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Effects of Immunostimulant (Levamisole hydrochloride) on the clinico-pathological changes in cockerel experimentally infected with very virulent infectious bursal disease virus (vvIBDV)

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ABSTRACT

The study was aimed to determine the effects of Levamisole hydrochloride (LMS HCl) on the clinico-pathological changes in cockerel experimentally infected with very virulent infectious bursal disease virus (vvIBDV). One hundred day old cockerels were purchased and randomly divided into 4 groups (A, B, C and D) with 25 chicks per group. 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 0.05 ml of vvIBDV intraocularly on day 28 without LMS HCl. Group D served as controls. Clinical signs observed were anorexia, weakness, recumbency, ruffled feathers, whitish diarrhea, drooped wings and depression. The clinical signs were milder in LMS treated groups. Mortality rates of 32%, 48% and 100% were observed in groups A, B and C respectively. There was mitigation in mortality rates in LMS treated groups. Pathological findings revealed slightly enlarged bursa of Fabricius, spleen and thymus, others are haemorrhagic breast, mucosal haemorrhages of proventriculus and thigh muscles, were observed in groups A, B and C. In conclusion, the study revealed that LMS HCl administration decreases the clinico-pathological changes in cockerels challenged with vvIBDV.

Keys: *Levamisole, clinico-pathological changes, cockerel, vvIBDV and immunostimulant.*

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INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious, immunosuppressive viral disease of young chickens that causes significant economic losses to the poultry industry worldwide [1].The disease was first recognized in 1962 in an outbreak in Gumboro, Delaware, U.S.A. and further outbreaks were subsequently referred to as" Gumboro disease" [2] .

The most prominent lesion of the disease is located in the bursa of Fabricius; hence the present name of the disease. The mode of transmission of IBD may be by direct contact between infected and susceptible flocks. IBD is caused by infectious bursa disease virus (IBDV) which is a small, non-enveloped virus and belongs to the Family Birnaviridae, characterized by a bi-segmented dsRNA genome [3]. Two serotypes of IBDV have been identified. Serotype I affects young chickens with high mortality and morbidity followed by immunosuppression [4]. Whereas serotype II, isolated from both chickens and turkeys, is non-pathogenic [5].The pathotypic serotype I IBDV strains can be grouped into classical virulent, antigenic variant and very virulent (vv) strains. The later strain is associated with high mortality, ranging from 60-100% [5]. The vvIBDV strains cause severe lesion in caecal tonsils, thymus, spleen, and bone marrow. Thus, it suppresses the immune response and indicates the need for immunomodulators.

Levamisole hydrochloride (LMS) is an imidazole-thiazole group derivative, which is an effective, safe and broad spectrum anthelmintic commonly used in animal and human medicine. It is used in poultry at recommended anthelmintic dosage of 25mg/kg body weight. Its immunomodulatory property has been demonstrated in diseased and stressed birds [6]. It was reported by [6] that it enhanced humoral immune response in clinically-immunosuppressed broilers.

The aim of the study was to determine the immuno-modulatory effects of LMS on clinico-pathological changes in cockerels experimentally infected with vvIBDV.

MATERIALS AND METHODS

Study Area

The study was carried out in Animal Rooms of the Department of Veterinary Public Health and Preventive Medicine and Veterinary Teaching Hospital of Ahmadu Bello University (A.B.U), Zaria (11 ^{0}10' N and 07 ^{0} 38' E), Kaduna State, Nigeria.

Source of Birds

The birds used for the study were purchased from Olam hatchery limited, Nigeria.

Housing and management

The birds were kept in deep litter system, fed with a commercial chick ration (Vital feed®) and were provided with portable drinking water *ad libitum.*

Acclimatization

Birds were acclimatized for 14 days prior to commencement of the experiment.

Source of challenge virus

The vvIBDV Nigerian isolate was provided by Department of Veterinary Medicine, A.B.U., Zaria, Kaduna State.

Ethical Considerations/Approval

Ethical approval for this work was obtained from Ahmadu Bello University Committee for Animal Use and Care (ABUCAUC), Zaria, with the approval number ABUCAUC/2018/020. All cockerels for this experiment were handled and conducted in strict compliance to the International standards for the care and use of animals for research purposes.

Experimental Design

One hundred cockerels were divided at random into four groups of 25 birds each as follows:

Group A; Chicks administered with LMS in drinking water at immuno-stimulating dose of 25 mg/kg as described by [7] on days 26 and 27,challenge 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, treated with LMS in drinking water on days 29 and 30 at dose of 25 mg/kg as described by [7] . The chicks were euthanized on day 32.

Group C; Chicks infected with 0.05ml of vvIBDV intraocularly on day 28. The chicks were euthanized on day 32.

Group D; Chicks neither administered with levamisole-HCl nor infected with vvIBDV (Control). The chicks were euthanized on day 32.

Clinical observations
Following inoculation of the birds with vvIBDV, the chicks were closely observed for clinical signs, morbidity and mortality.

Gross Pathological Examination
Dead and euthanized birds were examined for lesions of IBD. The bursa of Fabricius, spleen and thymus, alongside with other organs, such as gizzard, kidney, liver, proventriculus and skeletal muscles were also examined. The findings were recorded and photographed.

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

RESULTS

Clinical Signs

Clinical signs observed in group A
In group A (cockerels treated with levamisole-HCl prior to challenge with vvIBDV at day 28), had anorexia, weakness, reluctance to move, recumbency, ruffled feathers, drooped wings, depression, whitish diarrhea, morbidity and 32% mortality (Table I).

Clinical signs observed in group B
In group B (cockerels treated with levamisole-HCl post challenge with vvIBDV at day 28), showed anorexia, weakness, reluctance to move, recumbency, ruffled feathers, drooped wings, depression, whitish diarrhea, morbidity and 40% mortality (Table I).

Clinical signs observed in group C
In Group C (cockerels challenged with vvIBDV at day 28), had anorexia, weakness, reluctance to move, recumbency, ruffled feathers, drooped wings, depression, trembling, head hidden beneath wings, whitish diarrhea, morbidity and 100% mortality at day 3 pi (as shown in Table I).

Clinical Signs observed in group D
In Group D (cockerels neither treated with levamisole-HCl nor challenged with vvIBDV), did not show any clinical sign of illness.

Table I . Clinical signs and mortality among Levamisole hydrochloride immonomodulated cockerels challenged with very virulent infectious bursal disease virus at 4 weeks of age.

Clinical Signs	Day 1 (pi)			Day 2 (pi)			Day 3 (pi)			Day 4 (pi)		
	A	B	C	A	B	C	A	B	C	A	B	C
Anorexia n (%)	8 (32)	0	0	20(80)	15(60)	21(84)	7(28)	7(28)	0	3(12)	4(25)	-
Weakness n (%)	10(40)	0	0	20(80)	14(56)	25(100)	9(36)	9(36)	0	5(20)	5(20)	-
Recumbencyn(%)	0	0	0	14(56)	9(36)	15(60)	9(36)	7(28)	0	312	312	-
Depression n(%)	0	0	0	10(40)	8(32)	12(48)	6(24)	6(24)	0	3(12)	3(12)	-
Ruffled feathers n(%)	0	0	0	12(48)	10(40)	10(40)	9(36)	7{ 28)	0	5(20)	6(24)	-
Hurdling n(%)	9(36)	0	0	15(60)	13(52)	10(400	7(28)	8(32)	0	3(12)	3(120	
Somnolence n(%)	0	0	0	10(40)	3(12)	8(32)	5(20)	6(24)	0	2(8)	2(8)	-
Whitish diarrhea n(%)	0	0	0	4(16)	4(16)	6(24)	3(12)	3(12)	0	1(4)	1(4)	-
Morbidity n(%)	10(40)	0	0	20(8)	16(64)	24(96)	9(36)	10(40)	0	4(16)	6(24)	-
Mortality n(%)	0	0	0	6(24)	5(20)	15(60)	4(16)	2(8)	10(40)	2(8)	1(4)	-

Key:
n: Number of birds
%: Percentage
Pi : Post inoculation

Gross Pathological Examinations

In group A at days 2 and 3 pi (aged 30 and 31, respectively), gross lesions observed from birds that died naturally include slight haemorrhage, congestion in the breast and thigh muscles, enlarged BF and spleen, haemorrhagic kidney, trachea, thymus and proventriculus (Table II). The euthanized birds from this group had no gross changes.

The gross lesions observed from dead birds in Group B, at days 2 and 3 pi (aged 30 and 31 respectively) were slight congestion in the breast muscle; haemorrhagic leg and thigh muscles, enlarged BF with haemorrhagic bursal plicae; slightly congested caecal tonsils, slightly haemorrhagic proventriculus (Table II). On the other hand, the euthanized birds from this group had no relevant gross lesions.

In group C, the gross lesions observed were congested and hemorrhagic breast muscles (Plate 1).There were also enlarged, congested, and hemorrhagic kidneys; enlarged and congested liver; enlarged, congested and hemorrhagic lungs. There were mucosal haemorrhages in the proventriculus; congested, enlarged and turgid BF (Plate 2), haemorrhagic bursal plicae (Plate 3); congested and haemorrhagic caecal tonsils; congested and haemorrhagic spleens (Plate 4); enlarged and congested thymuses (Plate 5).

There was no gross pathology in the euthanized cockerels from the control group (Group D) during the period.

Table II : Summary of the gross lesions recorded at post mortem in groups A to C (birds challenged with vvIBDV at 4 weeks of age).

Organs	Gross lesions (A)	Gross lesions (B)	Gross lesion (C)
Bursa of Fabricius	Slightly enlarged in dead ones Normal in euthanized ones	Enlarged in dead ones Normal in euthanized ones	Enlarged, turgid , oedematous, Haemorrhagic and atrophy of bursa
Bursal plicae	Hemorrhagic in dead birds Normal in euthanized birds	Hemorrhagic in dead birds Normal in euthanized birds	Haemorrhagic
Spleen	Slightly hemorrhagic in dead ones. Normal in euthanized ones	Hemorrhagic in dead ones Normal in euthanized ones	Congested, Enlarged and Hemorrhagic
Thymus	Hemorrhagic in dead ones Normal in euthanized ones	Hemorrhagic in dead ones Normal in euthanized ones	Congested and Hemorrhagic
Breast muscle	Slightly congested in dead ones. Normal in euthanized ones	Slightly congested in dead ones. Normal in euthanized ones	Congested and Haemorrhagic
Gizzard	Slightly congested in dead ones. Normal in euthanized ones	Slightly congested in dead ones. Normal in euthanized ones	Enlarged and Haemorrhagic
Kidney	Slightly hemorrhagic in dead ones. Normal in euthanized birds	Hemorrhagic in dead ones Normal in euthanized birds	Congested
Liver	Slightly congested in dead birds. Normal in euthanized birds	Slightly congested in dead birds. Normal in euthanized birds	Congested
Lung	Slightly congested in dead ones. Normal in euthanized birds	Slightly congested in dead ones. Normal in euthanized birds	Congested and Haemorrhagic
Proventriculus	Hemorrhagic in dead ones Normal in euthanized ones	Hemorrhagic in dead ones Normal in euthanized ones	Haemorrhagic
Thigh and leg muscles	Slightly congested and hemorrhagic in dead birds Normal in euthanized birds	Slightly hemorrhagic in dead birds. Normal in euthanized birds	Hemorrhagic
Trachea	Slightly hemorrhagic in dead birds. Normal in euthanized birds	Slightly hemorrhagic in dead birds. Normal in euthanized birds	Congested and Hemorrhagic
Ureter	No urates deposits in both dead and euthanized birds	No urates deposits in both dead and euthanized birds	Deposits of urates

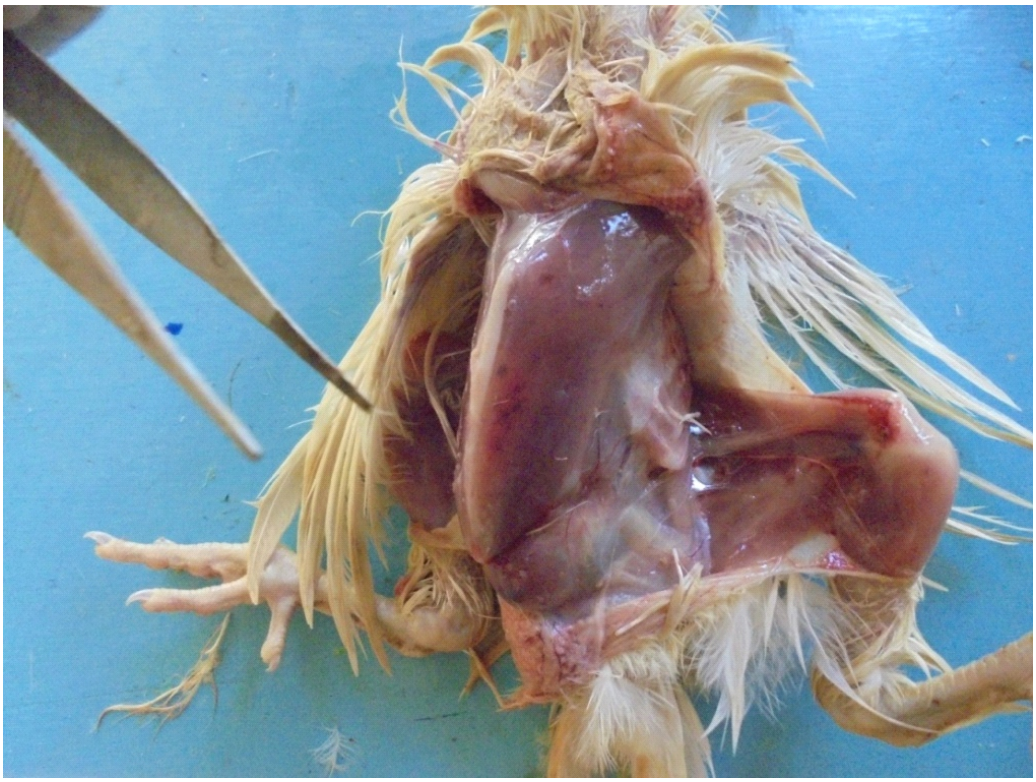

Plate 1: **Slight congestion and hemorrhagic breast muscle (arrow) of a cockerel in group C challenged with vvIBDV on day 28 at day 3 post infection.**

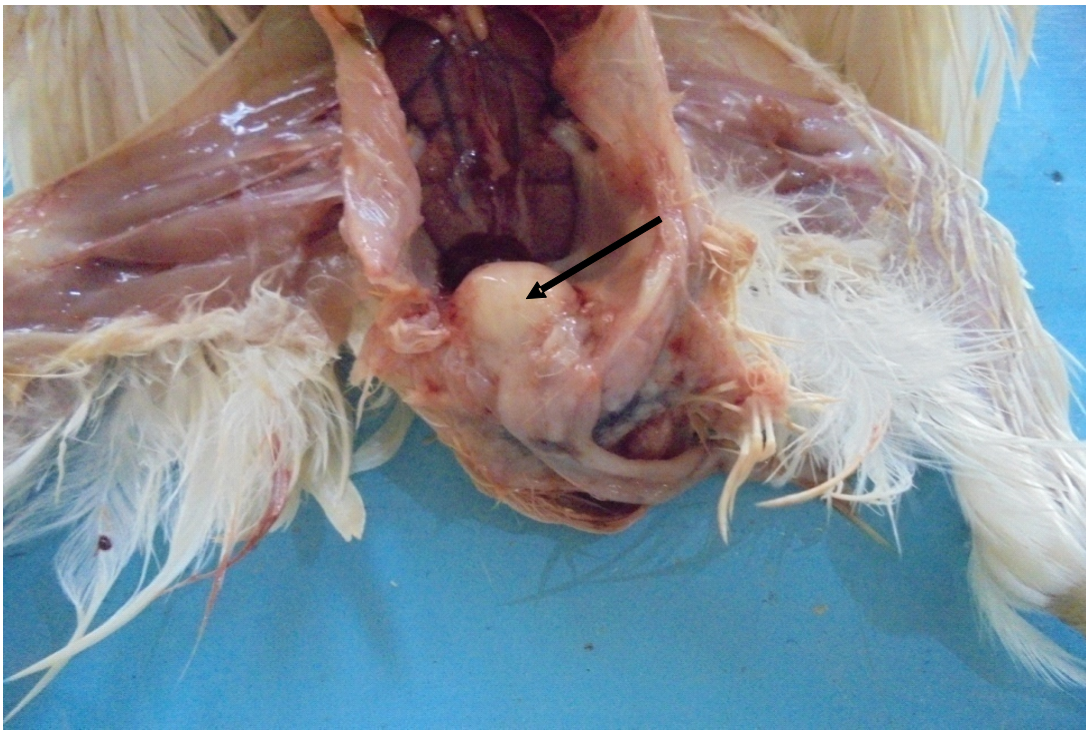

Plate 2: **Congested, enlarged and haemmorhagic bursa of Fabricius (arrow) of a cockerel in group C inoculated with vvIBDV on day 28 at day 3 post infection.**

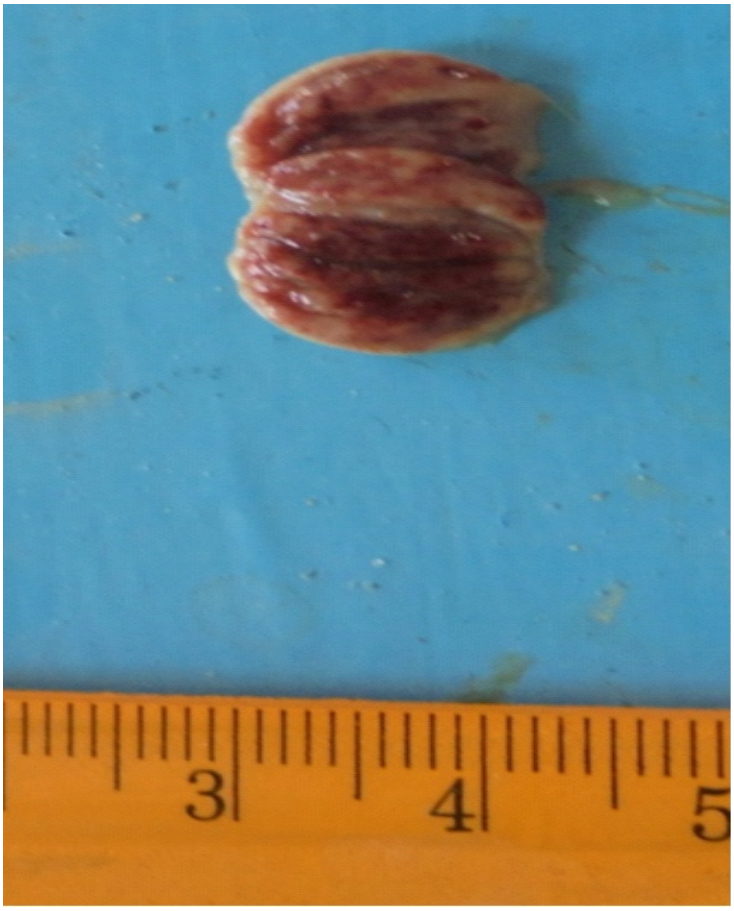

Plate 3: **Haemorrhages in the bursal plicae of a cockerel in group C challenged with vvIBDV on day 28 at day 3 post infection.**

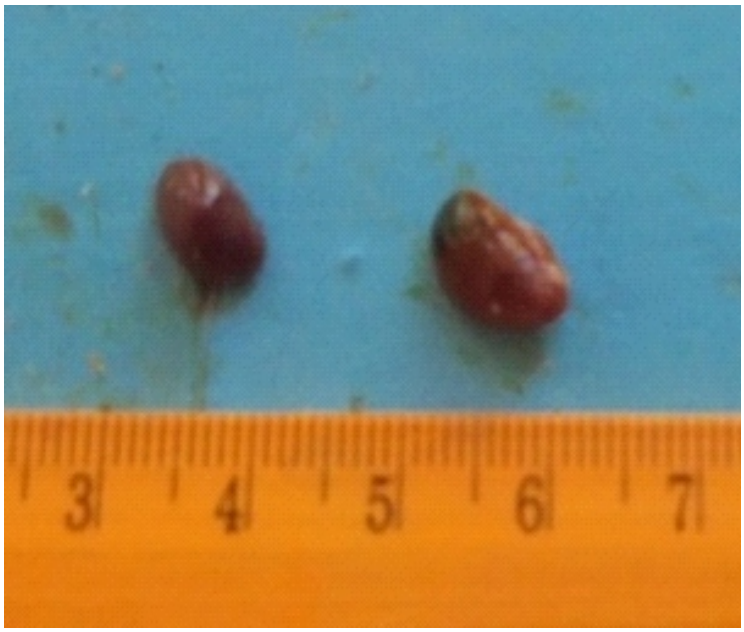

Plate 4: **Congested, enlarged and hemorrhagic spleens of cockerels in group C challenged with vvIBDV on day 28 at day 3 post infection.**

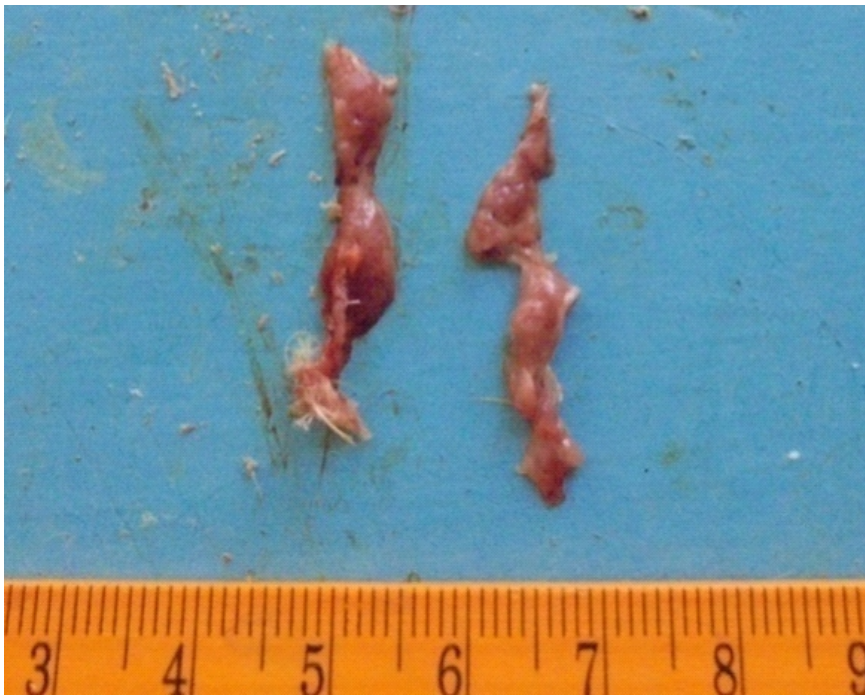

Plate 5: Congested and enlarged thymuses of cockerel in group C inoculated with vvIBDV on day 28 at day 3 post infection.

DISCUSSION

In this study, the clinical signs observed in the cockerels infected with vvIBDV without treatment with LMS HCl were ruffled feathers, drooped wings, depression, head hidden beneath wing, prostration, reluctant to move, recumbency, somnolence, trembling of muscle and whitish diarrhea. These clinical signs were consistent with the findings of [8] .There were differences in the clinical signs in vvIBDV inoculated groups treated with LMS-HCl pre- and post-inoculation. The clinical signs were milder in LMS treated groups, which might be attributed to the immunomodulatory effect of LMS-HCl. In previous investigation, LMS-HCl has been suggested to enhance immune response particularly in immuno-compromised state [9]. This suggests that the administration of LMS-HCl at an immunomodulatory dose could lead to reduction of the clinical signs of IBD. This is contrary to the finding of Suleiman that birds treated with LMS before and after challenge with IBDV generally do not come down with clinical signs of disease. This difference in the findings could be as a result of virulence in the virus used. This is in line with earlier reports by [4-8-11] that the degree of virulence of the infecting IBDV, type of birds, among other factors, may be responsible for the variability observed in the morbidity and mortality.

In this work, the mortality rates of 40%, 32%, and 100% were recorded in group A, B and C respectively. These were consistent with observations of other scientists who reported IBD mortality ranges between 5% - 57% [10-11] .There was mitigation in the mortality rates in vvIBDV inoculated groups treated with LMS pre and post inoculation which might be attributed to the immunomodulatory effect of

LMS. Also, variation in the rates of mortality could be associated to many other factors. It has been observed that the differences in age, breed, type of bird, size of bursae, climatic conditions, management practices, concurrent infection and degree of virulence of infecting IBDV, could be responsible for the variability in the mortality rate [4-8-11] .The enlargement observed in the BF, spleen and thymus early in the challenged cockerels were probably as a result of inflammatory response to vvIBDV. The oedema grossly observed on the organs might be attributed to the widening of the inter-follicular space distended by fluid accumulation and infiltration of massive heterophils to the organs. These have been documented in earlier reports on the pathogenesis of IBD [10-11].

Congestion and haemorrhage are among the major gross lesions noticed during post mortem examination in birds infected with IBDV. Ecchymotic and petechial haemorrhage in the bursa and proventicular-gizzard junction and paintbrush haemorrhage in breast, legs and thigh muscles conform to previous observations [12-13].The frequency of hemorrhage in bursa, skeletal muscles and the proventricular-gizzard mucosa was found to be higher in the dead than the euthanized birds. This observation might suggest that haemorrhage contributes to mortality in IBD.

In conclusion, the administration of levamisole HCl at immunomodulatory dose of 25 mg/kg pre- and post-inoculation with vvIBDV mitigated the clinical signs of IBD and also had significant effect on gross lesions in chicks infected with vvIBDV. The study recommends that administration of LMS at immuno-modulatory dose of 25 mg/kg can be of tremendous significance before, during and after an outbreak of infectious bursal disease; this would significantly decrease the rate of losses with reference to condemnation of carcass and mortality. This could assist the farmers as well as Veterinarians to remain in poultry business. Also, further research involving concurrent administration of LMS with IBD vaccine should be carried out.

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