC) values. The area under the concentration time curve is an integral parameter proportional to the total amount of a drug in the systemic blood (Kukes, 2009). The maximum concentration characterizes efficacy and safety of a drug, and its values should not go beyond a therapeutic range. The time-to-peak concentration with a "concentration - effect" linear relation allows to estimate the time of the maximum effect of a drug (Belolipetskaya and Sukhanov, 2005). The present study found that time to reach the maximum concentration of the active substance in all animals was 3 hours, and active ingredient maximum concentration of moxidectin averaged 307.232 ng/ml in cats, and 909.765 ng/ml in dogs.

One of the main factors that determines the effect of a drug is its concentration in the receptor area. Such concentration is determined rather difficult, therefore, in practice, drug concentration values in the blood plasma are used to describe processes that occur with such drug in the body. The movement of a drug in the body is usually depicted as a concentration time curve which is the dependence of the concentration of a drug or its metabolite in blood plasma on the time after drug administration.

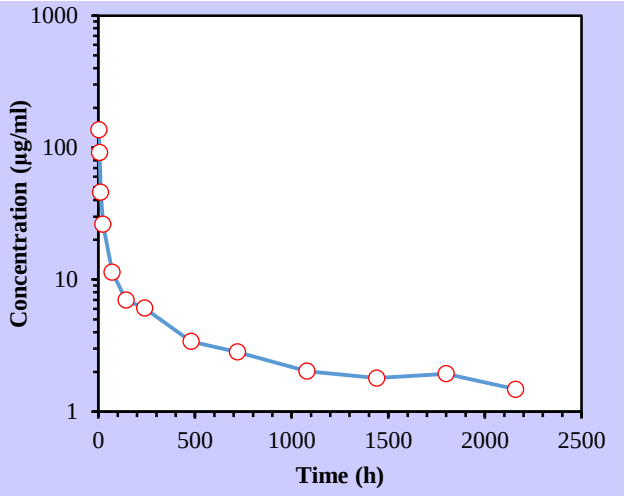

Figure 1. Dynamics of changes in the moxidectin concentration in the blood serum of the cats as a result of oral administration of the drug "Neoterica Protecto syrup" at a dose of 1.5 mg/kg

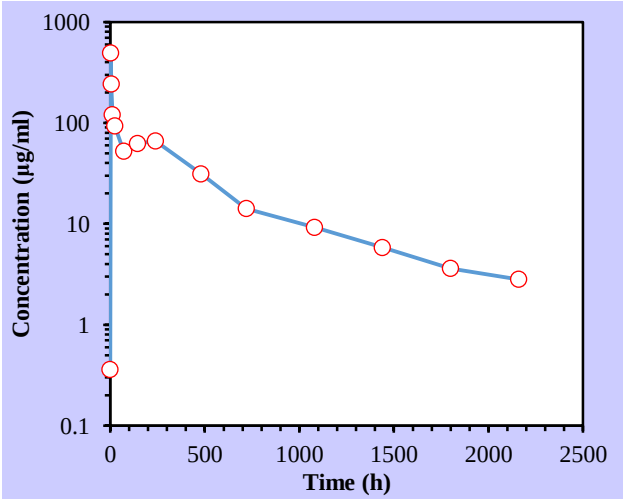

Figure 2. Dynamics of changes in the moxidectin concentration in the blood serum of the dogs as a result of oral administration of the drug "Neoterica Protecto syrup" at a dose of 1.5 mg/kg

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DISCUSSION

According to the literature, when studying the metabolism of moxidectin in various animal species after quantitative determination by HPLC, the 14C-moxidectin metabolism was the highest in the liver microsomes of sheep (32.7%), compared to cows (20.6%), deer (15, 4%), goats (12.7%), rabbits (7.0%), and rats (3.0%) while the smallest amount in metabolism occurred in microsomes of pigs, that is 0.8% of the total amount of detected metabolites (Dupuy et al., 2001). When studying the moxidectin distribution in tissues of cattle after a single subcutaneous administration, the highest content of the residues was found in the abdominal fat and the back, and the lowest content was found in the liver, kidneys, and muscles of the coupling (Zulalian et al., 1994).

Pharmacokinetic studies of moxidectin and ivermectin in horses showed a longer time for moxidectin presence, as demonstrated by a fourfold increase in mean values of the time, compared to ivermectin and longer time and higher concentrations of moxidectin, compared to ivermectin explain the longer anthelminthic effect of drug Equest (moxidectin) (Perez et al., 1999).

When studying the comparative kinetics of macrocyclic lactones 80 days after treatment of cattle, metabolites were found in the plasma as 5.75% of doramectin, 8.50% of ivermectin, and 13.8% of moxidectin of the total amount of their respective parent drugs excreted in plasma (Lanusse et al., 1997).

The studies by Belykh (2020) have indicated that moxidectin after being applied externally in the form of a solution is well absorbed into the systemic circulation of animals and reaches a maximum concentration after 4-10 days, and it also reaches significant concentrations of moxidectin in the blood serum of cats and dogs determined within 28 days after a single application. Regarding the pharmacokinetics of the active substances of Gelmintal Tablets (praziquantel and moxidectin) after oral administration, it was found that moxidectin remained in the blood of animals for 25 days (Arisov et al., 2016b).

Based on literature data of other authors and on the current research, it can be concluded that a lipophilic substance, moxidectin, reaches its maximum concentration at three hours after being administered orally in a high dose of 1.5 mg/kg, and begins to accumulate in adipose tissues of the animal body in large quantities, which explains the low level of apparent volume of distribution (Vss). Subsequently, considering a high level of metabolism in the liver, moxidectin is gradually released from fat depot into the blood plasma of animals, which ensures that the drug concentrations maintained at a therapeutic level for such a long time (up to three months).

The present study has confirmed the long-term presence of moxidectin in the blood serum of animals after a single oral administration of Neoterica Protecto Syrup in the minimum therapeutic dose (1.5 mg of moxidectin per 1 kg of animal weight). The results of this study showed that moxidectin was rapidly absorbed into the blood of animals after three to six hours reaching the maximum concentrations, and was found by the end of the experiment in the blood serum of animals, which indicates its long-term therapeutic effect up to 90 days. Based on the results obtained, it can be concluded that the maintenance of therapeutic concentrations of moxidectin for 90 days should ensure the protection of animals from ectoparasites and nematodes. To confirm this hypothesis, further clinical studies of the drug “Neoterica Protecto syrup” are needed when used for the treatment and prevention of parasitosis of cats and dogs on target animal species for 90 days. However, given that a therapeutic dose of moxidectin is quite high compared to other similar drugs used for animals, studies of toxicological properties of the drug and its tolerance in target animal species at increased therapeutic doses are necessary. Furthermore, allergic reactions, severe renal failure, and acute liver disorders are listed as contraindications of moxidectin. When studying the efficacy, the drug must not be used for depleted, sick, or infected animals, as well as animals weighing less than 2.0 kg. Consideration should be given to the poor tolerance of collies, bobtails, Shetland sheepdogs, and other breeds that are sensitive to macrocyclic lactones, and treatment of animals should be supervised by a veterinarian.

CONCLUSION

The study of pharmacokinetics is mainly based on the assessment of the active substance concentration at certain points in time after the application of the drug. Blood is the main object of research. The study of the drug concentration in the blood provides information on the drug circulation time in the body, drug bioavailability, the effect of concentrations on the pharmacological effect, therapeutic and lethal dosage, and the dynamics of active or toxic metabolites formation.

As a result of moxidectin pharmacokinetics study after a single use of an antiparasitic drug in syrup form at a therapeutic dose of 1.5 mg moxidectin per one kg of animal weight, it was found that active substance concentration in the blood serum has respectively reached levels of 136.211 - 467.116 ng/ml and 491.861-1370.217 ng / ml in cats and dogs in three hours. The active substance remained present in the blood circulation of both species for at least 90 days after the single oral administration of the drug. The findings indicated that a single administration of the drug at the recommended therapeutic dose ensures the maintenance of therapeutic concentrations of moxidectin in the blood for 90 days. Accordingly, it should provide protection of animals against parasites during this period.

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DECLARATIONS

Authors' Contribution

Gulnara B. Arisova and Mikhail V. Arisov planned the study, developed the experimental design, and were directly involved in the experiment. Irina A. Stepanova participated in the interpretation of the results and the writing of the scientific paper. All authors read and approved the final manuscript and analyzed data.

Competing interests

The authors state no conflict of interest.

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Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors. All the authors approved and agreed to publish the manuscript.

REFERENCES

Al-Azzam SI, Fleckenstein L, Cheng KJ, Dzimianski MT, and McCall JW (2007). Comparison of the pharmacokinetics of moxidectin and ivermectin after oral administration to beagle dogs. Biopharmaceutics and Drug Disposition, 28 (8): 431-438. DOI: https://doi.org/10.1002/bdd.572

Lanusse C, Lifschitz A, Virkel G, Alvarez L, Sanchez S, Sutra JF, Galtier P, and Alvinerie M (1997). Comparative plasma disposition kinetics of ivermectin, moxidectin and doramectin in cattle. Journal of Veterinary Pharmacology and Therapeutics, 20: 91-99. DOI: https://doi.org/10.1046/j.1365-2885.1997.00825.x

Alvinerie M, Sutra JF, Badri M, Galtier P (1995). Determination of moxidectin in plasma by high-performance liquid chromatography with automated solid-phase extraction and fluorescence detection. Journal of Chromatography B: Biomedical Sciences and Applications, 674 (1): 119-124. DOI: http://dx.doi.org/10.1016/0378-4347(95)00294-5

McCall JW (2005). The safety-net story about macrocyclic lactone heartworm preventives: A review, an update, and recommendations. Veterinary Parasitology, 133: 197-206. DOI: https://doi.org/10.1016/j.vetpar.2005.04.005

Perez R, Cabezas I, García M, Rubilar L, Sutra JF, Galtier P, and Alvinerie M (1999). Comparison of the pharmacokinetics of moxidectin (Equest) and ivermectin (Eqvalan) in horses. Journal of Veterinary Pharmacology and Therapeutics, 22: 174-180. DOI: https://doi.org/10.1046/j.1365-2885.1999.00200.x

Zulalian J, Stout SJ, daCunha AR, Garces T, and Miller P (1994). Absorption, tissue distribution, metabolism, and excretion of moxidectin in cattle. Journal Agricultural and Food Chemistry, 42: 381-387. DOI: https://doi.org/10.1021/jf00038a028

Zhang Y, Huo M, Zhou J, and Xie S (2010). PK Solver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Computer methods and programs in biomedicine, 99: 306-314. DOI: https://doi.org/10.1016/j.cmpb.2010.01.007

Jafarov MH, Vasilevich FI, and Mirzaev MN (2019). Evolution of antiparasitic substances. In Collection: Current issues of veterinary medicine, zoo technology and biotechnology Collection of scientific papers of the educational and methodology and scientific and practical conference dedicated to the 100th anniversary of the founding of the FSBEI HE Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after K.I. Skryabin. Moscow: 323-327. Available at: https://www.elibrary.ru/item.asp?id=41511027

Platonova AO, and Avsevieva VA (2018). Modern anthelmintic drugs for small pets. (Analysis of scientific literature). Alley of Science, 6 (22): 407-410. Available at: https://www.elibrary.ru/item.asp?id=35349138

Gokbulut C, Biligili A, Kart A, and Turgut C (2010). Plasma dispositions of ivermectin, doramectin, and moxidectin following subcutaneous administration in rabbits. Laboratory Animals, 44: 138-142. DOI: https://doi.org/10.1258/la.2009.009053

Korth-Bradley JM, Parks V, Patat A, Matschke K, Mayer P, and Fleckenstein L (2012). Relative bioavailability of liquid and tablet formulations of the antiparasitic moxidectin. Clinical Pharmacology in Drug Development, 1(1): 32-37. DOI: https://doi.org/10.1177/2160763X11432508

Arisov MV, Abramov VE, Balyshev AV, and Demin AI (2016a). The pharmacokinetics of the active substances of Gelmintal (syrup). Veterinary Medicine and Zootechnics, 12: 43-47. Available at: https://www.elibrary.ru/item.asp?id=27710597

Arisov MV, Abramov VE, Balyshev AV, and Smirnova ES (2016b). The pharmacokinetics of the active substances of Gelmintal in tablets. Veterinary Medicine, 8: 59-63. Available at: https://www.elibrary.ru/item.asp?id=26555535

Belykh IP (2020). Pharmacokinetic characteristics of the drug based on moxidectin for young stock and small breed of domestic animals. World's Veterinary Journal, 10(2): 231-236. DOI: https://www.dx.doi.org/10.36380/scil.2020.wvj30.

Dupuy J, Escudero E, Eeckhoutte C, Sutra JF, Galtier P, Alvinerie M (2001). In vitro metabolism of 14C-moxidectin by hepatic microsomes from various species. Veterinary Research Communications, 25: 345-354. DOI: https://doi.org/10.1023/A:1010686508307

Guidelines for the examination of medicines (2014). 1: 268-274. Available at: https://lit-review.ru/guides/expertise_vol1.pdf

Craven J, Hennessy DR and Friis C (2002). Does the rate of fat deposition influence the pharmacokinetic of subcutaneously administered moxidectin and ivermectin in pigs? Journal of veterinary pharmacology and therapeutics, 25 (5): 351-357. DOI: https://doi.org/10.1046/j.1365-2885.2002.00435.x

Oukessou M, Berrag B and Alvinerie M (1999). A comparative kinetic study of ivermectin and moxidectin in lactating camels (Camelus dromedarius). Veterinary parasitology, 83: 151-159. DOI: https://doi.org/10.1016/S0304-4017(99)00045-X

Sallovitz JM, Lifschitz A, Imperiale F, Virkel G and Lanusse C (2003). A detailed assessment of the pattern of moxidectin tissue distribution after pour-on treatment in calves. Journal of veterinary pharmacology and therapeutics, 26: 397-404. DOI: https://doi.org/10.1046/j.0140-7783.2003.00537.x

Belolipetskaya VG, Sukhanov YV (2005) Pharmacokinetic research and practical medicine. Rational Pharmacotherapy in Cardiology, 2: 43-47. Available at: https://cyberleninka.ru/article/n/farmakokineticheskie-issledovaniya-i-prakticheskaya-meditsina/viewer

Kukes VG (2009) Clinical Pharmacokinetics: Theoretical, Applied and Analytical Aspects: Guidelines: 14-19. Available at: https://medknigaservis.ru/wp-content/uploads/2018/12/Q0010079.pdf

Cotreau MM, Warren S, Ryan JL, Fleckenstein L, Vanapalli SR, Brown KR, Rock D, Chen CY and Schwertschlag US (2003) The Antiparasitic Moxidectin: Safety, Tolerability, and Pharmacokinetics in Humans. The Journal of Clinical Pharmacology, 43 (10): 1108-1115. DOI: https://doi.org/10.1177/0091270003257456

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The Effect of Different Dietary Energy and Protein Sources on Blood Profile of Crossbreed Holstein Dairy Cows Raised in Small Stake Holder Farms

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ABSTRACT

The study aimed to evaluate the effect of protein and energy supplementation on the biochemical blood parameters in Holstein cows. The effect of energy and protein supplementation used corn and soybean meal was evaluated on biochemical blood profile in three groups of Holstein cows raised in small stakeholder farmers in Yogyakarta from February to May 2020. Thirty multiparous Holstein cows were allocated to three treatment groups, namely T0 in which the cows fed by the basal diet from the local farmer as well as the T1 (3.5% energy and protein supplementation) and T2 (5% energy and protein supplementation), in which the cows were fed by added energy and protein supplementation. The diets designed for the treatment groups were different from the basal diet by adding two additional ingredients which were soybean meal and corn meal in purpose to depress the stress from adaptive feeding. The results showed that the treated cows (T1 and T2) had significantly higher serum concentrations of glucose (T1 = 2.12 ± 0.49 mmol/L, T2 = 1.86 ± 0.40 mmol/L) rather than T0 (0.98 ± 0.48 mmol/L). The total concentration of serum protein and urea in treated cows was significantly lower than those with the basal diet. Total serum protein and urea in T1 were 0.69 ± 1.37 mmol/L and 7.21 ± 1.99 mmol/L, respectively; which they were 0.63 ± 0.06 mmol/L and 7.69 ± 3.07 mmol/L in T2, compared to the T0 which were 0.82 ±0.05 mmol/L and 7.69 ± 3.07 mmol/L, respectively. There was no significant difference in blood cholesterol among all treatment groups. In conclusion, the supplementations that varied in the proportion of energy and protein intake affected some biochemical blood profiles, such as glucose, protein, and blood urea nitrogen.

Keywords: Biochemical blood parameters, Crossbreed Holstein cows, Energy supplementation, Protein supplementation, Traditional farmers

INTRODUCTION

In Indonesia, dairy farms are held mainly by smallholder farmers whose cows are only two to three with low field productivity (Sembada et al., 2016). As most farms have little or no land for foraging, the cows have inadequate nutritional status. This inadequate nutrition is a major constrain that negatively affects animal reproduction (Cordova Izquierdo, 2015). The effect of poor nutrition, which decreases reproductive performance in cows, is causally related to the blood metabolic profile (Ferraretto et al., 2014; Rutherford et al., 2016). The evaluation of blood metabolic profiles is required to monitor the animal's health, reproduction, and physiological conditions (Puppel and Kuczyńska, 2016), which can prevent metabolic and nutritional disorders in cows. Also, the simple method of feed supplementation which is more efficient for those traditional farmers which they could apply easily, when the farmers just put some additional feed ingredients which are available in their location to increase the nutritional values in purpose to increase the animal productivity, rather than changing the whole formulation or adding a significant ingredient which is difficult to find in the area.

Whenever the physiologically and hormonally managed mobilization of energy in cows is not balanced, biological consequences can be observed (Crowe et al., 2018), especially when the mobilization of the body results from Negative Energy Balance (NEB) had a significant effect on the parameter metabolic, milk nutrient values or subsequent health (Sheehy et al., 2016). Protein and energy supplementation are some of the nutritional strategies that have been found to decrease reproductive problems due to the inadequate nutritional status in smallholder farms (Hostens et al., 2011; Cools et al., 2014). Therefore, it is important to determine the most suitable feedstuff to obtain the efficiency of feed formulation. The important key about supplementation is to use it only without changing the whole formulation to enhance the nutritional intake. Protein supplementation, such as soybeans and energy supplementation with corn, could increase the intake and nutritional value of lower-quality feed used by smallholder farmers (Olson, 2015), which are abundant and easy to access, especially in Indonesia or tropical countries.

The present study aimed to evaluate the effects of diets supplemented with soybean meal as a source of protein and corn as a source of energy on the biochemical blood parameters (glucose, protein, cholesterol, and blood urea nitrogen) of Holstein cattle.

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MATERIALS AND METHODS

Ethical approval

The procedures applied throughout the current study have been approved by the Animal Care and Use Committee of Faculty of Veterinary Medicine UGM, Yogyakarta Indonesia, No. 002/EC.FKH/Ket/2019

Experimental animals, location, and period of research

The present study was conducted using 30 Holstein crossbreed cows (with an average age of three years old) raised by smallholder farmers in Yogyakarta, Indonesia with the Body Condition Score (BCS) ranged two to three in two to four periods of lactation. The research was carried out from February to May 2020. The cows were under semi-intensive maintenance, housed in communal pens.

Feeding methods

The basal diet for the control group (T0, n = 10) was formulated based on the farmers' daily feed given to the cows with consideration of the BCS (two to three) and milk production (8 kg/days on average of 75% forages and 25% commercial concentrate, Table 1). In the treatment groups T1 and T2, the cows were fed with added energy and protein supplementation obtained from soybean and corn meal so the feed formulation was not totally changed (Cools, 2014) which had a purpose to suppress the stress effect from feed factors in animals, so it prevents animal welfare through the study. The treatment diet in T1 (n = 10) was formulated by replacing 14% of commercial concentrate in total with an added supplementation of energy and protein which was seven percent of corn meal and seven percent of soybean meal as fed (3.5% energy and protein supplementation in dry matter (DM). The second treatment (T2; n = 10) was formulated by replacing 20% of commercial concentrate in total with an added supplementation of energy and protein by 10% of corn meal and 10% of soybean meal as fed (five percent energy and protein supplementation in DM), which all the nutrient intakes and chemical composition of the feeds are shown in Tables 1 and 2 in Dry Matter (DM). The cows were fed individually at 06.00 and 15.00 with *ad-libitum* water intake.

Table 1. The compositions of ingredient and chemical of the diets

Ingredient (% DM)	T0	T1	T2
Pennisetum Purpureum	73.3	69.7	69.7
Commercial concentrate	26.7	25.0	21.0
Corn	-	2.65	4.65
Soybean meal	-	2.65	4.65
Dry matter (kg)	7.78	8.08	8.83
Crude protein (kg)	1.36	1.56	1.71
TDN (kg)	5.20	5.48	6.06

Reference: Hartadi (1980). T0: Cows were fed by the basal diet from the local farmer, T1: Cows were fed by added energy supplementation, T2: Cows were fed by added protein supplementation.

Table 2. Chemical composition of the forages and concentrates

Ingredients	Proximate analysis (%)						
	Dry Matter	Anorganic Matter (Ash)	Etyl Ether	Crude Fibre	Crude Protein	Nitrogen Free Extract	Total Digestible Nutrient
Pennisetum Purpureum	21.74	13.02	8.42	29.65	13.88	13.29	86.24
Commercial concentrate	90.45	23.80	6.75	13.26	20.07	36.12	52.83
Corn	87.54	2.04	7.24	2.83	10.27	77.62	83.89
Soybean Meal	87.40	7.22	4.28	5.21	56.44	26.85	80.42

Sampling methods

The samples of feed and feed residual were collected daily during the data collection period, dried at 55°C in the oven for 48 hours, grounded to pass through a 1 mm screen using a Wiley mill. The feeds and residual feed samples were analyzed for Dry Matter (DM), ash, Crude Protein (CP), Organic Matter (OM), Total Digestible Nutrient (TDN, Nakano et al., 2018).

Blood preparation and blood analysis

The concentration of biochemical blood profiles, such as glucose, protein, cholesterol, and blood urea nitrogen was determined in the serum samples from 30 cows. The blood samples were collected by tubes via vena jugularis every

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week and centrifuged at 3000 rpm for 15 minutes. Upon complete serum separation, it was transferred to microtubes and stored at -20°C until the analysis. The total amounts of glucose, protein, cholesterol, and urea of the serum were measured using a UV spectrophotometer (Microlab 300, Indonesia; Sitaresmi et al., 2017).

Statistical analysis

The obtained values expressed as Mean ± SEM with 95% ($p < 0.05$) of the confidence interval for the significant results in each treatment, which were analyzed using univariate linear regression by SPSS, version 26 (IBM, USA).

RESULTS

As shown in Table 3, cows fed with protein and energy supplementation (T1 and T2) had significantly ($p < 0.05$) higher serum concentrations of glucose (T1 = 2.12 ± 0.49 mmol/L, T2 = 1.86 ± 0.40 mmol/L) than cows who consumed only the basal diet provided by the smallholder farmers (T0 = 0.98 ± 0.48 mmol/L). However, there was no significant difference in blood glucose levels in the serum between the T1 and T2 treatment groups ($p > 0.05$). The treated cows (T1 and T2) had significantly ($p < 0.05$) lower concentrations in serum protein and urea rather than the cows fed by the basal diet (T0). The protein concentration in T0, T1 and T2 were 0.82 ± 0.05, 0.69 ± 0.1.37, and 0.63 ± 0.06 mmol/L, respectively. The urea concentrations in T0, T1, and T2 were 12.23 ± 2.22, 7.21 ± 1.99, and 7.69 ± 3.07 mmol/L, respectively (Table 3). However, there was no significant difference ($p > 0.05$) in blood protein and urea serum levels between T1 and T2. There was no significant difference ($p > 0.05$) in blood cholesterol among all the treatment groups.

Table 3. Biochemical blood concentrations of the cows fed with diets containing different amounts of energy and protein

Parameter	T0	T1	T2
Glucose (mmol/L)	0.98 ± 0.48^{c}	2.12 ± 0.49^{ab}	1.86 ± 0.40^{ab}
Cholesterol (mmol/L)	4.84 ± 0.94	4.69 ± 1.59	5.49 ± 0.62
Protein (g/L)	0.82 ± 0.05^{a}	0.69 ± 1.37^{bc}	0.63 ± 0.06^{bc}
Urea (mmol/L)	12.23 ± 2.22^{a}	7.21 ± 1.99^{bc}	7.69 ± 3.07^{bc}

[a, b, c] Total means with different superscripts within a row differ significantly ($p < 0.05$). T0: cows were fed with the basal diet from the local farmer, T1: cows were fed with added energy supplementation, T2: cows were fed with added protein supplementation.

DISCUSSION

The result showed that the energy and protein supplementation affected some of the blood profiles of cows. These findings were similar to those reported in previous studies (Ferraretto et al., 2014). Supplementation in lactating cows can enhance physiological and reproduction parameters (McLean et al., 2018). The supplementation used in the present study has affected the levels of insulin and IGF-1 which can lead to differences in the blood profile glucose, which is an indication of supplementation efficiency (Lents et al., 2005). An increased concentration of serum glucose with protein and energy supplementation increased nutrient availability. The data about glucose were similar to the current results (Table 3), the addition of feed supplementation increased serum glucose due to the increased gluconeogenesis in the tissue owing to raise insulin levels. In cows, blood glucose is mainly produced via hepatic gluconeogenesis in the liver (Goselink et al., 2013), so the supplementation of feed protein and energy could increase the gluconeogenesis in cells using the excess of energy and protein feed. It has been previously reported that protein and energy supplementation decreased liver triglyceride deposition (Elek et al., 2013), serum β-hydroxybutyrate (BHBA), Non-Esterified Fatty Acids (NEFA) concentration, and increased liver health in cows; however, supplementation did not significantly ($p > 0.05$) increase the energy and protein levels in T1 and T2 groups (Sun et al., 2016). The nutrient content notably the protein and energy from the basal diet by the farmers (T0) was inadequate which were 1.36 kg for protein and 5.20 kg for total digestible nutrient (TDN) meanwhile the minimum requirement for those nutrients were 1.38 kg for protein and 5.6 kg for TDN (NRC, 2005). In this condition can be concluded that those cows were in deficiency status or the negative energy balance (NEB). This negative status was indicated by the low glucose levels recorded in serum, and the creation of ketone bodies in blood from the lipid metabolic cycle. These findings were similar to those of other studies (Wu et al., 2013; Sun et al., 2016). Higher glucose levels in the blood serum, within the normal range, could be a noble indicator of energy. In addition, glucose is a key nutrient that affects ovarian activity in ruminants, which influences the rates of steroidogenesis and gonadotropin synthesis as well as their secretion (Sitaresmi et al., 2017). Therefore, protein and energy supplementation could increase the reproduction in cows. Protein and energy supplementation could increase the breakdown of protein synthesis via methionine and choline mechanisms to increase milk production (Ardalan et al., 2011), which likely influenced the tendency observed in the current study toward a lower protein serum level in the

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treatment diets than that of the basal diet. The lower results of protein serum in treatment cows (T1 and T2), compared to the control group, were due to the supplemented protein. In other words, soybean meal was given freshly without any protein protection treatments or agents, such as aldehydes or tannins, which could lead to high bypass potetionals through direct absorption by the small intestine as amino acids. However, those proteins were easily breakdown by rumen microbes to volatile fatty acids (VFAs) as the main ingredients for gluconeogenesis later and produced glucose as the main energy which was proven significantly by increased glucose serum in treatment cows as the main products of gluconeogenesis (Widyobroto et al., 2008). The supplementation of energy and protein feed could diminish serum ketones or blood urea (BUN) concentrations by varying NEFA and BHBA serum concentrations (Ardalan et al., 2011). Present results indicated that supplementation of protein and energy in feed significantly reduced the BUN levels in cows (Table 3). The basal diet, which did not meet the energy requirements, tended to have a nutritional imbalance of urea at levels higher than 12.5 mmol/L and the BUN limit was below 7.14 mmol/L for normal reproduction. If the cows possess a higher level of BUN, these conditions could lead to reproductive impairment, such as repeat breeding, caused by the obstruction of gonadotropins hormones or other factors due to high BUN levels (Widayati et al., 2019). This condition was not caused by a high protein intake from the feed, but rather by an imbalance or deficiency in nutrients. Such imbalance leads to increased levels of triglycerols being mobilized from adipose tissue to generate the necessary energy and the production of urea as a side metabolite (Humer et al., 2016). The obtained results of the present study showed that the supplementation of energy and protein in feed tended to be in the normal reported range of 12.5-18 mmol/L (Umar et al., 2015). The data from the current study indicated that the addition of protein and energy as supplements could increase the ability of cows to utilize nutrients effectively and efficiently.

Cholesterol is a source of energy and a precursor of steroid reproductive hormones, which are also required for cell function. Similar to this study, supplementation protein and energy has been found to decrease the serum lipid and cholesterol content in the blood (Goselink et al., 2013; Sun et al., 2016, Table 3). The reason is that the protein could enhance complete hepatic oxidation of serum NEFA and acetyl-CoA from mitochondria β-oxidation of CO_2 and H_2O. Protein and energy supplementation increased lipid transfer from the blood to the liver, and stimulated further activation of gluconeogenesis (Tharwat et al., 2012; Goselink et al., 2013; Sun et al., 2016). The decrease in cholesterol concentration in blood serum during feed supplementation, although not significant, was a good indicator of reproductive potential (Sitaresmi et al., 2017). As reported in a previous study, cholesterol serum was positively correlated with ovarian disorders, which could cause impairment of reproductive status in ruminants (Samarutel et al., 2008).

CONCLUSION

The basal diet provided by the smallholder farmers resulted in a negative energy balance in cows, which was shown by the lower glucose levels and higher blood urea concentrations in the biochemical blood profile of Holstein crossbred cows. Feed supplementations that varied in the proportion of energy and protein intake affected some biochemical blood profile markers such as glucose, proteins, and urea. The protein and energy supplementation were increased blood glucose, and decreased protein and urea of the blood serum, therefore the cholesterol level was not different in all treatments.

DECLARATION

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Authors' contribution

Hudaya and Widayati conceived, conducted the fieldwork, administrated, drafted the manuscript and performed the statistical analysis, and reviewed the manuscript. Sitaresmi conducted a literature search. Widayati designed and supervised the study. All authors conducted data interpretation, edited the manuscript, read and approved the final manuscript.

Competing interests

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

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.

REFERENCES

Ardalan M, Dehghan-Banadaky M, Rezayazdi K, and Ghavi Hossein-Zadeh N (2011). The effect of rumen-protected methionine and choline on plasma metabolites of Holstein dairy cows. The Journal of Agricultural Science 149: 639-646. DOI: https://www.doi.org/10.1017/S0021859610001292

Cools S, Van den Broeck W, Vanhaecke L, Heyerick A, Bossaert P, Hostens M, and Opsomer G (2014). Feeding soybean meal increases the blood level of isoflavones and reduces the steroidogenic capacity in bovine corpora lutea, without affecting peripheral progesterone concentrations. Animal Production Science 144: 79-89. DOI: https://www.doi.org/10.1016/j.anireprosci.2013.12.008

Cordova Izquierdo A (2015). Best Practices in animal reproduction: impact of nutrition on reproductive performance livestock. Advances in Dairy Research, 4: 2-5. DOI: https://www.doi.org/10.4172/2329-888x.1000152

Crowe MA, Hostens M, and Opsomer G (2018). Reproductive management in dairy cows - The future. Irish Veterinary Journal, 71: 1-13. DOI: https://www.doi.org/10.1186/s13620-017-0112-y

Elek P, Gaál T, and Husvéth F (2013). Influence of rumen-protected choline on liver composition and blood variables indicating energy balance in periparturient dairy cows. Acta Veterinaria Hungarica, 61: 59-70. DOI: https://www.doi.org/10.1556/AVet.2012.053

Ferraretto LF, Gencoglu H, Hackbart KS, Nascimento AB, Dalla Costa F, Bender RW, Guenther JN, Shaver RD, and Wiltbank MC (2014). Effect of feed restriction on reproductive and metabolic hormones in dairy cows. Journal of Dairy Science, 97: 754-763. DOI: https://www.doi.org/10.3168/jds.2013-6925

Goselink RMA, van Baal J, Widjaja HCA, Dekker RA, Zom RLG, de Veth MJ, and van Vuuren AM (2013). Effect of rumen-protected choline supplementation on liver and adipose gene expression during the transition period in dairy cattle. Journal of Dairy Science, 96: 1102-1116. DOI: https://www.doi.org/10.3168/jds.2012-5396

Hartadi, H (1980). Tables of feed composition for Indonesia. International Feedstuffs Institute. Utah Agriculture Experiment Station. Utah University. Logan. Utah.

Hostens M, Fievez V, Vlaeminck B, Buyse J, Leroy J, Piepers S, De Vliegher S, and Opsomer G (2011). The effect of marine algae in the ration of high-yielding dairy cows during transition on metabolic parameters in serum and follicular fluid around parturition. Journal of Dairy Science, 94: 4603-4615. DOI: https://www.doi.org/10.3168/jds.2010-3899

Humer E, Khol-Parisini A, Metzler-Zebeli BU, Gruber L, and Zebeli Q (2016). Alterations of the lipid metabolome in dairy cows experiencing excessive lipolysis early postpartum. PLoS One, 11: 1-18. DOI: https://www.doi.org/10.1371/journal.pone.0158633

Lents CA, Wettemann RP, White FJ, Rubio I, Ciccioli NH, Spicer LJ, Keisler DH, and Payton ME (2005). Influence of nutrient intake and body fat on concentrations of insulin-like growth factor-I, insulin, thyroxine, and leptin in plasma of gestating beef cows. Journal of Animal Science, 83: 586-596. DOI: https://www.doi.org/10.2527/2005.833586x

McLean KJ, Boehmer BH, Spicer LJ, and Wettemann RP (2018). The effects of protein supplementation of fall calving beef cows on pre-and postpartum plasma insulin, glucose and IGF-I, and postnatal growth and plasma insulin and IGF-I of calves. Journal of Animal Science, 96: 2629-2639. DOI: https://www.doi.org/10.1093/jas/sky173

Nakano M, Matoba K, and Togamura Y (2018). An estimation for total digestible nutrients in fresh herbage from a perennial ryegrass - White clover mixed pasture. Japan Agricultural Research Quarterly, 52: 155-161. DOI: https://www.doi.org/10.6090/jarq.52.155

NRC (2005). National Science Education Standards. Washington DC. National Academy Press.

Olson KC (2015). Cow Supplementation : Getting the Best Bang for Your Buck. Proceedings, Range Beef Cow Symposium, 24: 163-175. Available at https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1352&context=rangebeefcowsymp

Puppel K, and Kuczyńska B (2016). Metabolic profiles of cow's blood; a review. Journal of the Science of Food and Agriculture, 96: 4321-4328. DOI: https://www.doi.org/10.1002/jsfa.7779

Rutherford AJ, Oikonomou G, and Smith RF (2016). The effect of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle. Journal of Dairy Science, 99: 4808-4815. DOI: https://www.doi.org/10.3168/jds.2015-10154

Samarutel J, Ling K, Waldmann A, Jaakson H, Kaart T, Leesma A, and Samaru J (2008). Field trial on progesterone cycles , metabolic profiles , body condition score and their relation to fertility in estonian holstein dairy cows. Reproduction in Domestic Animals, 43: 457-463. DOI: https://www.doi.org/10.1111/j.1439-0531.2007.00935.x

Sheehy MR, Fahey A, Aungier SPM, Carter F, Crowe MA, Mulligan FJ (2016). A comparison of serum metabolic and production profiles of dairy cows that maintained or lost body condition 15 days before calving. Journal of Dairy Science, 100: 1–12. DOI: https://www.doi.org/10.3168/jds.2016-11206

Sembada P, Duteurtre G, Purwanto BP, and Suryahadi (2016). Improved milk production performance of smallholder

farms in West Java (Indonesia). Tropical Animal Health and Production, 48: 793-799. DOI: https://www.doi.org/10.1007/s11250-016-1029-2

Sitaresmi PI, Widyobroto BP, Bintara S, and Widayati DT (2017). Progesterone and biochemical profile of ettawa-saanen crossbreed goats in Turi Area, Yogyakarta-Indonesia. International Journal of Dairy Science, 12: 289-294. DOI: https://www.doi.org/10.3923/ijds.2017.289.294

Sun F, Cao Y, Cai C, Li S, Yu C, and Yao J (2016). Regulation of nutritional metabolism in transition dairy cows: Energy homeostasis and health in response to post-ruminal choline and methionine. PLoS One, 11: 1-27. DOI: https://www.doi.org/10.1371/journal.pone.0160659

Tharwat M, Endoh D, and Oikawa S (2012). Hepatocyte apoptosis in dairy cows with fatty infiltration of the liver. Research in Veterinary Science 93: 1281-1286. DOI: https://www.doi.org/10.1016/j.rvsc.2012.03.011

Umar M, Kurnadi B, Rianto E, Pangestu E, and Purnomoadi A (2015). The effect of energy level of feeding on daily gain, blood glucose and urea on Madura cattle. Journal of the Indonesian Tropical Animal Agriculture 40: 159-166. DOI: https://www.doi.org/10.14710/jitaa.40.3.159-166

Widayati DT, Adiarto, Widyobroto BP, and Suranindyah YY (2019). Cortisol and blood urea nitrogen profiles in fertile and repeat-breeder Holstein-Friesian crossbred cows. Pakistan Journal of Biological Sciences 22: 356-360. DOI: https://www.doi.org/10.3923/pjbs.2019.356.360

Widyobroto BP, Priyono S, and Agus A (2008). Effect of Underaded Protein and Energy Level on Intake and Digestibility of Nutrient and Blood Metabolite in Dairy Cows. Animal Production, 5(10): 96-101. https://media.neliti.com/media/publications/64743-ID-none.pdf

Wu F, Jin Z, and Jin J (2013). Hypoglycemic effects of glabridin, a polyphenolic flavonoid from licorice, in an animal model of diabetes mellitus. Molecular Medicine Reports, 7: 1278-1282. DOI: https://www.doi.org/10.3892/mmr.2013.1330

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The Grass Was Greener - Climate Change, One Health, and the High Hopes to Mitigate COVID-19, Avian Influenza, and other Zoonotic Emerging Diseases

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EDITORIAL
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Over the last decades, global warming has significantly affected the world's climate, negatively impacting numerous ecosystems (Cardenas et al., 2006; Beyer et al., 2021; Dupraz and Burnand, 2021). The accumulating influences of climate change, including the rise of the earth's surface temperature and sea level as well as melting glaciers among many other direct and indirect effects (Calel et al., 2020; Harvey et al., 2020), are reshaping, not only the ecological landscape of many world regions, but also setting the stage for emerging diseases sceneries. Floods, droughts, hurricanes (Zambrano et al., 2021), heat waves and surging fires across all continents (Bonilla-Aldana et al., 2019) are all part of the human-driven fingerprint that has led to climate change (Figure 1). These effects have also resulted in a massive reduction of vegetation across many regions around the globe. As the legendary British rock 'n' roll band Pink Floyd once sang in their most celebrated song "*High Hopes*", *"…the grass was greener…"* (Pink Floyd, *dixit*), framed in an environmental context these lyrics should call for a reflection on how climate change is leaving its mark on earth's landscape.

Accelerating climate change is not only affecting human health but also animal health in ways that if left uncontrolled could trigger the emergence/reemergence of climate-sensitive pathogens, vector-borne, and zoonotic diseases (Rodriguez-Morales, 2013; Chowdhury et al., 2018; Chowdhury et al., 2020) (Figure 1). Potential emerging pathogens include most importantly viruses, like the recently evolving Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) pandemic for which a number of environmental and climate-related changes appear to have paved its way from animals to human transmission (Yan Yam, 2020).

Possible causes influencing climate-related pathogen emergence include human transgression on wildlife habitats and wildlife exploitation which may lead to increased human-animal, and animal-animals interactions (Figure 1), creating opportunities for pathogens to spill over among species (Yan Yam, 2020). Additionally, the loss of biodiversity compounded by climate change reduces the interface between animals for disease transmission, which increases chances for pathogen exposure and spread to humans, and consequently, potential outbreaks (Escalera-Antezana et al., 2020), epidemics, and even pandemics, as recently seen with the SARS-CoV-2 causing the Coronavirus Disease 2019 (COVID-19) (Dhama et al., 2020).

On the other hand, the impact of environmental deleterious human activities, such as deforestation can lead to an increase or shift of selection pressures on different pathogens, particularly viruses (Kalbus et al., 2021; Laporta et al., 2021), Figure 1). Such is the case of SARS-CoV-2, whose origin remains largely unknown (Mohammed, 2021; The Lancet Infectious, 2021) although recent findings have revealed that 96.2% of its genome shares similarities with bat-

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related coronaviruses; thus, suggesting its probable origin from Chiroptera (Zhou et al., 2020; Bonilla-Aldana et al., 2021). A similar scenario was previously recorded in 1997-1998 with the emergence of the Nipah virus (Uppal, 2000; Chua et al., 2002) following an event of slash-and-burn deforestation that led to a severe haze across much of Southeast Asia and consequent bat (*Pteropus*) invasions to fruit orchards lying in close proximity to swineherds, which ultimately led to spread amongst pigs followed by cross-species transmission to humans (Breed et al., 2010; Bonilla-Aldana et al., 2019).

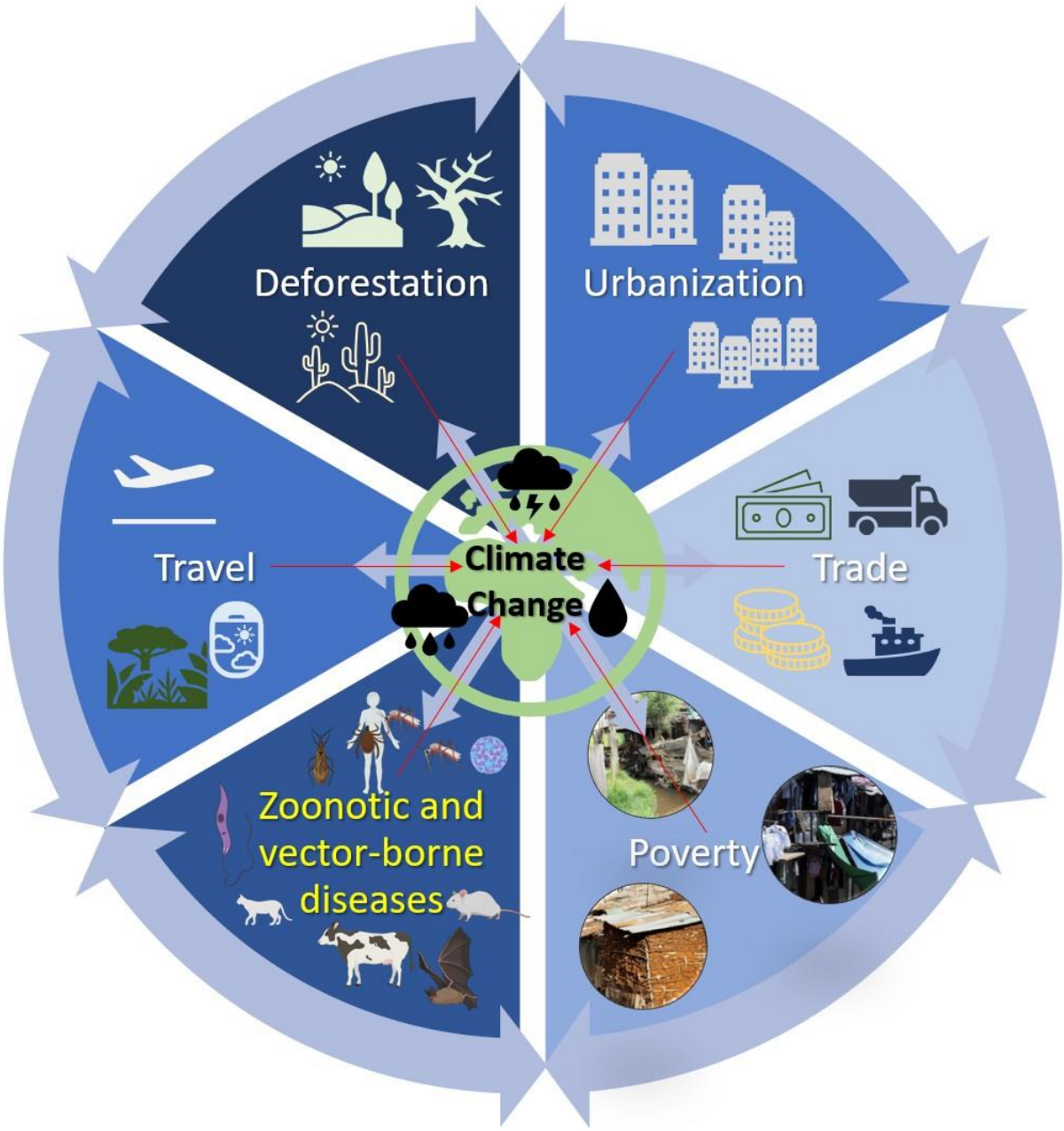

Figure 1. Some selected factors interacting with climate change, and their potential impacts on zoonotic and vector-borne diseases.

In addition, climate change can also influence the incidence and severity of multiple infectious diseases by affecting vector ecology and competency as well as host immune responses. Certain zoonotic respiratory infections may arise in previously spared geographical areas, influenced by a number of climatic causes derived from global warming (Mirsaeidi et al., 2016). An example of this includes avian influenza viruses, which are raising red flags after recent reports on the circulation H5N6, H5N8 (Bonilla-Aldana et al., 2020a), and recently H10N3 (ProMedMail, 2021b). In particular, avian influenza H5N8 has been linked to recent outbreaks in poultry farms and amongst wild birds and humans. More recently, the World Health Organization (WHO) alerted about the detection of avian influenza (H5N8) in seven poultry farm workers from Astrakhan Oblast in the Russian Federation (ProMedMail, 2021a; European Food Safety et al., 2021). Likewise, in December 2020, experts from the UK Plant Health Agency (APHA) laboratory isolated

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H5N8 influenza virus during post-mortem analysis performed on common seals (*Phoca vitulina*), a grey seal (*Halichoerus grypus*), and a red fox (*Vulpes vulpes*) from a wildlife rehabilitation center (Zambrano et al., 2021; ProMEDmail, 2021c). Now, in June 2021, a human case due to H10N3 has been reported in China (ProMedMail, 2021b).

Climate change is affecting all areas of society by negatively modulating environmental determinants of both human and animal health, and will continue to do so for generations. The current COVID-19 pandemic is probably associated with the impacts of climate change, due to land use and changes in the interactions between bats and intermediate hosts of coronaviruses. This emphasizes the importance of a One Health approach to tackle the numerous serious ongoing environmental challenges (Bonilla-Aldana et al., 2020b; Bonilla-Aldana et al., 2020c). Given the broad diversity of pathogens affecting wildlife and their continuous evolution, forecasting pathogen emergence through interdisciplinary networking has become the best strategy to reduce the risk of future outbreaks. Efforts to prevent disease emergence should also be emphasized in study areas, such as pathogen surveillance, pathogen-human interaction, and drivers of cross-species transmission. This is the only way we will be able to transit "... along the long road and on down the causeway" (Pink Floyd, *dixit*) of the multiple challenges imposed by climate change and its impact on human-animal disease ecology. Looking into the future, we have "high hopes" that humankind will manage to restore its carbon footprint while allowing to mitigate further impacts on climate change and reducing the risk of future pandemics of zoonotic origin.

DECLARATIONS

Authors' contributions

DKBA and AJRM conceived the review, developed the first draft of the manuscript. ÁAFM, DAVT, FMBV, JRS, AEPM, and JAS, critically reviewed the manuscript for relevant intellectual content. All authors have read and approved the final version of the paper.

Competing interests

All authors report no potential conflicts.

REFERENCES

Beyer RM, Manica A and Mora C (2021). Shifts in global bat diversity suggest a possible role of climate change in the emergence of SARS-CoV-1 and SARS-CoV-2. Science of the Total Environment, 767: Article number 145413. DOI: https://www.doi.org/10.1016/j.scitotenv.2021.145413

Bonilla-Aldana DK, Aguirre-Florez M, Villamizar-Pena R, Gutierrez-Ocampo E, Henao-Martinez JF, Cvetkovic-Vega A, Dhama K, Rabaan A, Sah R, Rodriguez-Morales AJ, et al. (2020a). After SARS-CoV-2, will h5n6 and other influenza viruses follow the pandemic path? Infezioni in Medicina, 28(4): 475-485. Available at: https://pubmed.ncbi.nlm.nih.gov/33257621/

Bonilla-Aldana DK, Dhama K and Rodriguez-Morales AJ (2020b). Revisiting the one health approach in the context of covid-19: A look into the ecology of this emerging disease. Advances in Animal and Veterinary Sciences, 8(3): 234-237. Available at: https://search.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov/resource/en/covidwho-822918

Bonilla-Aldana DK, Holguin-Rivera Y, Perez-Vargas S, Trejos-Mendoza AE, Balbin-Ramon GJ, Dhama K, Barato P, Lujan-Vega C, and Rodriguez-Morales AJ (2020c). Importance of the one health approach to study the sars-cov-2 in latin america. One Health, 10: Article number 100147. DOI: https://www.doi.org/10.1016/j.onehlt.2020.100147

Bonilla-Aldana DK, Jimenez-Diaz SD, Arango-Duque JS, Aguirre-Florez M, Balbin-Ramon GJ, Paniz-Mondolfi A, Suarez JA, Pachar MR, Perez-Garcia LA, Delgado-Noguera LA et al. (2021). Bats in ecosystems and their wide spectrum of viral infectious potential threats: Sars-Cov-2 and other emerging viruses. International Journal of Infectious Diseases, 102: 87-96. DOI: https://www.doi.org/10.1016/j.ijid.2020.08.050

Bonilla-Aldana DK, Suarez JA, Franco-Paredes C, Vilcarromero S, Mattar S, Gomez-Marin JE, Villamil-Gomez WE, Ruiz-Saenz J, Cardona-Ospina JA, Idarraga-Bedoya SE et al. (2019). Brazil burning! What is the potential impact of the amazon wildfires on vector-borne and zoonotic emerging diseases? - a statement from an international experts meeting. Travel Medicine and Infectious Diseases, 31: Article number 101474. DOI: https://www.doi.org/10.1016/j.tmaid.2019.101474

Breed AC, Yu M, Barr JA, Crameri G, Thalmann CM, and Wang LF (2010). Prevalence of henipavirus and rubulavirus antibodies in pteropid bats, Papua new guinea. Emerging Infectious Diseases, 16(12): 1997-1999. DOI: https://www.doi.org/10.3201/eid1612.100879

Calel R, Chapman SC, Stainforth DA, and Watkins NW (2020). Temperature variability implies greater economic damages from climate change. Nature Communications, 11(1): Article number 5028. DOI: https://www.doi.org/10.1038/s41467-020-18797-8

Cardenas R, Sandoval CM, Rodriguez-Morales AJ, and Franco-Paredes C (2006). Impact of climate variability in the occurrence of leishmaniasis in northeastern Colombia. American Journal of Tropical Medicine Hygiene, 75(2): 273-277. Available at: https://pubmed.ncbi.nlm.nih.gov/16896132/

Chowdhury FR, Ibrahim QSU, Bari MS, Alam MMJ, Dunachie SJ, Rodriguez-Morales AJ, and Patwary MI (2018). The association between temperature, rainfall and humidity with common climate-sensitive infectious diseases in Bangladesh. PLoS ONE, 13(6): e0199579. DOI: https://www.doi.org/10.1371/journal.pone.0199579

Chowdhury FR, Ibrahim QSU, Bari MS, Alam MMJ, Dunachie SJ, Rodriguez-Morales AJ, and Patwary MI (2020). Correction: The association between temperature, rainfall and humidity with common climate-sensitive infectious diseases in Bangladesh. PLoS ONE, 15(4): e0232285. DOI: https://www.doi.org/10.1371/journal.pone.0232285

Chua KB, Chua BH, and Wang CW (2002). Anthropogenic deforestation, El Nino and the emergence of Nipah virus in Malaysia. Malaysian Journal of Pathology, 24(1): 15-21. Available at: https://pubmed.ncbi.nlm.nih.gov/16329551/

Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, and Rodriguez-Morales AJ (2020). Coronavirus disease 2019-covid-19. Clinical Microbiology Reviews, 33(4): e00028-20. DOI: https://www.doi.org/10.1128/CMR.00028-20

Dupraz J, and Burnand B (2021). Role of health professionals regarding the impact of climate change on health-an exploratory review. International Journal of Environmental Research and Public Health, 18(6): 3222. DOI: https://www.doi.org/10.3390/ijerph18063222

Escalera-Antezana JP, Rodriguez-Villena OJ, Arancibia-Alba AW, Alvarado-Arnez LE, Bonilla-Aldana DK, and Rodriguez-Morales AJ (2020). Clinical features of fatal cases of Chapare virus hemorrhagic fever originating from rural la Paz, bolivia, 2019: A cluster analysis. Travel Medicine and Infectious Diseases, 36: Article number 101589. DOI: https://www.doi.org/10.1016/j.tmaid.2020.101589

European Food Safety Authority, European Centre for Disease Prevention and Control and European Union Reference Laboratory for Avian Influenza, Adlhoch C, Fusaro A, Gonzales JL, Kuiken T, Marangon S, Niqueux E Staubach C, Terregino C, et al. (2021). Avian influenza overview December 2020 - February 2021. EFSA Journal, 19(3): e06497. DOI: https://www.doi.org/10.2903/j.efsa.2021.6497

Harvey JA, Heinen R, Gols R, and Thakur MP (2020). Climate change-mediated temperature extremes and insects: From outbreaks to breakdowns. Global Changes and Biology, 26(12): 6685-6701. DOI: https://www.doi.org/10.1111/gcb.15377

Kalbus A, de Souza Sampaio V, Boenecke J, and Reintjes R (2021). Exploring the influence of deforestation on dengue fever incidence in the Brazilian Amazonas State. PLoS ONE, 16(1): e0242685. DOI: https://www.doi.org/10.1371/journal.pone.0242685

Laporta GZ, Ilacqua RC, Bergo ES, Chaves LSM, Rodovalho SR, Moresco GG, Figueira EAG, Massad E, de Oliveira TMP, Bickersmith SA et al. (2021). Malaria transmission in landscapes with varying deforestation levels and timelines in the amazon: A longitudinal spatiotemporal study. Scientific Reports, 11(1): Article number 6477. DOI: https://www.doi.org/10.1038/s41598-021-85890-3

Mirsaeidi M, Motahari H, Taghizadeh Khamesi M, Sharifi A, Campos M, and Schraufnagel DE (2016). Climate change and respiratory infections. Annals of the American Thoracic Society, 13(8): 1223-1230. DOI: https://www.doi.org/10.1513/AnnalsATS.201511-729PS

Mohammed MEA (2021). The percentages of sars-cov-2 protein similarity and identity with SARS-CoV and batcov ratg13 proteins can be used as indicators of virus origin. Journal of Proteins and Proteomics, pp. 1-11. DOI: https://www.doi.org/10.1007/s42485-021-00060-3

ProMedMail (2021a). Avian influenza, human (02): Russia, h5n8, 1st case. Available at: Https://promedmail.Org/promed-post/?Id=8204014

ProMEDmail (2021b). Promedmail - avian influenza, human (09): China (JS) H10N3, 1st rep. Available at: Https://promedmail.Org/promed-post/?Id=20210602.8416833

ProMEDmail (2021c). Promedmail - avian influenza (45): Europe (UK, Croatia) seal, fox, wild bird, hpai h5n8, oie. Available at: Https://promedmail.Org/promed-post/?Id=20210317.8252821

Rodriguez-Morales AJ (2013). Climate change, climate variability and brucellosis. Recent Patents in Antiinfective Drug Discovery, 8(1): 4-12. DOI: https://www.doi.org/10.2174/1574891x11308010003

The Lancet Infectious D (2021). In an ocean of ashes, islands of order: Who's SARS-CoV-2 origin report. Lancet Infectious Diseases, 21(5): Article number 579. DOI: https://www.doi.org/10.1016/S1473-3099(21)00213-9

Uppal PK (2000). Emergence of Nipah virus in Malaysia. Annans of the New York Academy of Sciences, 916: 354-357. DOI: https://www.doi.org/10.1111/j.1749-6632.2000.tb05312.x

Yan Yam EL (2020). Climate change and the origin of SARS-CoV-2. Journal of Travel Medicine, 27(8): 224. DOI: https://www.doi.org/10.1093/jtm/taaa224

Zambrano LI, Fuentes-Barahona IC, Henriquez-Marquez KI, Vasquez-Bonilla WO, Sierra M, Munoz-Lara F, Luna C, Bonilla-Aldana DK, and Rodriguez-Morales AJ (2021). Covid-19 and hurricanes: The impact of natural disasters during a pandemic in honduras, central america. Prehospital and Disaster Medicine, 36(2): 246-248. DOI: https://www.doi.org/10.1017/S1049023X21000182

Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579(7798): 270-273. DOI: https://www.doi.org/10.1038/s41586-020-2012-7

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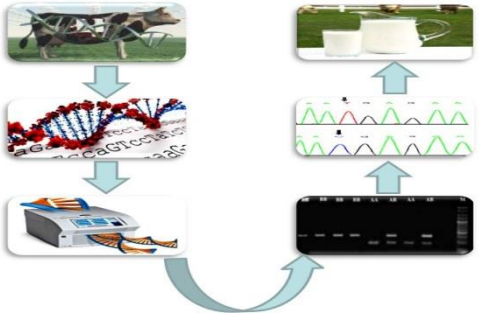

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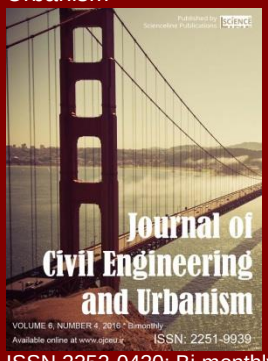

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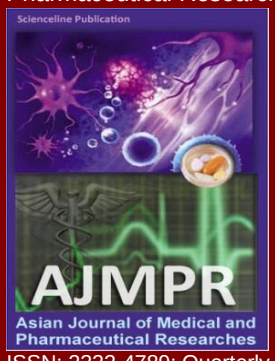

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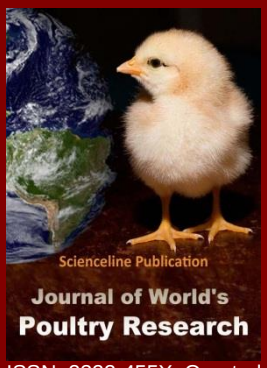

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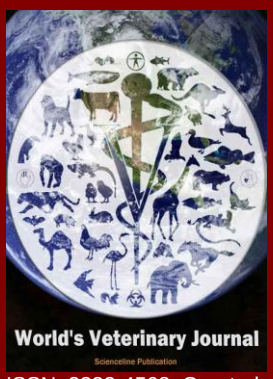

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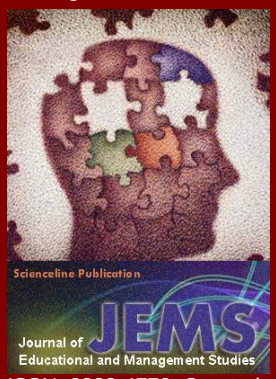

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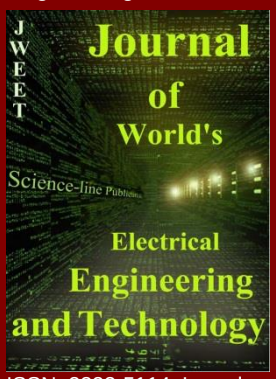

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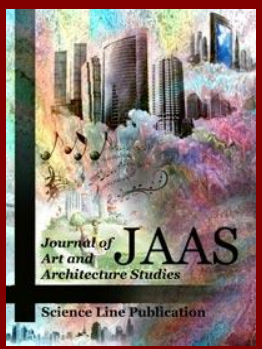

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