RESEARCH ARTICLE

ASSESSMENT OF HAEMATOLOGICAL PROFILE AND OTHER PATHOLOGICAL CHANGES IN FARM CHICKENS NATURALLY INFECTED WITH CHICKEN ANAEMIA VIRUS IN MAIDUGURI

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ABSTRACT

The haematology and histopathological changes in Chicken anaemia virus (CAV) infection among apparently healthy village chickens in Maiduguri, Nigeria, were investigated using conventional PCR. A total of one hundred blood and tissue samples were obtained from the study area. Blood samples were collected in EDTA tubes for haematological analysis, while tissue samples (thymus, liver, and spleen) were collected and pooled from each of the birds in duplicates into 10% neutral buffered formalin for histopathology, and the other sample was frozen at -20oC for chicken anaemia virus DNA extraction. A total of 42/100 (42%) of the pooled tissue samples were positive for CAV DNA with the expected band size of 387 bp. Haematological findings showed no significant difference between CAV-positive and CAV- negative chickens in all the parameters examined. Microscopic changes observed in the CAV- positive samples were focal aggregations of inflammatory cells, primarily in lymphoid cells and binucleate hepatocytes in the liver, lymphoid depletion in the white pulp, arising as a result of lymphocytolysis of the lymphoid follicular cells of the spleen, and lymphoid depletion in the cortical area of the thymus. These findings are the first of their kind in this region, underscoring the need to further explore CAV epidemiology in Nigeria. The study highlights the necessity for ongoing development and implementation of reliable diagnostic methods, including molecular techniques, to inform appropriate preventive measures against this economically significant avian disease.

Keywords: Chicken anaemia virus, haematological parameters, histopathology, molecular diagnostic, village chicken

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INTRODUCTION

Diseases are the main factor limiting the viability of the village chicken production system in underdeveloped nations (Hamer et al., 2013; Sehgal, 2015). However, the studies on infectious poultry diseases in developing African nations have mostly concentrated on bacterial, viral, and protozoan infections; other studies have also examined ectoparasites and gastrointestinal parasites (Letebrhan et al., 2015; Weyuma et al., 2015). There has not been much focus about Chicken anaemia virus infections and their effects. In much of Nigeria, village chickens are usually raised under complex management systems (Opara et al., 2014). According to Chepkemoi et al. (2017) and Lawal et al. (2021), they have access to an environment where they hunt for food, even on unclean rubbish dumps and near unclean pools of water. Where the chickens are kept unconfined, disease control becomes difficult, and outbreaks of infectious diseases such as Chicken anaemia virus (CAV) cause substantial losses (Shettima et al., 2017).

Chicken anaemia virus disease is an economically significant poultry disease that causes infectious anaemia. It is one of the important poultry diseases in developing regions, including Nigeria (Fatoba and Adeleke, 2019). The virus has been classified as a single-stranded DNA virus with icosahedral symmetry initially belonging to the family *Circoviridae* (Alkateb and Gerish, 2019; Sreekala et al., 2020). However, CAV has recently been classified in the *Gyrovirus* genus of the *Anelloviridae* (Tanget al., 2016; Li et al., 2017a; Rosario et al., 2017). Chicken infectious anaemia, also known as blue wing disease or anaemia-dermatitis syndrome, is a viral infection of chicks mostly 2–4 weeks old (Gowthaman, 2019; Schatand Van Santen, 2020; Kamdi et al., 2020). The virus is known for its high mortality in chickens. It also causes immunosuppression, subcutaneous hemorrhage, and anaemia (Orakpoghenor, 2019; Chandrashekaraiah et al., 2020). Other reported clinical signs and lesions included lymphoid depletion, atrophy of the bursa of Fabricius and thymus, hemorrhages in thigh and breast muscles and yellowish to whitish bone marrow (Abdallah et al., 2022; Mounika et al., 2023).

Since its initial discovery in 1979, Chicken anaemia virus disease has become widely distributed and prevalent worldwide. The disease was first documented in Ibadan, Nigeria, by Oluwayeluet al. (2005) in Ibadan, Nigeria. The Chicken anaemia virus is thought to be ubiquitous in Nigeria because its presence has been identified in commercial flocks of birds (Oluwayelu, 2010; Schatand Van Santen, 2020; Adedeji et al., 2024), including village chickens, ducks, turkeys, and geese (Shettima et al., 2017).

To the best of our literature search, there is a paucity of information about the Chicken anaemia virus in the study area. Therefore, the purpose of this study was to determine the prevalence of CAV using conventional PCR techniques, and to evaluate the possible haematological and microscopic changes induced by the disease in village chickens.

MATERIALS AND METHODS

Ethical Consideration

All applicable national and international guidelines for the care and use of animals were followed. All procedures performed in the studied animals were following the ethical standards of the University of Maiduguri, Faculty of Veterinary Medicine, Committee on Animal Use and Care (AUP No.: AUP-R003/2023).

Study Area

This investigation was conducted in Maiduguri Metropolis, Borno State, Nigeria. Borno State is located in the northeastern part of Nigeria (Elijah et al., 2022). The state shares borders with three West African countries, namely, the Republic of Chad to the northeast, Niger Republic to the north and Cameroon Republic to the east. Within the country, it neighbors were Bauchi State to the south, Yobe State to the west and Gombe State to the southwest (Elijah et al., 2022).

Experimental Design

The study utilized a convenience sampling method. One hundred blood and tissue samples (including thymus, liver, and spleen) were collected from village chickens in Maiduguri. The study took into consideration gender (cocks and hens). Chickens sampled were classified as growers (3–4 months) and adults (over 5 months), according to Addass et al. (2012) age descriptions for chickens. Blood samples obtained at the point of slaughter were placed into sterile vacutainer tubes containing EDTA and transported to the Veterinary Pathology Laboratory at the University of Maiduguri for haematological analysis. The tissue samples were collected in duplicate, one aliquot was directly placed into sample bottles and stored at -20oC for subsequent investigation of CAV presence using a conventional method. The other aliquot was fixed in 10% buffered formalin for histopathological analysis. A Chicken anaemia virus (Cux-1 DNA)-positive control, indicative of Chicken anaemia virus, was sourced from a virus research laboratory at the National Veterinary Research Institute (NVRI) in Vom, Nigeria. This control was utilized for PCR optimization and viral replication monitoring.

Evaluation of Haematological Parameters

For haematological analysis, blood samples were collected into EDTA sample tubes from the jugular vein during slaughter. Blood samples were assessed for packed cell volume (PCV), hemoglobin concentration (HbC), total red blood cell (RBC) count, white blood cells (WBC) count and WBC differentials, according to Campbell (2010). The mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated, based on the PCV, RBC count, and Hb, respectively.

Tissue Preparation for Histology

The 10% buffered formalin fixed thymus, spleen, and liver were dehydrated in graded alcohol (70, 80, 90, and 100%), while xylene and paraffin wax were used for clearing and embedding, respectively. Serial sections 5 µ thick were obtained using a rotator microtome. Deparafinised sections were stained with hematoxylin and eosin, as described by Majama and associates (2024). Slides were examined using light microscope at different magnifications. Photomicrographs of lesions were obtained using a digital camera (AmScope) mounted on the microscope.

PCR

Viral DNA extraction was carried out from homogenized pooled tissue samples using a high pure template preparation kit (innuPREP DNA Mini Kit 2.0), according to the manufacturer's instructions (Ajinnuscreen, GmbH, Berlin, Germany). The extracted DNA concentration was measured and kept at -20°C prior to use. Briefly, a general master mix (MyTaqTMMix, Bioline GmbH, Germany) was prepared, which contained all the reaction components in one cocktail without the DNA template. The PCR tubes were labeled according to the number of samples, one each of the negative and positive controls was included. The reagents were thawed and left on ice. The PCR tubes were transferred into the heating block of the thermocycler (Eppendorf Mastercycler Nexus, Hamburg, Germany), and the tubes were properly closed to prevent evaporation during PCR. According to the DNA polymerase provider, there was an initial denaturation phase, followed by 35 cycles of one denaturation, one annealing, and one extension step each. Denaturation was observed at 95°C for 30 seconds, annealing at 64°C for 45 seconds, and elongation was set at 65°C for 5 minutes.

Statistical Analysis

The data generated from the study were subjected to statistical analysis using SPSS version 16.0 statistical software and the data were expressed as mean±standard deviation. Chi-square test was used to perform categorical comparison and determine significance at 95% confidence interval. P-value of 0.05 or less was considered statistically significant.

RESULTS

Detection of CAV Infection by Conventional PCR

The conventional PCR used for the detection of CAV DNA from village chickens tissue samples in Maiduguri using the CUX-1 set of primers produced the expected band of 387bp on Agarose gel electrophoresis. Out of the 100 tissue samples analyzed by conventional PCR, 42 (42%) samples were positive for CAV nucleic acid.

The effects of natural CAV infection on haematological parameters of village chickens in Maiduguri

Table 1 summarizes the effects of natural CAV infection on the haematological parameters of village chickens in Maiduguri, Nigeria. No significant difference was seen in all the haematological parameters of CAV-positive birds as compared to the CAV-negative birds, with the exception of MCH, MCHC, and mean heterophil counts. The mean MCH, MCHC, and heterophil counts were significantly ($P < 0.05$) higher in CAV-positive chickens as compared to CAV-negative birds.

The mean values of the haematological parameters for CAV-positive and CAV-negative male and female village chickens are presented in Table 2. There were no significant differences between male and female village chickens, both CAV-positive and CAV-negative, in the mean values of the PCV, Hb, RBC, WBC, MCV, heterophil, lymphocyte and basophil counts. However, CAV-positive female chickens demonstrated significantly higher MCH and MCHC values ($P < 0.05$) compared to CAV-positive male village chickens and CAV-negative male and female village chickens. On the other hand, the CAV-positive male village chickens exhibited significantly higher mean monocyte and eosinophil counts ($P < 0.05$) than CAV- positive female and CAV-negative male and female village chickens.

The mean values of haematological parameters of CAV-positive and CAV-negative chicks and grower village chickens are presented in Table 3. No significant differences ($P > 0.05$) were observedbetween the chicks and growers of both CAV-positive and CAV-negative village chickens in the mean values of PCV, Hb, RBC, WBC, MCV, heterophil, lymphocyte, and basophil counts. However, CAV-positive grower village chickens demonstrated significantly higher MCH and MCHC values ($P < 0.05$) compared to CAV-positive chicks, as well as both chicks and grower CAV-negative village chickens. Additionally, mean monocyte and eosinophil counts were to be significantly higher ($P<0.05$) higher in CAV-positive village chicks as compared to CAV-positive grower chickens, and both CAV-negative chicks and grower village chickens.

Microscopic lesions

Histopathological examination of naturally infected CAV-positive chicken tissues confirmed by PCR assay showed focal aggregations of inflammatory cells primarily in lymphoid cells and binucleate hepatocytes in the liver (Figure 1). The spleen showed severe lymphoid depletion in the white pulp arising as a result of lymphocytolysis of the lymphoid follicular cells (Figure 2). The thymus also showed lymphoid depletion in the cortical area (Figure 3).

Table 1 Effect of natural CAV infection on haematological parameters of village chickens in Maiduguri, Nigeria

Parameters	CAV-Positive (n=42) village chickens	CAV-Negative (n=58) village chickens
PCV (%)	31.8±3.8a	31.2±3.6a
Hb(g/dl)	10.8± 5.1a	10.5 ± 3.2a
RBC(x106/µl)	2.6 ± 0.5a	2.5 ± 0.4a
WBC(x103/µl)	18.2 ± 5.7a	17.0 ± 5.3a
MCV(fl)	127.4 ± 15.6a	125.8 ± 18.8a
MCHC(g/dl)	35.8 ± 15.0a	32.0 ± 7.1b
MCH(pg)	45.8 ± 22.1a	39.8 ± 8.3b
Heterophil(x103/µl)	6.1 ± 5.7a	5.8 ± 2.3b
Lymphocyte (x103/µl)	10.4 ± 3.2a	9.8 ±3.1a
Monocyte (x103/µl)	0.9 ± 0.5a	0.8 ± 0.3a
Eosinophil(x103/µl)	0.8 ± 0.4a	0.6 ± 0.3a
Basophil (x103/µl)	0.2 ± 0.6a	0.2 ± 0.8a

a,bMeans±standard deviations with different superscripts are significantly different at $p<0.05$ along rows

Table 2 Effects of sex on the haematological parameters of village chickens naturally infected with CAV in Maiduguri Nigeria

Parameters	CAV-Positive		CAV-Negative	
	Male (n=20)	Female (n=22)	Male (n=24)	Female (n=26)
PCV(%)	32.5 ± 3.5a	31.2 ± 4.0a	30.6 ± 2.7a	32.5 ± 4.0a
Hb(g/dl)	11.0 ± 5.1a	10.6 ± 5.1a	10.7 ± 4.1a	10.8 ± 2.4a
RBC(x106/µl)	2.6 ± 0.5a	2.5 ± 0.5a	2.4 ± 0.4a	2.7 ± 0.4a
WBC(x103/µl)	19.1 ± 5.7a	17.4 ±5.6a	15.9 ± 4.8a	18.6± 5.6a
MCV(fl)	127.2±15.5a	127.6+16.0a	125.8±19.6a	124.5±17.6a
MCHC (g/dl)	28.3 ± 4.9a	42.5 ± 54.2b	31.2±6.0a	34.0±8.3a
MCH(pg)	35.8 ±5.9a	55.0 ±27.1b	38.7±6.1a	41.9±10.3a
Heterophil (103/µl)	6.4 ± 2.3a	5.9 ± 2.6a	5.5 ± 2.3a	6.2 ± 2.4a
Lymphocyte (103/µl)	10.9 ± 3.5a	10.1 ± 3.1a	9.1 ± 2.7a	10.7 ± 3.6a
Monocyte (103/µl)	1.0 ± 0.5b	0.8 ±0.4a	0.7 ± 0.3a	0.9 ±0.3a
Eosinophil(x103/µl)	0.9 ± 0.5b	0.7 ± 0.3a	0.6 ± 0.3a	0.7 ± 0.3a
Basophil (x103/µl)	0.3 ± 0.7a	0.0 ± 0.0a	0.2 ±0.8a	0.0 ± 0.1a

a,bMeans±standard deviations with different superscripts are significantly different at $p<0.05$ along rows

Table 3 Effect of age on the haematological parameters of village chickens naturally infected with CAV in Maiduguri

Parameters	CAV-Positive		CAV-Negative	
	(Chicks)	(Growers)	(Chicks)	(Growers)
PCV (%)	31.3 ± 4.4a	32.1 ± 3.5a	31.1 ± 2.9a	31.1 ± 4.2a
Hb(g/dl)	9.0 ± 1.7a	11.6 ± 5.9a	10.4 ± 2.7a	10.7 ± 3.6a
RBC(x106/µl)	2.7 ± 0.4a	2.5 ± 0.5a	2.5 ± 0.4a	2.5 ± 0.4a
WBC(x103/µl)	20.0 ± 5.0a	17.4 ±5.9a	17.3 ± 4.4a	16.7 ± 5.9a
MCV(fl)	126.1±15.0a	128.0+16.0a	127.0±11.6a	124.5±14.6a
MCHC (g/dl)	30.5 ± 6.3a	38.2 ± 17.1b	31.8±8.7a	32.3±5.2a
MCH(pg)	37.9 ±6.0a	49.4 ±25.6b	39.5±9.5a	40.0±7.0a
Heterophil (103/µl)	6.9 ± 2.3a	5.8 ± 2.6a	6.0 ± 1.9a	5.6 ± 2.6a
Lymphocyte (103/µl)	11.1 ± 3.0a	10.1 ± 3.4a	9.7 ± 3.0a	9.7 ± 3.3a
Monocyte (103/µl)	1.1 ± 0.5b	0.8 ±0.4a	0.8 ± 0.3a	0.8 ±0.3a
Eosinophil(x103/µl)	0.9 ± 0.5b	0.7 ± 0.4a	0.7 ± 0.4a	0.5 ± 0.3a
Basophil (x103/µl)	0.0 ± 0.1a	0.0 ± 0.1a	0.0 ±0.1a	0.0 ± 0.0a

a,b Means±standard deviations with different superscripts are significantly different at $p<0.05$ along rows

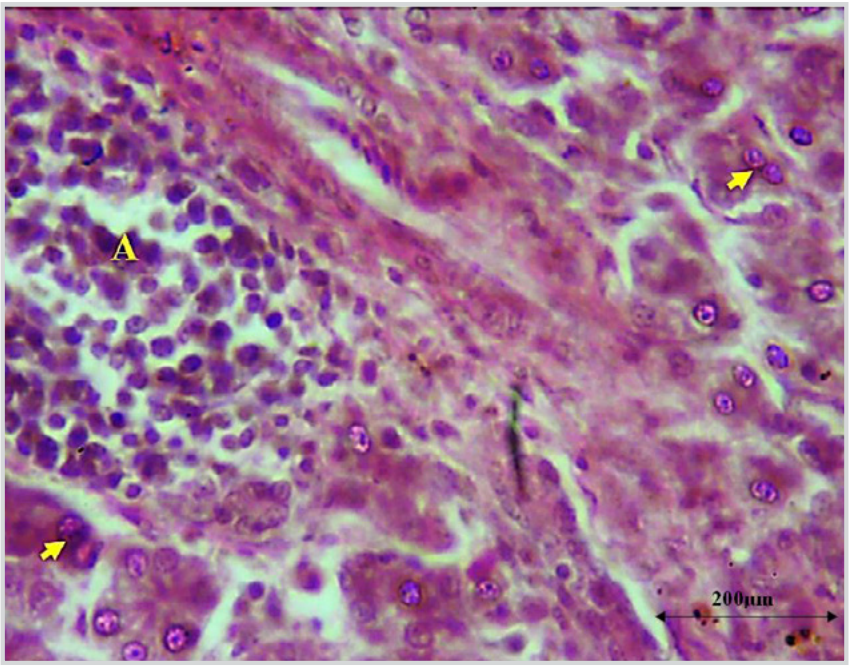

Figure 1
Photomicrograph of a section of the liver in CAV-positive village chicken showing focal aggregations of lymphoid cells (A) and binucleate hepatocytes (arrow heads), H & E X400

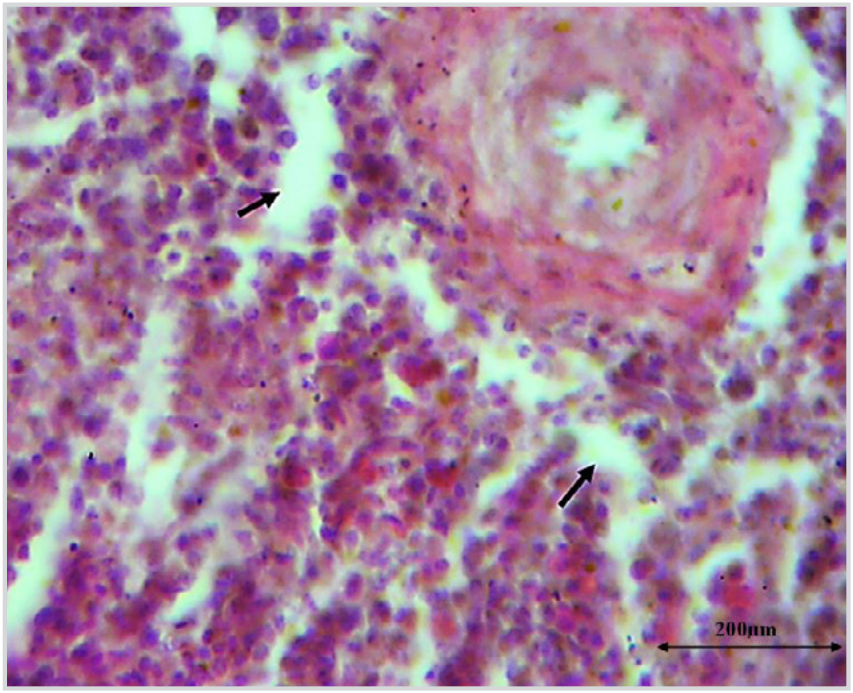

Figure 2

Photomicrograph of a section of the spleen in CAV-positive chicken showing severe depletion of the lymphoid follicle leaving empty spaces (black arrow), H & E, X400

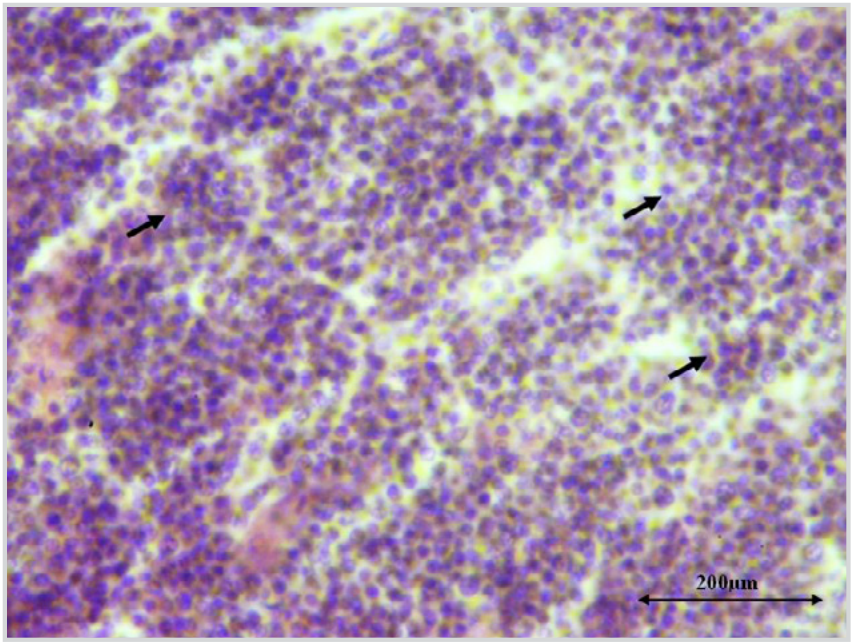

Figure 3

Photomicrograph of a section of the thymus in CAV-positive chickens showing cortical depletion of lymphocytes (black arrows), H & E, X400

DISCUSSION AND CONCLUSION

Chicken anaemia virus has been identified using different serological (Abdelwahab and Mansour, 2019; Fatoba et al., 2019) and molecular techniques (Yaoet al., 2019; Tan et al., 2020). Molecular techniques,such as polymerase chain reaction (PCR), offer significant advantages by providing faster and more specific identification of more fastidious viral pathogens (Mustafa et al., 2020). Therefore, the identification of CAV DNA in village chicken tissues by PCR in this investigation confirm the susceptibility of hens to CAV infections, as the presence of DNA indicates active infection. Given the vaccination against CAV is not commonly practiced among backyard chickens in Nigeria, the detection of CAV DNA in thee ostensibly healthy, free-roaming chickens suggests natural exposure to the virus and identifies them as potential reservoirs for transmission to commercial poultry. Furthermore, it was observed in this study that there was no statistically significant difference between the haematological values (PCV, Hb, RBC, WBC, MCV, heterophil, lymphocyte, monocyte, and basophil counts, MCH, and MCHC) of CAV-positive village chickens, between gender or across age groups. This aligns with the findings of Haridy et al. (2012), who reported that no significant difference between the PCV of CAV- experimentally- infected chickens and their uninfected controls. The higher mean values of WBC, heterophil, lymphocyte, observed in this study tallies with the findings of Wani et al. (2015) reported elevated heterophil and lymphocyte values in CAV-infected flocks compared to normal ones. Notably, heterophils significantly increased, whereas lymphocytes showed a significantly decreased in CAV-infected flocks compared to the normal flock. In contrast, eosinophils and monocytes did not significantly change except in some flocks (Krishan et al., 2015). The findings of the present study may be attributed to co-infection of the CAV with other pathogens. It is also considered that village chickens could act as carriers for facilitating transmission within the flocks (Oluwayelu et al., 2005; Pauly et al., 2019; Conteh et al., 2020).

The microscopic changes observed in the thymus, liver, and spleen of CAV-infected village chickens in the present study are in general agreement with earlier reports by Mounika et al. (2023). This observation also supports the report of Hegazy et al. (2014) that the Chicken anaemia virus leads to atrophy of the thymus gland. Hussein et al. (2016) and Govindhasamy (2022) reported a similar pattern of lymphoid reduction and proliferation of ellipsoidal reticular cells in the spleen and thymus in certain CAV-infected birds. It has also been observed that CAV produces significant shrinkage and loss of zonal architecture, with no clear distinction between the cortex and medulla of the thymus (Abdallah et al., 2022). CAV generates a protein (VP3 or apoptin) that triggers apoptosis, and it is this apoptotic mode of thymocyte death that most likely accounts for lymphoid depletion in the absence of severe inflammation (Hussein et al., 2016). The significant death of lymphocytes seen in all analyzed lymphoid organs in the current investigation is consistent with the findings of Hegazy et al. (2014), Lai et al. (2017) and Feng et al. (2020) all reported that the Chicken anaemia virus VP3 protein triggered apoptosis in vitro and in vivo. These show that apoptosis, a phenomenon that has been observed for a few other viruses, is also an important phenomenon during the pathogenesis of CAV (Hussein et al., 2016; Feng et al., 2020). Backyard chickens' hardiness, which is the outcome of natural challenge selection, shows that they are more disease resistant and less susceptible to CAV infection. Furthermore, these chicks may develop into healthy reservoirs, a theory that calls for additional research into the potential of subclinical chicken anaemia.

In conclusion, there were no statistically significant changes among the haematological parameters of CAV-positive and CAV-negative village chickens. Histopathological examination of the naturally infected tissues, which were positive for CAV DNA by PCR assay, confirmed microscopic lesions associated with CAV infections.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

CONTRIBUTION

YMS-Conception, Design, Funding and writing; HIG-Supervision, Data interpretation/Analysis, and critical review; TMH-Critical review; MUS-Data collection and Literature review; MBM-Supervision; YBM-Materials; AMW-Writing.

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PROCJENA HEMATOLOŠKOG PROFILA I DRUGIH PATOLOŠKIH PROMJENA KOD FARMSKIH PILIĆA PRIRODNO INFICIRANIH SA VIRUSOM INFEKTIVNE ANEMIJE PILIĆA U MAIDUGURIJU

SAŽETAK

Korištenjem konvencionalne PCR metode istraživali smo hematološke i histopatološke promjene kod infekcije sa virusom infektivne anemije pilića (CAV) naizgled zdravih seoskih pilića u Maiduguriju, u Nigeriji. Na istraživanom području smo prikupili ukupno stotinu krvnih i tkivnih uzoraka. Krvni uzorci za hematološku analizu su prikupljeni u EDTA epruvete, dok su uzorci tkiva (timus, jetra i slezena) prikupljeni dvostruko i stavljeni u 10% neutralni puferski formalin za histopatologiju, dok je drugi uzorak zamrznut na -20oC radi ekstrakcije DNA virusa infektivne anemije pilića. Ukupno 42/100 (42%) uzetih tkivnih uzoraka je bilo pozitivno na CAV DNA s očekivanom veličinom trake od 387 bp. Hamatološki nalazi nisu pokazali signifikantnu razliku između CAV-pozitivnih i CAV-negativnih pilića za sve ispitivane parametere. Mikroskopske promjene uočene na CAV-pozitivnim uzorcima su bile u obliku fokalnih nakupina upalnih stanica, prvenstveno kod limfoidnih stanica i binuklearnih hepatocita, limfoidna deplecija u bijeloj srži kao rezultat limfocitolize limfoidnih folikularnih stanica slezene te limfoidna deplecija kortikalnog područja timusa. Ovi rezultati su prvi ovakve vrste u regiji, naglašavajući potrebu za daljnjim istraživanjem epidemiologije CAV-a u Nigeriji. Istraživanje naglašava potrebu za stalnim razvojem i implementacijom pouzdanih dijagnostičkih metoda, uključujući i molekularne tehnike, kako bi se kreirale odgovarajuće preventivne mjere protiv ove ekonomski signifikantne ptičje bolesti.

Ključne riječi: Hematološki parametri, histopatologija, molekularna dijagnostika, seoski pilići, virus infektivne anemije pilića