

Sequential Hematological Study of Experimental Infectious Bursal Disease Virus Infection in Chickens, Turkeys and Ducks

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Keywords

Chicken – Turkey – Duck – Infectious bursal disease virus – Experimental infection – Blood – Nigeria.

Summary

Following experimental infectious bursal disease virus infections in four-week-old broiler chicks, turkey poults and ducklings, blood samples were chronologically collected and analyzed for postinfection (pi) changes. Although there was a net increase in packed cell volume values in chicks reaching a peak of 31% from 12 h to 144 h pi, there was, on the contrary, a general decline in the values in turkey poults and ducklings to minima of 26.5% at 12 h pi and 28.2% at 48 h pi, respectively. Leukocyte counts in chicks significantly increased ($p < 0.05$) to a peak of $66.83 \times 10^3/\mu\text{l}$ at 120 h, while counts in poults decreased to a minimum of $26.75 \times 10^3/\mu\text{l}$ at 96 h pi. Lymphocyte counts in chicks were reduced to a minimum of $5.9 \times 10^3/\mu\text{l}$ at 48 h pi after an initial reduction between three and six hours postinfection. A similar decline occurred in poults with a minimum of $7.81 \times 10^3/\mu\text{l}$ at 48 h pi. The trend of changes in heterophil counts for the three species was similar to those observed for leukocyte counts. While eosinophil counts in chicks initially increased to a peak of $1.93 \times 10^3/\mu\text{l}$ at 6 h pi and subsequently declined, eosinophil values in poults declined to a minimum of $0.88 \times 10^3/\mu\text{l}$ at 6 h pi, followed by an increase to a maximum of $5.7 \times 10^3/\mu\text{l}$ at 72 h pi. However, all hematological values in ducklings remained relatively unchanged. These results showed that there was biphasic lymphopenia, eosinophilia and heterophilia in chicks, lymphopenia and delayed eosinophilia in poults, and relatively unchanged values in ducklings. This emphasizes the fact that different levels of susceptibility exist in the three poultry species studied.

■ INTRODUCTION

The infectious bursal disease (IBD) is recognized as an important disease of young chickens worldwide. It causes unthriftiness, anorexia, ruffled feathers, diarrhea and mortality in affected flocks. The infectious bursal disease virus (IBDV) infection of chickens less than three weeks of age causes immunosuppression with resultant susceptibility to other diseases and lack of humoral response to vaccinations (9).

Although chickens are highly susceptible to IBD, other poultry species such as turkeys and ducks show minimal or no susceptibility to the clinical disease under natural conditions (9). Serological evidence of infection has been established in turkeys, even though neither of the two IBDV serotypes has produced clinical disease in this species (3, 10, 11). In addition, IBDV has been isolated from clinically healthy ducks which were negative for IBD antibody (10). Thus, these three poultry species appear to have different levels of susceptibility to IBDV infection, from the highest in chickens to none in ducks.

Hematological studies have helped in the diagnosis of infectious and other diseases for many years. Hemograms determined during the course of disease conditions coupled with history and

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clinical signs, often assist in the making of a diagnosis. Their use as a diagnostic aid in avian species, especially pet birds, is becoming increasingly popular. Quantitative changes in a particular leukocyte type indirectly reflect the nature of the disease process and the body's response (7). Leukocytosis and heterophilia are characteristic features of bacterial infections, e.g. fowl typhoid (2), while leukopenia is a characteristic of viral diseases (7). Chicken infectious anemia particularly causes pancytopenia (14). Eosinophilia is observed in parasitic and allergic conditions as well as during inflammatory reactions (7). Avian hematopoietic neoplasias, e.g. Marek's disease and lymphoid leukosis, may result in leukocytosis due to increased lymphocyte counts (16). Limited hematological studies have been carried out in this respect in chickens suffering from IBD. Chineme and Cho (5) reported increased mean hematocrit values, as well as lymphocytopenia, in IBDV-infected chickens. In addition, it has been suggested that hemocoagulopathy is involved in the pathogenesis of IBD (5, 8, 13, 12).

The two distinct phases of viremia described by Weiss and Kaufers-Weiss (15) during IBDV infections and the speculated hemocoagulopathy in the pathogenesis of IBD in chickens as well as the differences in susceptibility of chickens, turkeys and ducks to the disease provide a basis for a comparative hematological study in chickens, turkeys and ducks.

■ MATERIALS AND METHODS

Experimental birds

Twenty-seven broiler chicks, 20 turkey poults and 18 ducklings were obtained from commercial sources and reared in isolation cages until they were four weeks of age (age at which chickens are susceptible to IBD). The experimental birds were tested and confirmed to be IBD-seronegative.

Experimental infection and blood sampling

The virus inoculum used was derived from a bursal homogenate from an IBD outbreak in which the flock mortality rate was over 90%. Each bird was infected with 40 µl of virus inoculum equivalent to 16 x 10^{4.6} ELD₅₀ via conjunctival instillation when they were four weeks of age. Blood samples were collected from two or three birds per species, at each sampling time, before they were infected and chronologically at 3, 6, 12, 24, 48, 72, 96, 120 and 144 h postinfection (pi).

Hematology

Packed cell volume

PCV of each blood sample was determined immediately after collection by the microhematocrit method.

Leukocyte counts

The Coulter counter method was used for the determination of total leukocyte counts. A 25 µl volume of whole blood was mixed with 5 ml of avian diluting fluid consisting of sodium citrate 3.8 g, neutral formalin 0.2 ml, brilliant cresyl blue 0.5 g, and 100 ml of distilled water. Leukocytes were counted using a light microscope and differential leukocyte counts were determined according to the standard described by Zinkl (16).

Statistical analysis

Mean values obtained postinfection were compared with preinfection mean values using the Student-Newman-Keuls test.

■ RESULTS

PCV values of chicks, poults and ducklings pre- and post-IBDV infection are presented in Table I. These values in chicks slightly increased generally from a preinfection level of 27.0% to 30.5% at 6 h pi and to 31% at 12 h, 120 h and 144 h pi. However, PCV values in turkey poults and ducklings generally declined from a preinfection level of 33% to 26.5% on day 4 pi in poults and from a preinfection level of 38% to 28% on day 2 pi in ducklings.

Total leukocyte counts in chicks decreased from a preinfection value of 27.7 x 10³/µl to 17.33 x 10³/µl (p < 0.05) at 24 h pi (Figure 1). It subsequently increased above the preinfection level and peaked at 120 h pi with a value of 66.83 x 10³/µl (p < 0.05), followed by 50.5 x 10³/µl at 144 h pi. In poults, there was a general decrease in leukocyte counts from a preinfection level of 37.25 x 10³/µl to 26.75 x 10³/µl at 96 h pi followed by a rise to 36.00 x 10³/µl at 144 h pi. Total leukocyte counts postinfection in ducklings ranged between 1.95 x 10³/µl and 4.15 x 10³/µl, and were not significantly different (p > 0.05) from the preinfection level of 2.0 x 10³/µl.

Lymphocyte counts (Figure 2) in chicks decreased from a preinfection level of 17.17 x 10³/µl to 9.27 x 10³/µl at 6 h pi, followed by a non-significant recovery to 11.71 x 10³/µl at 12 h pi. There was another decrease to a minimum of 5.90 x 10³/µl at 48 h pi. There was subsequently an increase in lymphocyte counts, which rose sharply to a peak of 16.7 x 10³/µl at 120 h, followed by 12.7 x 10³/µl at 144 h pi.

In turkey poults, lymphocyte counts initially increased from a preinfection level of 13.78 x 10³/µl to about 16.00 x 10³/µl at 3 to 6 h pi, followed by a sharp decline to a minimum level of 7.81 x 10³/µl at 48 h pi. From 72 h pi, there was an increase in counts up to 15.12 x 10³/µl at 144 h pi. However, lymphocyte counts in ducklings ranged from 1.32 x 10³/µl to 3.06 x 10³/µl during the period of the experiment.

Heterophil counts (Figure 3) in chicks postinfection remained fairly constant up to 48 h pi and then rapidly increased to a maximum of 48.08 x 10³/µl at 120 h pi (p < 0.05), followed by 31.90 x 10³/µl at 144 h pi. In turkey poults, heterophil counts were fairly

Table I

Packed cell volume (%) of IBD virus infected chicks, turkey poults and ducklings

Sampling time	Chicks	Turkey poults	Ducklings
Preinfection control	27.0 ± 0	33.0 ± 1.41	38.0 ± 1.41
3 h pi	26.5 ± 2.12	31.5 ± 2.12	ND
6 h pi	30.5 ± 0.71	30.5 ± 3.54	35.0 ± 1.41
12 h pi	31.0 ± 1.41	26.5 ± 3.54	29.5 ± 0.71
24 h pi	29.0 ± 2.65	28.5 ± 4.95	33.0 ± 1.41
48 h pi	27.0 ± 1.41	27.5 ± 0.71	28.0 ± 5.66
72 h pi	28.3 ± 1.53	26.7 ± 2.83	35.0 ± 4.24
96 h pi	22.5 ± 3.54	26.5 ± 2.12	35.0 ± 1.41
120 h pi	31.0 ± 1.00	29.5 ± 0.71	32.5 ± 2.12
144 h pi	31.0 ± 1.41	30.0 ± 0	33.5 ± 0.71

IBD: infectious bursal disease

pi: postinfection

ND: not done

stable but for 24 h and 96 h pi when slight declines were recorded. In ducklings, heterophil counts postinfection were stable with no significant change during the 144 hours of observation.

Eosinophil counts (Figure 4) in chicks increased from a preinfection level of $0.83 \times 10^3/\mu\text{l}$ to a peak of $1.93 \times 10^3/\mu\text{l}$ at 6 h pi. Thereafter, there was no appreciable difference in counts, which ranged from $0.55 \times 10^3/\mu\text{l}$ to $0.86 \times 10^3/\mu\text{l}$ during 24 to 144 h pi. In turkey poults, there was a reduction in eosinophil counts from a preinfection level of $3.72 \times 10^3/\mu\text{l}$ to a minimum level of $0.88 \times 10^3/\mu\text{l}$ at 6 h pi. Thereafter, there was a sharp rise in the counts to peaks of $5.20 \times 10^3/\mu\text{l}$ and $5.71 \times 10^3/\mu\text{l}$ at 24 h and 72 h pi, respectively. Eosinophil counts in ducklings were again relatively stable with no appreciable change from 6 to 144 h pi.

DISCUSSION

This study was carried out to determine and compare chronologically the hematological changes associated with IBDV infection

in chickens, turkeys and ducks as part of an investigation into the pathogenesis of IBD. Of these three poultry host species, the chicken is the only one known to be naturally susceptible to IBD.

Increased hematocrit values, as observed in infected chickens in this study, have previously been observed by Chineme and Cho (5). It is believed that dehydration, which is one of the clinical and postmortem findings in IBD, is a major cause of the increase in the hematocrit value.

Preinfection (control) values obtained for total leukocyte, lymphocyte and eosinophil counts in chickens were within the normal ranges reported by Zinkl (16), i.e. $12\text{-}30 \times 10^3/\mu\text{l}$ for total leukocyte, $7\text{-}17.5 \times 10^3/\mu\text{l}$ for lymphocyte, and $0\text{-}1 \times 10^3/\mu\text{l}$ for eosinophil. Lymphopenia was observed in chicks and turkey poults from 24 to 96 h pi. According to Campbell and Coles (4), lymphopenia occurs in acute viral diseases due to glucocorticoid excesses. Specifically, IBDV causes destruction of B-lymphocytes within the bursa of Fabricius, which is the organ of maturation and differentiation of B-lymphocytes, before

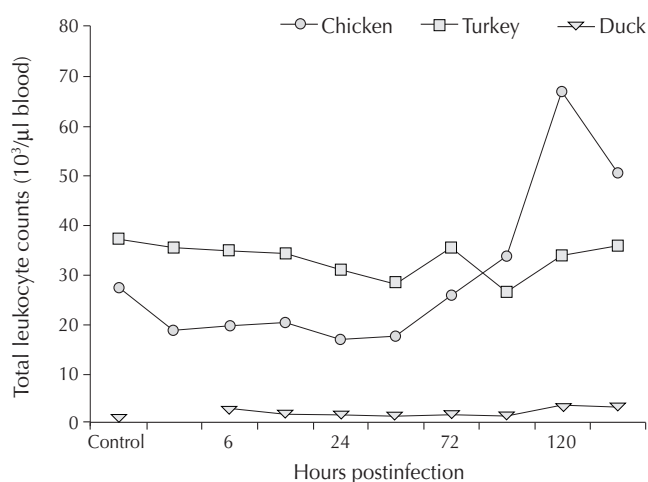


Figure 1: Effect of an infectious bursal disease virus infection on the total leukocyte count in chickens, turkeys and ducks.

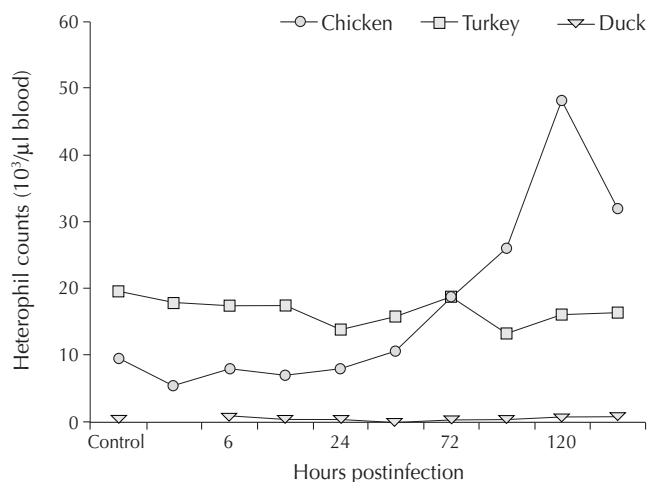


Figure 3: Effect of an infectious bursal disease virus infection on the heterophil count in chickens, turkeys and ducks.

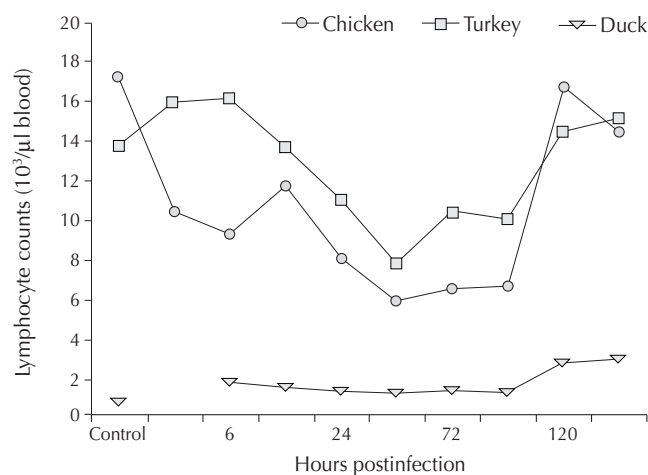


Figure 2: Effect of an infectious bursal disease virus infection on the lymphocyte count in chickens, turkeys and ducks.

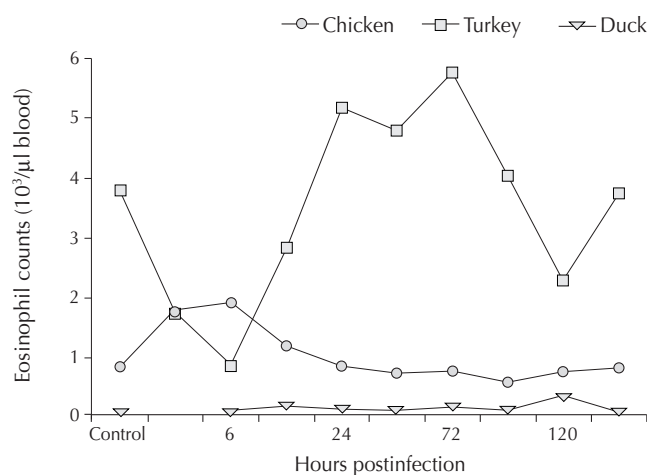


Figure 4: Effect of an infectious bursal disease virus infection on the eosinophil count in chickens, turkeys and ducks.

they migrate into the blood stream. Weiss and Kaufer-Weiss (15) detected IBDV antigen in the bursa of Fabricius of chickens 11 h pi. Thus, 24 hours provide an adequate period for the effect of B-lymphocyte destruction to be evident in the blood in cases where an IBDV infection has been established. Indeed, the two-phase lymphopenia in the chicks between 3 to 96 h pi helps to explain the pathogenesis of biphasic viremia in IBD described by Weiss and Kaufer-Weiss (15).

The marked heterophilia observed in the chicks in this study is evidence of massive tissue destruction in this species. Heterophils are known to phagocytize tissue debris (6). Absence of a heterophilic response in poults and ducklings is therefore a noteworthy finding, which is consistent with the hypothetical IBD-susceptibility ranking of these two species.

Eosinophilia was observed in both chicks and turkey poults but occurred earlier in chicks. Eosinophilia is associated with tissue destruction, especially tissues such as skin, lungs, gastrointestinal tract and female genitalia. These tissues have high concentrations of mast cells, and during tissue destruction these cells degranulate, resulting in histamine release, which is chemotactic to eosinophils (7). Thus, the findings of this study indicate early destruction of tissues (i.e. B-lymphocytes within the bursa of Fabricius) in chicks and imply a delayed or aborted IBD infectivity in poults, especially with the absence of external and internal (gastrointestinal and blood) parasites in the experimental birds.

The leukopenia that occurred in the chicks at 24 and 48 h pi could be due to the decrease in lymphocyte counts that occurred during

this period. Leukocyte counts, however, started to increase from 72 h pi due to heterophilia, which began at 72 h and peaked at 120 h pi.

The absence of postinfection heterophilia and lymphopenia in ducklings showed that infection was not established in this species. This refractoriness to IBD infection was further illustrated by the minimal (delayed) and total absence of tissue damage (as measured by eosinophilia) in turkey poults and ducklings, respectively.

In summary, after infection, increased PCV, eosinophilia, biphasic lymphopenia and heterophilia developed in chicks, while a single-phase lymphopenia and delayed eosinophilia occurred in turkey poults. Importantly, the absence of an appreciable hematological change in ducklings provides an explanation at the cellular level, for the non-susceptibility of this species to IBDV infection.

■ CONCLUSION

The results in this study presented hematological parameters which were consistent with the pathogenesis of IBD in chicks. These included the prompt or natural susceptibility to the infection, biphasic lymphopenia and the subsequent heterophilic response to tissue damage. On the other hand, a single-phase lymphopenia together with minimal heterophilic change in turkey poults implied delayed or unsuccessful IBDV infectivity in this species. The hematological stability in ducklings portrayed or explained the unresponsiveness or refractoriness of this species to IBDV infection.

REFERENCES

1. ABDU P.A., 1987. A retrospective study of infectious bursal disease of chickens in Zaria. In: Proc. 11th Annual conference of the Nigerian Society for Animal Production, Ahmadu Bello University, Nigeria, 23-27 March 1986, p. 65-69.
2. ALLAN D., DUFFUS W.P., 1971. The immunopathology in fowls (*Gallus domesticus*) of acute and subacute *Salmonella gallinarum* infection. *Res. vet. Sci.*, **12**: 140-151.
3. BARNES H.J., WHEELER J., REED D., 1982. Serological evidence of infectious bursal disease virus infection in Iowa Turkeys. *Avian Dis.*, **26**: 560-565.
4. CAMPBELL T.W., COLES E.H., 1986. Avian clinical pathology. In: Coles E.H. Ed., *Veterinary clinical pathology*. Philadelphia, PA, USA, WB Saunders, p. 289-290.
5. CHINEME C.N., CHOY., 1984. Clinicopathological and morphological changes in chickens experimentally infected with infectious bursal (Gumboro) disease virus. *Trop. Vet.*, **2**: 218-224.
6. COLES H.E., 1986. *Veterinary clinical pathology*, 4th Edn. Philadelphia, PA, USA, WB Saunders, p. 17-23.
7. JAIN N.C., 1986. *Schalm's Veterinary hematology*, 4th Edn. Philadelphia, PA, USA, Lea and Febiger, p. 747-748.
8. KOSTERS J., BETCH H., RUDOLPH R., 1972. Properties of infectious bursal agent of chicken (IBA). *Med. Microb. Immun.*, **157**: 291-298.
9. LUKERT P.D., SAIF Y.M., 1991. Infectious bursal disease. In: Calnek B.W., Barnes H.J., Beard C.W., Reid W.M., Yoder H.W., Eds, *Diseases of poultry*, 9th Edn. Ames, IA, USA, Iowa State University Press, p. 648-663.

10. MCFERRAN J.B., MCNULTY M.S., MCKILLOP E.R., CONNER T.J., MCCracken R.M., COLLIN D.S., ALLAN G.M., 1980. Isolation and serological studies with infectious bursal disease viruses from fowl, turkey and duck: Demonstration of a second serotype. *Avian Pathol.*, **9**: 395-404.
11. SANDER J.E., 1995. IBD invades turkeys, but does not cause disease. *Poult. Times* (Dec. 4): 3.
12. SKEELES J.K., LUKERT P.D., DE BUYSSCHER E.V., FLETCHER O.J., BROWN J., 1979. Infectious bursal disease virus infections. I. Complement and virus-neutralizing antibody response following infection of susceptible chickens. *Avian Dis.*, **23**: 95-106.
13. SKEELES J.K., SLAVIK M.F., BEASLEY J.N., BROWN A.H., MEINECKE C.F., MARUCA S., WELCH S., 1980. An age-related coagulation disorder associated with experimental infection with infectious bursal disease virus. *Am. J. vet. Res.*, **41**: 1458-1461.
14. VON BULOW V., 1991. Infectious anaemia. In: Calnek B.W., Barnes H.J., Beard C.W., Reid W.M., Yoder H.W., Eds, *Diseases of poultry*, 9th Edn. Ames, IA, USA, Iowa State University Press, p. 690-699.
15. WEISS E., KAUFER-WEISS I., 1994. Pathology and pathogenesis of infectious bursal disease. In: Proc. International symposium on infectious bursal disease and chicken infectious anaemia, Rauschholzhausen, Germany, 21-24 June 1994.
16. ZINKL J.G., 1986. Avian hematology. In: Coles H.E., Ed., *Schalm's Veterinary hematology*, 4th Edn. Philadelphia, PA, USA, Lea and Febiger, p. 260-263.

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Résumé

Oladele O.A., Adene D.F., Obi T.U., Nottidge H.O., Aiyedun A.I. Etude hématologique séquentielle d'une infection expérimentale induite par le virus de la maladie de Gumboro chez des poulets, des dindes et des canards

Des poulets de chair, des dindonneaux et des cannetons âgés de quatre semaines ont été infectés expérimentalement avec le virus de la maladie de Gumboro, puis des échantillons sanguins ont été prélevés chronologiquement pour analyser les changements sanguins après l'infection. Bien qu'il y ait eu une nette augmentation des valeurs de l'hématocrite chez les poulets, atteignant un pic de 31 p. 00, 12 à 144 h postinfection, il y a eu, au contraire, une baisse générale de ces valeurs chez les dindonneaux et les cannetons avec des valeurs minimales respectivement de 26,5 p. 100 à 12 h postinfection et de 28,2 p. 100 à 48 h postinfection. La numération leucocytaire a augmenté chez les poulets de manière significative ($p < 0,05$) pour atteindre un pic de $66,83 \times 10^3/\mu\text{l}$ à 120 h postinfection, alors que chez les dindonneaux, elle a diminué jusqu'à $26,75 \times 10^3/\mu\text{l}$ à 96 h postinfection. Chez les poulets, une première diminution s'est produite entre trois et six heures après infection, pour atteindre la valeur la plus basse de $5,9 \times 10^3/\mu\text{l}$ à 48 h postinfection. Une baisse semblable a été enregistrée chez les dindonneaux avec comme valeur minimale $7,81 \times 10^3/\mu\text{l}$ à 48 h postinfection. Les nombres d'hétérophiles ont évolué chez les trois espèces d'une manière semblable à celle observée pour la numération leucocytaire. Alors que la numération des éosinophiles a d'abord augmenté chez les poulets pour atteindre un pic de $1,93 \times 10^3/\mu\text{l}$ à 6 h postinfection, puis a diminué, ces valeurs ont d'abord diminué chez les dindonneaux, jusqu'à un minimum de $0,88 \times 10^3/\mu\text{l}$ à 6 h postinfection, puis ont augmenté, atteignant un maximum de $5,7 \times 10^3/\mu\text{l}$ à 72 h postinfection. Toutes les valeurs hématologiques sont cependant restées relativement inchangées chez les cannetons. Les résultats ont montré que les poulets étaient affectés de lymphocytopenie en deux phases, d'éosinophilie et d'hétérophilie, les dindonneaux de lymphocytopenie et d'éosinophilie retardée, et que les cannetons avaient été peu affectés. Ceci renforce le fait qu'il existe des niveaux de sensibilité différents chez ces trois espèces de volaille.

Mots-clés : Poulet – Dindon – Canard – Virus bursite infectieuse – Infection expérimentale – Sang – Nigeria.

Resumen

Oladele O.A., Adene D.F., Obi T.U., Nottidge H.O., Aiyedun A.I. Estudio hematológico secuencial del virus de la bursitis infecciosa experimental en pollos, pavos y patos

Después de una infección experimental con el virus de la bursitis infecciosa en pollos de 4 semanas, pavos y patos, se recolectaron cronológicamente muestras de sangre y se analizaron las cargas post infección (pi). A pesar de un aumento neto en el hematocrito en pollos, alcanzando un pico de 31% de 12 a 144 h pi, hubo por el contrario una disminución de los valores en pavos y patos hasta un mínimo de 26,5% a 12 h y 28,2% a 48 h pi respectivamente. Los conteos leucocitarios en pollos aumentaron significativamente ($p < 0,05$) hasta un pico de $66,83 \times 10^3/\mu\text{l}$ a 120 h, mientras que en pavos disminuyeron a un mínimo de $26,75 \times 10^3/\mu\text{l}$ a 96 h pi. Los conteos linfocitarios en pollos se redujeron a un mínimo de $5,9 \times 10^3/\mu\text{l}$ a 48 h pi después de una reducción inicial entre tres y seis horas pi. Una disminución similar ocurrió en pavos, con un mínimo de $7,81 \times 10^3/\mu\text{l}$ a 48 h pi. La tendencia de los cambios en conteos de heterófilos para las tres especies fue similar a la observada para los conteos leucocitarios. Mientras que los conteos de eosinófilos en pollos aumentaron inicialmente hasta un pico de $1,93 \times 10^3/\mu\text{l}$ a 6 h pi y subsecuentemente declinaron, los valores de eosinófilos en pavos declinaron hasta un mínimo de $0,88 \times 10^3/\mu\text{l}$ a 6 h pi seguido por un aumento hasta un máximo de $5,7 \times 10^3/\mu\text{l}$ a 72 h pi. Sin embargo, todos los valores hematológicos en patos se mantuvieron relativamente estables. Estos resultados muestran que hubo una linfopenia bifásica, eosinofilia y heterofilia en pollos, una linfopenia y una eosinofilia tardía en pavos y relativamente ningún cambio en patos. Esto enfatiza el hecho de que existen diferentes niveles de susceptibilidad entre las tres especies estudiadas.

Palabras clave: Pollo – Pavo – Pato – Virus bursitis infecciosa – Infección experimental – Sangre – Nigeria.