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Prophylactic effect of ascorbic acid and levamisole on clinical signs and lesions of infectious bursal disease in five-week-old cockerels

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ABSTRACT

Aim: The study was aimed to evaluate prophylactic effect of ascorbic acid (AA) and levamisole combination on clinical signs and lesions of infectious bursal disease was investigated.

Method and materials: A total of Eighty (80) two-weeks old cockerels were purchased from RNT poultry production unit, Yobe state, Nigeria. The birds were fed with pelletized chicks' mash from Vital feeds, Nigeria and clean drinking water was provided ad-libitum. The chicks were vaccinated with Newcastle disease vaccine (Lasota) at 3 weeks of age and raised upto4 weeks before used for the experiment. The birds were divided into 4 groups (A to D; n= 20). Group A (pretreated infected), Group B (infected treated), Group C (infected untreated) and Group D (uninfected untreated).Pre-treatment and treatment of the birds following infection with IBDV was done with AA and Levamisole. On days 0,2,3,5 and 7 post inoculation, birds were sacrificed for evaluation of gross and microscopic lesions.

Results: Birds in Groups A to C manifested clinical signs of depression, ruffled feathers, whitish diarrhea and loss of appetite, which are more severe in group C. On days 0,2,3,5 and 7 post inoculation, the birds were sacrificed for evaluation of gross and histopathology. Gross lesions observed included intramuscular haemorrhages, congested and enlarged spleen, enlarged and haemorrhagic bursa of fabricius, enlarged liver with pale areas; while microscopically, lesions included moderate lymphocytolysis in intact bursal follicles, congestion of central veins, lymphoid depletion and red pulp hyperplasia in the spleen which were milder in group A and B when compared to group C.

Conclusion: It was concluded that AA and levamisole combination treatment during infectious bursal disease outbreaks can modify the manifestation and course of the disease leading to reduced morbidity and mortality as well as reduce or milder clinical manifestation and lesions of the disease.

Keywords: Ascorbic acid, cockerels, infectious bursal disease, levamisole.

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Introduction

The poultry industry has grown tremendously in Nigeria in terms of production and marketing in recent times (Achoja *et al.*, 2006). However, incidence of diseases has been a major setback to poultry industry (Chibuzo *et al.*, 1999). Infectious bursal disease (IBD) or Gumboro disease, among top five poultry diseases, has been a socio-economically important and immunosuppressive disease of poultry industry throughout world (Orakpoghenor *et al.*, 2020; Adino and Bayu, 2022; Kapoor *et al.*, 2022; Shah *et al.*, 2022; Zhang and Zheng, 2022; Hayajneh and Araj, 2023). In 1957, it was reported for the first time in broiler flocks around Gumboro, Delaware, USA (Kegne and Chanie, 2014; Liew *et al.*, 2016; Adino and Bayu, 2022; Zhang *et al.*, 2022). The disease is acute and also an extremely infectious and transmissible disease of poultry caused by IBD virus (IBDV) (Mwenda *et al.*, 2018) in young chickens (Damairia *et al.*, 2023).

In Nigeria, the disease occurs among commercial and occasionally village chickens (Akoma and Baba, 1995; El-Yuguda and Baba, 2002; El-Yuguda and Baba 2004) and with equal frequency throughout the year. IBD is characterised by mucoid diarrhoea, dehydration, depression and rapid weight loss, morbidity of close to 100% and mortality of 20-30% (Shettima *et*

al., 2018). Recovery is in 5-7 days (El-Yuguda and Baba, 2004). The post mortem lesions usually seen in IBD include enlarged and edematous bursae, hemorrhages in legs, thighs and breast muscles which may extend to other visceral organs like the kidneys and the proventriculus (Awolaja and Adene, 1995; El-Yuguda and Baba, 2004). The disease is highly immunosuppressive rendering infected birds poorly responsive to vaccination (El-Yuguda *et al.,* 2005) and more susceptible to infections (Okoye and Ezema, 2004).

The principal method of control is by active immunization of birds (Orakphogenor *et al.,* 2020; Du *et al.,* 2023). However, outbreaks are still common even in vaccinated flocks (Abdu, 1986; Igbokwe *et al.,* 1996; Ibrahim *et al.,* 2003; Lawal *et al.,* 2014). Consequently, many workers have tried to find additional ways of controlling IBD along with vaccination. The use of non-specific factors such as treatment with immune modulators like vitamin E and K (Ofoegbu, 2003) has given some promising results. Ascorbic Acid or vitamin C is important in the optimum function of the immune system through enhancement of leucocytes production and also through protection against free radical injury (Wu *et al.,* 2000). Ascorbic acid is also said to be present in high concentrations in leucocytes and is utilized at a higher rate during infections and phagocytosis (Wu *et al.,* 2000). Levamisole is a broad-spectrum synthetic imidazothiazole derivative, which are nicotine-like (Oladele *et al.,* 2012). As an immune modulator (Oladele *et al.,* 2012), it has been established to restore depressed T-lymphocyte function and potentiates the activity of phagocytes. Treatment with AA and levamisole combination may enhance the fibrous insulation of bursal vessels in the chicken which will boost the immune system thereby providing protection against IBD virus. Therefore, the aim of this study was to investigate the possible beneficial effects of a combination of AA and levamisole on the course of clinical IBD with regards to clinical signs and post-mortem lesions.

Materials and Methods

Experimental birds: A total of Eighty (80) two-weeks old cockerels were purchased from RNT poultry production unit, Yobe state, Nigeria. They were raised on a clean and disinfected deep liter. The birds were fed with commercially prepared pelletized chicks' mash from Vital feeds (Grand cereals and oil mills limited Bukuru Jos, Nigeria) and clean drinking water was provided ad-libitum. The chicks were vaccinated with Newcastle disease vaccine (Lasota) at 3 weeks of age and raised up to the age of 4 weeks before they were used for the experiment.

Ascorbic Acid: Ascorbic acid powder of high analytical grade was obtained from National Agency for Food, Drugs Administration and Control (NAFDAC) Maiduguri area laboratory, Nigeria and was administered at a dose rate of 500mg per kg OS (orally) for 8 days to each bird.

Levamisole: Levamisole injection was also obtained from a reputable Veterinary Pharmaceutical outlet at Gamboru market in Maiduguri, Nigeria and was administered at a dose rate of 0.2 mg per kg subcutaneously (SC) for 8 days to each bird.

Virus Antigen: A package of IBD viral antigen containing both positive and negative serum was purchased from NVRI (VOM), Jos Plateau state. Nigeria. This was used to inoculate the bird at the viral dilution of 1:16. Each bird was given a drop of the preparation (0.025mls) orally.

Experimental Design: The birds were divided into four groups (A to D) of twenty (20) each and subjected to the following treatments: -

Group A: were pretreated with ascorbic acid and levamisole, orally and subcutaneously at a dose rate of 500mg per kg orally and 0.2mg per kgSC respectively.

Group B: were infected with IBDV and treated with ascorbic acid and Levamisole as in group A

Group C: were infected with IBDV and left untreated.

Group D: were the uninfected untreated group.

Each bird in group A was administered 0.5ml of 25% (500mg) ascorbic acid per OS daily followed by levamisole 0.2mg per kg subcutaneously for 8 days prior to inoculation. The treatment continued throughout course of the infection.

Postmortem Examination: On days 0,2,3,5 and 7 post inoculation birds were sacrificed for evaluation of gross and microscopic lesions. Post-mortem examination was performed based on the standard procedure of Zander *et al.* (1991) and the gross lesions observed were duly recorded. Tissue samples of bursa of Fabricius (BF), spleen, muscles and liver were collected and fixed in 10% neutral buffered formalin

Tissue Preparation for Histology: The 10% buffered formalin fixed lungs were dehydrated in graded alcohol (70, 80, 90 and 100 %); while Xylene and Paraffin wax were used for clearing and embedding

respectively. Serial sections of 5 µ thick were obtained using a rotator microtome. Deparaffinized sections were stained with haematoxylin and eosin as described by Bancroft and Gamble, (2002). Slides were examined using light microscope at different magnifications. Photomicrographs of lesions were taken using Amscope digital camera for microscope version (3.0 China).

Results and Discussion

The birds in group A to C showed different clinical signs after inoculation with IBD virus, including depression, ruffled feather, weakness, whitish diarrhea and loss of appetite (Table 1).

The gross lesions observed including intramuscular haemorrhages which was more severe in group A and C than in group B. These lesions were seen on the 3rd and 5th day post-infection. Haemorrhages were seen in the spleen which were severe on day 5 in group C and moderate on day 3 in groups B and C. Bursa of Fabricius was enlarged and haemorrhagic. The haemorrhages in the bursa of Fabricius was more severe on days 3 and 5 in group C and on day 5 in group B but moderate on day 3 in groups A and B (Fig 1-3).

The microscopic lesions observed included moderate lymphocytolysis of the Bursal follicles (Fig 4), red pulp hyperplasia or severe congestion with white pulp depletion in the spleen (Fig 5) and congestion of central veins in the liver (Fig 6).

Table 1: Clinical observation of cockerel chicks pre-treated and treated with combination of ascorbic acid and levamisole following infection with IBDV, infected untreated and uninfected untreated chicks

Observations	Group A (Pretreated infected)	Group B (Infected treated)	Group C (Infected untreated)	Group D (Uninfected untreated)
Incubation period	3 days	3 days	2 days	-
Mortality	0/20 (0%)	0/20 (0%)	0/20 (0%)	-
Morbidity	12/20 (60%)	15/20 (75%)	19/20 (95%)	-
Duration of Clinical signs	2 days	3 days	4 days	-
Clinical signs	Weakness, ruffled feathers, whitish diarrhea	Weakness, whitish diarrhoea, ruffled feathers	Whitish diarrhea, ruffled feathers, loss of appetite, depression	-

KEY: IBDV = Infectious bursal virus.

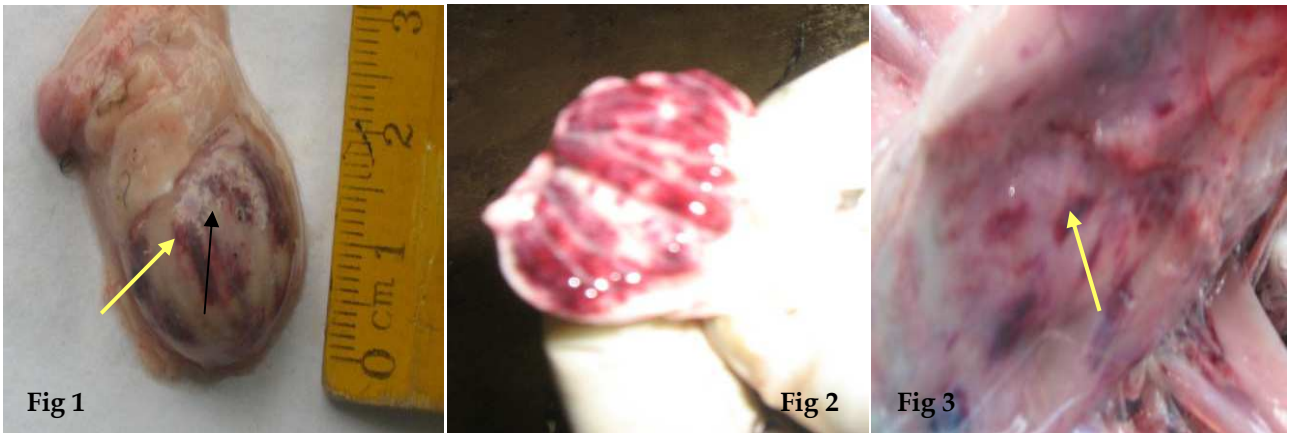

Fig 1: Gross picture of the bursal of Fabriciusin 5 weeks old cockerels showing enlargement and haemorrhages on the serosal surface (arrow). Fig 2: Gross picture of an enlarged bursa of Fabricius in 5 weeks old cockerel showing haemorrhages on the mucosal surface. Fig 3: Gross picture of the pectoral muscle in 5 weeks old cockerel showing intramuscular haemorrhages (arrow).

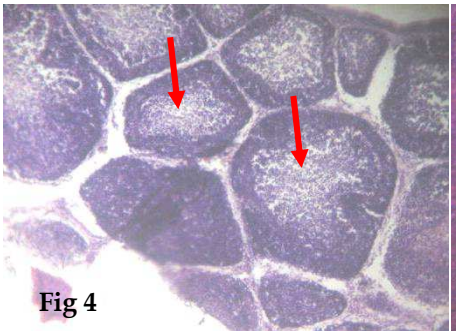
Fig 4

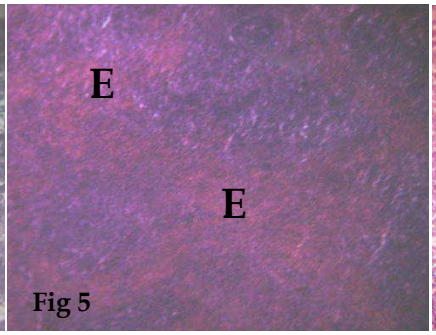

Fig 5

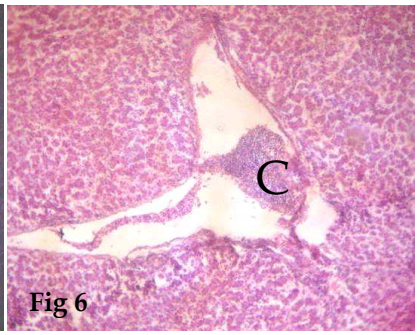

Fig 6

Fig 4 : Photomicrograph of the bursa of Fabricius of 5 weeks old cockerels pretreated with a combination of AA and levamisole and infected with IBD showing lymphocytolysis of the bursal follicles(arrows). H&E (X200). **Fig 5 :** Photomicrograph of the spleen of 5 weeks old cockerels pretreated with a combination of AA and levamisole and infected with IBD showing red pulp hyperplasia (E). H&E (X100). **Fig 6:** Photomicrograph of the liver of 5 weeks old cockerels infected with IBD and treated with a combination of AA and levamisole showing congestion of the central veins (C). H&E (X200).

Ascorbic acid is important in the optimum function of immune system through enhancement of leucocyte production and also protection against free radical injury (Otanwa *et al.* 2023). Levamisole has been established to have an immunomodulating effect by restoring depressed T-Iymphocyte function and potentiation of the activities of phagocytes (Oladele *et al.* 2012). Hence, the combination of ascorbic acid and levamisole may enhance the immune system by providing cellular protection against infectious bursal disease virus infection.

The clinical signs observed in this study were consistent with those reported elsewhere (Oluwayelu *et al.* 2002; Liang *et al.* 2015; Zannah *et al.* 2020; Omer and Khalafalla, 2022; Du *et al.* 2023). Chickens in group C (infected untreated) exhibit ruffled feathers, whitish diarrhea, loss of appetite and depression. These clinical symptoms were distinct from those of similar diseases, such as inclusion bodies hepatitis (IBH), reported by Wibowo *et al.* (2019), or experimental study post challenge with IBDV (Xu *et al.* 2020). These signs were moderate in groups A and group B, and could be attributed to the combine immunoprotective and modulating effect of AA and levamisole in the infected chickens (Oladele *et al.*, 2012). The morbidity in group C (infected untreated) was 95% which was later reduced to 75% and 60% in pretreated infected (group A) and infected treated (group B) respectively. It suggested a possible immunosuppressive effect of the combined drugs used as pretreatment before infection and treatment after infection (El-Zanaty 1994). The incubation period was delayed by one day in both groups A and B, with no mortality recorded. These all indicated that ascorbic acid and levamisole combination enhances the immune system by increasing resistance against IBD virus infection.

The observed gross lesion of intramuscular haemorrhages in group C (infected untreated) could arise from minute endothelial injuries in small blood vessels which lead to diapedesis into extravascular spaces of the muscles. These muscles were active muscles in chickens prone to strenuous activities of leg movement while carrying of the body weight and sapping the wings, respectively. This muscular activity could enhance the hemorrhage by diapedesis by generating increased capillary pressure. The hemorrhages were reported to be associated with thrombocytopenia and clotting abnormalities which might be elicited by depletion of blood coagulation factors (Skeeles *et al.*, 1979a, b, 1980; Zeryehum *et al.*, 2012). Similarly, haemorrhages was observed in the Bursa of fabricius been the major immune organ, in addition to the spleen. This was consistently reported by Damairia *et al.* 2023. The lesions of haemorrhages were also reported in other organs including kidney, thymus, proventricular-ventricular junction (Islam and Samad, 2004). The microscopic lesions of congested hepatic central vein, lymphocytolysis of the bursal follicles and red pulp hyperplasia observed the pretreated and infected, and the IBDV infected and treated group are suggestive of inflammatory reactions induced by the viral agent. This is in agreement with the report of previous researchers during their studies on IBDV infection (Islam *et al.* 2008; Kulsum *et al.* 2018). The result of the present study indicated that pretreatment and treatment following infection with IBDV is beneficial in chickens to some extent from the adverse effect (tissue damages) of disease.

Conclusion

It was concluded that ascorbic acid and levamisole combination treatment during infectious bursal disease outbreaks can modify the manifestation and course of the disease leading to reduced morbidity and mortality as well as reduce or milder clinical manifestation of the disease. It can therefore be recommended for the management of IBD outbreaks.

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