EFFECT OF LEVAMISOLE HYDROCHLORIDE ON HAEMATOLOGICAL PARAMETERS IN COCKEREL EXPERIMENTALLY INFECTED WITH VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS (VVIBDV)

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ABSTRACT
The study was carried out to determine the effect of levamisole hydrochloride (LMS-HCl) on hematological parameters in cockerel experimentally infected with very virulent infectious bursa disease virus (vvIBDV). One hundred day old cockerels randomly divided into 4 groups (A, B, C and D) with 25 chicks per group were used for this study. Group A Chicks were administered LMS HCl orally at dose of 25 mg/kg on days 26 and 27, challenged with 0.05 ml of vvIBDV intraocularly on day 28. Group B infected with 0.05 ml of vvIBDV intraocularly on day 28, treated with LMS HCl orally on days 29 and 30 at same dose. Group C Chicks infected with 0.05ml of vvIBDV intraocularly on day 28 without LMS HCl, Group D served as controls. Blood sample was aseptically collected from the chicks randomly selected from each group, via the brachial vein using tuberculin syringes for haematological analysis on day 1, 10, 28, 30, 31 and 32. Significant increase ($p > 0.05$) in PCV were observed in groups A, B and C. Significant increase ($p > 0.05$) in total white blood cell count (TWBC) was observed in group B from day2 to day 4 post-infection. This significant increase in group B is suggestive of the ability of LMS HCl to enhance the immune system in an immunocompromised state of the cockerels. In CONCLUSION, the study revealed that LMS-HCl administration at immunomodulatory dose of 25 mg/ kg influenced lymphocytic response post inoculation with vvIBDV.

Keywords: Cockerel, hematological parameters, immumomodulator, levamisole, vvIBDV.

INTRODUCTION
Infectious bursal disease (IBD) commonly known as Gumboro disease is a disease of global economic importance (Lukert and Saif, 2003). The disease is characterized by the destruction of the lymphoid cells in the bursa of Fabricius as the virus replicates in differentiating B- lymphocytes (Kibenge *et al*., 1988). The disease was reported to occur in broiler chicks between 3-6 weeks of age and immuno-suppression is the main consequence in infected chicks (Abdul *et al*., 2001).The disease is caused by infectious bursal disease virus (IBDV) which is classified in the *Avibirnavirus* genus of the family *Birnaviridae* (Abdul *et al*., 2001). The very virulent IBDV (vvIBDV) causes mortality exceeding 50% in susceptible chickens (Mahgoub, 2012).The vvIBDV strains are able to cause greater decrease in thymic weight index and more severe lesion in caecal tonsils, thymus, spleen, and bone marrow, but the bursal lesions are similar (Lukert and Saif, 2003).Thus, it suppresses the immune response and indicates the need for immunomodulators. These are substances that enhance the immune response, with increased use particularly in poultry production around the world (Oladele *et al*., 2012).The aim of the work thus was to determine the immunomodulatory effect of LMS-HCl on haematological parameters of cockerels experimentally infected with vvIBDV.

MATERIALS AND METHODS
The study was carried out in Animal Rooms of the Department of Veterinary Medicine, Ahmadu Bello University (A.B.U.), Zaria, Kaduna State, Nigeria. The birds used for the study were purchased from reputable commercial hatchery, kept in deep litter system, fed with a commercial chick ration

(Vital feed®) and provided with portable drinking water *ad libitum.* Birds were acclimatized for 14 days prior to commencement of experiment. Ethical clearance was obtained from A.B.U. Committee for Animal Use and Care (ABUCAUC), with approval number ABUCAUC/2018/020. The vvIBDV Nigerian isolate was provided by the Department.

Experimental design

A total of 100 cockerels were divided at random into four groups of 25 birds each as follows: Group A; Chicks administered with LMS-HCl in drinking water at immuno-stimulating dose of 25 mg/kg as described by Hamed *et al*. (2017) on days 26 and 27,challenged with 0.05 ml of vvIBDV intraocularly on day 28. The chicks were euthanized on day 32 (4 days post inoculation).Group B; Chicks infected with 0.05 ml of vvIBDV intraocularly on day 28, LMS-HCl in drinking water on days 29 and 30 at dose of 25 mg/kg (Hamed *et al*., 2017). The chicks were euthanized on day 32.Group C; Chicks infected with 0.05ml of vvIBDV intraocularly on day 28 without LMS-HCl. The chicks were euthanized on day 32. Group D; Chicks neither administered with LMS-HCl nor infected with vvIBDV (Control).

Blood sample collection

About 1 ml of blood each from 4 chicks selected at random from each group was aspirated aseptically via the brachial vein using tuberculin syringe, emptied into a plain non-vacuum plastic tube containing 1.0mg of Ethylene diamine tetra acetic acid (EDTA) powder for haematology on day 1, to establish baseline data for haematological parameters. The blood sampling procedures were repeated on days 10, 28, 30, 31 and 32 of the experiment.

Haematological evaluations

The packed cell volume (PCV), total leucocyte count (TLC) and differential leucocytes count(DLC) were carried out using a standard haematological procedure as described by Coles *et al.* (1986).

Statistical analyses

The data obtained were expressed as mean ± SEM. Data were analyzed by one way ANOVA using Graph Pad prism Version 6.0. Tukeys post-hoc test was employed to determine difference between groups. Values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The mean pre-infection values of PCV in the cockerels were 23 ± 0.67%, 25 ± 1.40%, 24 ± 1.30% and 23 ± 0.85% in groups A, B, C and D respectively. On day 2pi, the mean PCV of the cockerels rose to 30 ± 1.30%, 31 ± 1.10% and 34 ± 2.00% in groups A, B and C respectively with no significant ($p > 0.05$) differences among the groups. The mean PCV values on day 3pi in groups A (33 ± 0.63%) and B (32 ± 0.41%) did not show a significant difference ($p > 0.05$). Cockerels in group D maintained mean PCV values with insignificant ($p > 0.05$) variation throughout the 4 days of the experiment (Figure I). In this work, the PCV was increased in groups A, B and C from day 2 post infections (pi) and this was accompanied by decrease in total red blood cell (RBC). Oladele *et al.* (2005) reported similar increase in PCV for up to 5 days pi following experimental IBDV infection in broiler chickens.. The increased PCV accompanying decreased RBC was probably due to dehydration as reported by Chineme and Cho (1984) and Oladele *et al.* (2005).

The mean pre-infection values of RBC in the cockerels were 4.6 ± 0.39 x 10^{12}/L, 4.1 ± 0.10 x10^{12}/L, 4.3 ± 0.42 x10^{12} /L and 4.4 ± 0.28 x10^{12} /L in groups A, B, C and D respectively. The mean RBC count significantly decreased ($p < 0.05$) in groups A, B and C on day 2pi with values of 3.7 ± 0.34x10^{12}/L, 3.5 ± 0.18x10^{12}/L and 3.6 ± 0.41x10^{12}/L respectively, compared to pre-inoculation values and that of group D (Figure II). The low RBC counts were maintained up to day 4pi (post infection) in groups A and B while group D cockerels did not show any significant ($p > 0.05$) alterations in RBC counts during the 4 days of experiment.

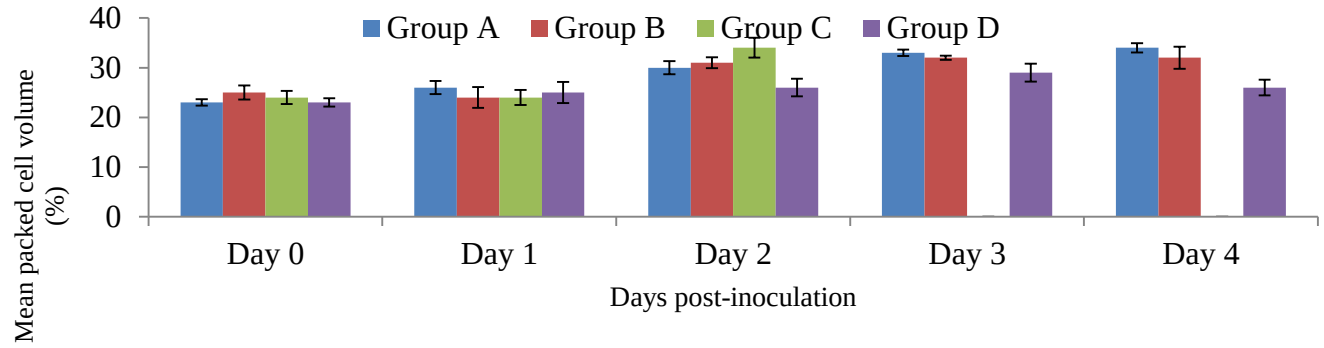

Figure I. Mean packed cell volume of vvIBDV infected and non-infected cockerels during the experiment.

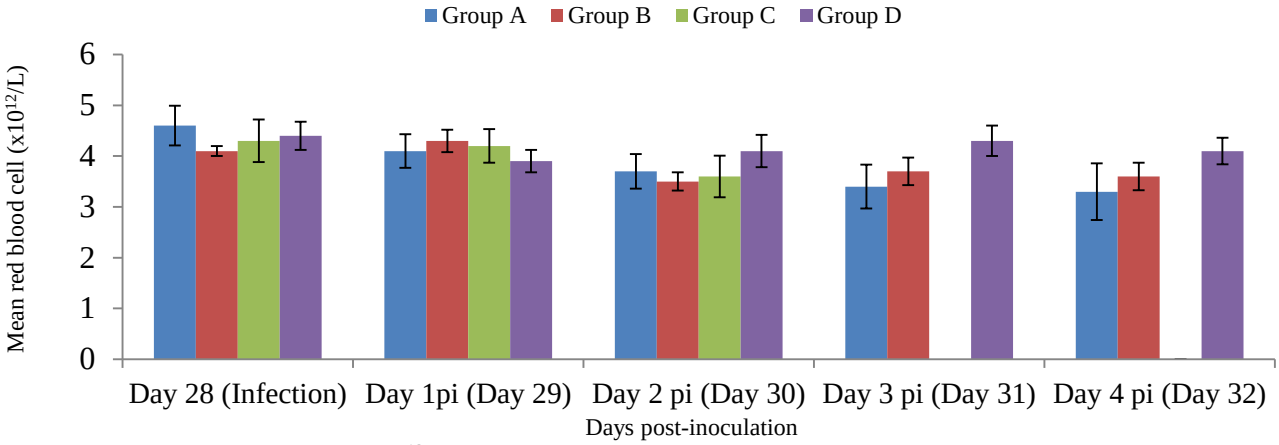

Figure II: Mean red blood cell($x10^{12}$/L) counts of vvIBDV infected and non-infected cockerels during the experiment.

Mean total leukocyte count (TLC) decreased from pre-inoculation values of 27 ± 1.10 $x10^9$/L, 26 ± 0.53 $x10^9$/L and 27 ± 0.61 $x10^9$/L to 19 ± 1.00 $x10^9$/L, 21 ± 0.85 $x10^9$/L and 14 ± 1.30 $x10^9$/L in groups A, B and C respectively on day 2pi (figure III). The mean TLC in group A then increased on day 3 and 4pi to values of 22 ± 0.43 $x10^9$/L and 24 ± 0.89 $x10^9$/L respectively but the difference was not significant ($p > 0.05$). Mean TLC in group B increased significantly ($p < 0.05$) to values of 34 ± 0.46 $x10^9$/L and 38 ± 0.90$x10^9$/L on days 3 and 4pi respectively. Group D cockerels had constant mean TLC up to day 4pi which were significantly ($p < 0.05$) different from those of groups A, B and C on day 2pi; and from groups A and B on days 3 and 4pi (figure III).The decreased TLC observed in groups A, B and C at day 2 pi could be due to increase in multiplication of IBDV, with consequent destruction of lymphocyte forming tissues leading to decrease in lymphocyte following infection. This agrees with the observation of Oladele *et al*. (2005) who reported decreased TLC due to decreased lymphocyte at 24 hours post-inoculation with IBDV. There was insignificant increased in mean TLC in group A on day 3 and 4 pi while in group B, a significant increase in TLC was observed from day 2 pi to day 4 pi. This significantly increased TLC in group B is suggestive of the ability of LMS-HCl to enhance the immune system in an immunocompromised state of the cockerels (Hamedi *et al*., 2017).

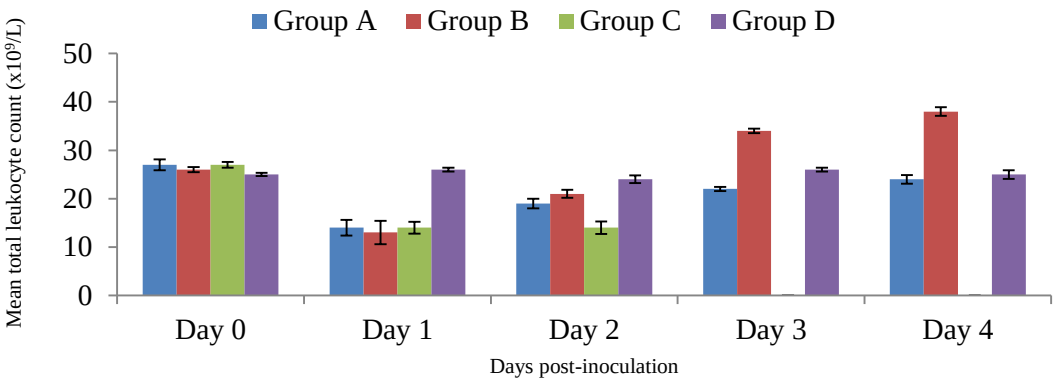

Figure III: Mean total leukocyte count ($x10^9$/L) of vvIBDV infected and non-infected cockerels during the experiment.

CONCLUSION

In CONCLUSION, following administration of levamisoleHCl at immunomodulatory dose rate of 25 mg/kg pre- and post-inoculation with vvIBDV, there was greater lymphocytic response post-inoculation with vvIBDV. It is thus recommended for use before and after an infection of birds with IBD to lessen the rate of losses with regard to condemnation of carcass and mortality.

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