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ORIGINAL ARTICLE

Comparative Study of Genetic Influence on the Susceptibility of Exotic Cockerels, Pullets and Broilers to Infectious Bursal Disease Virus

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SUMMARY
This study investigated comparatively the genetic influence on the susceptibility of exotic cockerels, pullets and broilers to natural infection with infectious bursal disease (IBD) virus in a flock of 150 seven-week-old exotic breed of chickens comprising of 50 Black Harco cockerels, 50 Black Harco pullets and 50 White Marshall broilers. Evaluation of possible genetic resistance was based on clinical signs, mortality and pathological changes in affected chicks. The virus was highly pathogenic for cockerels and pullets as clinical signs were evident on day 1 after the onset of the infection with hundred percent (100%) of the cockerels and pullets showing severe clinical disease on day 2 of the infection, while the broilers had the shortest timing as clinical signs were evident with 8% morbidity on day 2 of the infection only. The clinical signs were severe depression, diarrhoea, anorexia, prostration followed by death. Mortality was 92%, 78% and 6% for cockerels, pullets and broilers, respectively, within 3 days of the infection followed by recovery. Severe haemorrhages were present in the skeletal muscles, bursa, proventriculus-gizzard junction and caecal tonsils of dead cockerels and pullets only, while dead broilers showed only swollen bursae. Histologic lesions showed marked oedema, congestion of blood vessels, haemorrhages and necroses in the skeletal muscles, kidney, liver, and thymus of cockerels and pullets. Lymphocytic necrosis and depletion were marked in the spleen and caecal tonsils of the cockerels and pullets. Marked lymphoid depletion, oedema and heterophilic infiltrations were observed at day 2 of the infection in the bursae of cockerels, pullets and broilers. Assessing the clinical signs and lesions observed from affected chickens revealed that broiler is the least susceptible. It also revealed that within the Black Harco breed, cockerels are more susceptible to clinical IBD than the pullets. The low morbidity and mortality, and differential lesions observed in broilers indicated probable genetic resistance to clinical IBD.

Key words: Infectious bursal disease, chickens, breeds, susceptibility disease pattern, pathology.

INTRODUCTION
Infectious bursal disease (IBD) was described by Cosgrove (Cosgrove, 1962) as an acute, highly contagious viral infection of young chickens. The disease is caused by infectious bursal disease virus (IBDV), a double-stranded RNA virus that has

bisegmented genome; it belongs to the genus *Avibirnavirus* in the family *Birnaviridae* (Müller *et al*., 1979). The virus has tropism for the lymphoid tissues destroying immature B lymphocytes primarily in the bursa of Fabricius (Eterradossi and Saif, 2013). McFerran *et al*. (1980) designated several IBDV isolates from chickens and commercial IBDV vaccines as serotype 1, and two IBDV isolates from turkeys as serotype 2 on the basis of virus neutralization tests. Serotype 1 viruses have been reported to be pathogenic to chickens. In the late 1980s, classical serotype 1 IBDV evolved into very virulent (vvIBDV) forms in Europe, Asia, USA and other major parts of the world (van den Berg *et al*., 1991). These forms constituted a devastating disease for both small- and large-scale farmers in Nigeria (Adamu *et al*., 2013; Aliyu *et al*., 2016). The economic importance is characterized by high mortality of chickens 3 weeks of age and older, and severe, prolonged immunosuppressive effects in chickens infected at an early age that consequently result in poor immunological response to vaccination, high susceptibility to other infections and poor performance (Eterradossi and Saif, 2013).

Studies have shown that the virus is ubiquitous in commercial chicken operations (Jackwood *et al*., 2009; Adamu *et al*., 2013; Aliyu *et al*., 2016). The clinical signs and distinct lesions have been reported to vary considerably depending on the virulence of the strain, breed or genetic lineage, age and immune status of the chickens in experimental infections (Eterradossi and Saif, 2013). Higher mortality and pathological effects in pullets than in broilers or heavy breeds of chickens with virulent IBDV infections have been studied experimentally suggesting a difference in susceptibility between these two breeds of chickens (Bumstead *et al*., 1993; Tippenhauer *et al*., 2013; Silva *et al*., 2016). However, relatively little information is available regarding the difference in susceptibility between the breeds in natural IBDV infection. The purpose of the present study is to evaluate comparatively the possible genetic resistance based on clinical signs, mortality and pathological changes in unvaccinated cockerels, pullets and broilers naturally affected with IBDV.

MATERIALS AND METHODS

The experimental protocols were reviewed and approved by the Michael Okpara University of Agriculture, Umudike Committee on Medical and Scientific Research Ethics. General care of the birds was provided in accordance with the Institutional Animal Care and Use Committee as outlined in the *1998 Code of Federal Regulations, Animals and Animal Products (Animal and Plant Health Inspection Service, USDA, 1998).*

Flock History

A total of one hundred and fifty (150) of seven-week-old exotic breed of chickens comprising of fifty (50) Black Harco cockerels, 50 Black Harco pullets, and 50 White Marshall broilers were used for this study. They were purchased from a reputable local commercial hatchery at day-old and reared on deep litter. The chicks were kept in isolation in the Poultry Experimental Unit of the Department of Veterinary Pathology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, under strict biosecurity measures for experimental purposes. They were not vaccinated against any poultry disease. They were given vitamins and prophylactic antibiotics Gentadox WS® (Gentamycin & Doxycycline water-soluble powder) and anticoccidal Amprocox® (Amprolium 20% soluble powder with Vitamin K3) for 5 days at 2 days and 4 weeks of age, respectively in accordance with the manufacturer's recommendations. Feed and water were provided *ad libitum*. At six-week-old all the

birds were screened for maternally-derived antibodies to IBDV in the agar gel immunodiffusion (AGID) test and Newcastle disease virus by haemagglutination inhibition test and there was no detectable antibody in the groups in both tests.

General care of the birds was provided in accordance with the Institutional Animal Care and Use Committee as outlined in the *1998 Code of Federal Regulations, Animals and Animal Products* (Animal and Plant Health Inspection Service, USDA, 1998).

Experimental Protocol

Prior to commencement of the experiment, natural infection with IBDV occurred in the flock of 150 seven-week-old chicks.

Clinical Signs

The affected chicks were observed for clinical signs from days 1 to 8 after the onset of the natural infection. The daily percentage (%) morbidity and mortality rates were recorded.

Pathological Examinations

All the dead cockerels, pullets and broilers were necropsied and examined for gross lesions as described by Libby and Humphreys (1975) throughout the period of mortalities. The distribution of gross lesions in the dead chicks was recorded. The quantitative evaluations of gross lesion were statistically performed based on the mean number of birds with lesions in all the organs. Samples of the bursa, thymus, spleen, caecal tonsils, kidneys, liver, pancreas, proventriculus, muscles of the breast and thigh were collected from chicks that recently died of the disease and fixed immediately in 10% neutral buffered formalin for 48 hours. On day 6 of the infection, two chicks from the few chicks that survived from each type and breed of chicks were randomly selected and euthanized for sample collection. Fixed tissues were trimmed, embedded in paraffin wax, and cut into 5 μm thick sections. The sections were stained with haematoxylin and eosin (H&E), cover-slipped and examined by light microscopy.

IBDV antigen Detection and Confirmation by Agar Gel Immunodiffusion Test

This test was carried out at the Virology Department of National Veterinary Research Institute, Vom, Plateau State. The bursae were removed from ten recently dead chicks from each type and breed of chickens, minced, and 50% homogenate suspension of the organs was made in phosphate buffered saline containing antibiotics (penicillin, streptomycin, gentamycin and amphotericin B) (1000 μg/ml each). The homogenate was centrifuged at 3000*g* for 10 minutes. The supernatant fluid was harvested and tested for IBDV antigen by AGID test using a known positive antiserum as described in the OIE manual of diagnostics (OIE, 2016).

Bacteriological Examination

Samples of the bursa, spleen and heart were submitted for bacteriological examination. They were cultured aerobically at 37^{o} C on 5% sheep's blood and McConkey agar plates (Remel, Lenexa, KS) (Carter, 1990; Hoerr, 2008). Cultures were considered negative if no growth occurred after 48 hr of incubation.

Statistical Analyses

The data generated from this study were subjected to analysis of variance (ANOVA). The morbidity and mortalities data were analysed with the Fisher's Exact test. All tests were performed with a 5% level ($P < 0.05$) of significance using statistical products for service and solution (SPSS) at $P < 0.05$.

RESULTS

Clinical Signs

Sudden death of five cockerels and a pullet was observed on the first day of the natural

infection. The types of affected chickens, daily morbidity and mortality rates after the onset of the infection are shown in Tables I and II. Clinical signs in the remaining birds include: depression, reluctance to move, sleepiness, ruffled feathers, greenish-whitish diarrhoea and droopy appearance. In addition, drastic drop in feed and water consumption was observed. By day 2 of the infection, morbidity was 100% in the remaining cockerels and pullets, but 8% in broilers; prostration was commonly followed by death. Also, peak mortality was recorded in the remaining birds involving 38/45 (84.4%) of cockerels, 31/49 (63.3%) of pullets and 3 /50 (6%) of broilers. On day 3 of the infection, mortality was lowest in the remaining chicks and involved only 3 (42.9%) cockerels and 7 (38.9%) pullets (Figure 2); bringing the total mortalities to 46/50 (92%) cockerels, 39/50 (78%) pullets and 3/50 (6%) broilers excluding the sacrificed birds throughout the period of the infection. There were statistical differences ($P < 0.05$) between the morbidity and mortality rates (Tables I and II). Mortality lasted for 3 days from the first day after the onset of the natural infection and receding in a period of 5 to 7 days.

Table I: The daily morbidity of cockerels and pullets naturally infected with IBDV, compared with those of the broilers

Days PI	Cockerels	Pullets	Broilers	Statistics
1	15/50 [a]	8/50 [b]	3/50 [c]	P = 0.008; (P < 0.05)
2	45/45 [a]	49/49 [a]	4/50 [b]	P = 0.000; (P < 0.05)
3	3/4 [a]	7/11 [a]	1/47 [b]	P = 0.000; (P < 0.05)
4	3/4 [a]	5/11 [a]	0/47 [b]	P = 0.000; (P < 0.05)
5	2/4 [a]	5/11 [a]	0/47 [b]	P = 0.000; (P < 0.05)
6	1/4 [a]	4/11 [a]	0/47 [b]	P = 0.001; (P < 0.05)
7	0/2	1/9	0/45	P = 0.196; (P > 0.05)
8	0/2	0/9	0/45	No Stat

[a b c] Alphabetical superscripts in a row indicate significant differences in the morbidity between the groups, $P < 0.05$ (Fisher's Exact test)

Table II: The daily mortality of cockerels and pullets naturally infected with IBDV, compared with those of broilers

Days PI	**Cockerels**	**Pullets**	**Broilers**	**Statistics**
1	5/50 [a]	1/50 [b]	0/50 [b]	P = 0.048; (P < 0.05)
2	38/45 [a]	31/49 [b]	3/50 [c]	P = 0.000; (P < 0.05)
3	3/7 [a]	7/18 [a]	0/47 [b]	P = 0.000; (P < 0.05)
4	0/4	0/11	0/47	No Stat
5	0/4	0/11	0/47	No Stat
6	0/4	0/11	0/47	No Stat
7	0/4	0/11	0/47	No Stat

[a b c] Alphabetical superscripts in a row indicate significant differences in the mortality between the groups, $P < 0.05$ (Fisher's Exact test)

Gross Lesions

The distribution of gross lesions in the dead birds of each type of chickens is shown in Figures 1 to 4 and Table III. In the cockerel and pullet chicks, the major gross lesions were marked paint-brush haemorrhages on the thigh and breast muscles (Plate 1), petechial haemorrhages on the mesentery and other connective tissues on days 1 to 3 after the onset of the infection. The mean number of birds with muscle lesions was significantly ($P < 0.05$) higher in cockerels and pullets than in broilers (Figure 1). The bursae of the chicks that died on the first day after onset of the infection were enlarged and turgid with pale yellow discoloration. The bursae were also covered by gelatinous material and had clear fluid in the lumen. Bursae from chicks that died on days 2 and 3 after onset of the infection were completely swollen and haemorrhagic (Plate 1); while those that were euthanized on day 6 of the infection had atrophic bursae. The mean number of birds with bursal lesions was significantly ($P < 0.05$) higher in cockerels and pullets than in broilers (Figure 2). The spleen of birds that died on days 1 to 3 of the infection were swollen, with small pale foci while those that were euthanized on day 6 of the infection were normal in size. The mean number of birds with splenic lesions was significantly ($P < 0.05$) higher in cockerels and pullets than in broilers (Figure 3). Thymus from early mortalities was markedly and diffusely congested and haemorrhagic, while those from euthanized chicks were atrophic. The mean number of birds with thymic lesions was significantly ($P < 0.05$) higher in cockerels and pullets than in broilers (Figure 4). The kidneys were markedly swollen, congested and haemorrhagic, with whitish deposits in the distended tubules on days 1 to 3 of the infection. The mean number of birds with kidney lesions was significantly ($P < 0.05$) higher than those of the pullets and broilers on day 1 post outbreak, while those of the cockerels and pullets were significantly ($P < 0.05$) higher than that of the broilers on day 2 (Table III). The proventricular-gizzard junction had haemorrhages on the mucosal surface; the caecal tonsils were swollen and haemorrhagic, while the intestines showed evidence of catarrhal enteritis on days 1 to 3 of the infection. The cockerels had significantly ($P < 0.05$) higher haemorrhages at the proventricular-gizzard junction and caecal tonsils, and catarrhal enteritis than those of the pullets and broilers on day 1 of the infection, while those of the cockerels and pullets were significantly ($P < 0.05$) higher than those of the broilers on day 2 of the infection (Table III). On day 3 of the infection, the mean number of birds with lesions on the proventricular-gizzard junction, caecal tonsils and intestine was significantly ($P > 0.05$) higher in pullets than in broilers (Table III).

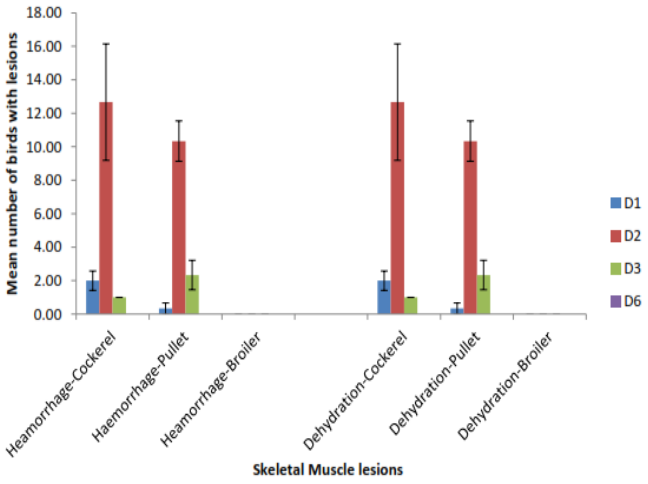

Figure 1: The daily mean number of birds with skeletal muscle lesions

In contrast, only 3 broilers died on day 2 of the infection and had swollen and mild haemorrhages in the bursae (Plate 2) and kidneys, while those euthanized on day 6 of the infection were normal in size.

Histopathological Changes

Histologic lesions in the bursa of cockerel and pullet chicks that died on days 1 and 2 after the onset of the infection consisted of generalized marked inflammatory oedema, heterophilic

infiltrations, accumulations of necrotic cellular and tissue debris in the spaces between the epithelia of the plicae and follicles and the interfollicular spaces. All the follicles showed marked lymphocytic depletion and some of the medulla contained cystic cavities containing eosinophilic fluid, necrotic lymphocytes, abundance of infiltrated heterophils and few macrophages. The bursal muscle layer was oedematous and many degenerating heterophils, marked haemorrhages and congestion of the blood vessels were seen in the inter- and intrafollicular spaces (Plate 3). The cortico-medullary junction was mostly lined by hyperplastic reticular cells. On day 3 of the infection, there was invagination of the hyperplastic bursal epithelium at the epithelial tufts which was forming some gland-like follicles. The bursae were devoid of necrotic debris and heterophils, while those euthanized on day 6 of the infection showed marked atrophy of the follicles, and inter and intrafollicular fibroplasias. In the spleen, congestion of the sinuses, hyperplasia of reticular cells of the sheathed capillaries (ellipsoid sheaths) and depletion of lymphoid cells were seen in chicks that died at days 1 to 3 of the infection (Plate 3). There were also heterophilic infiltrations with few macrophages and plasma cells. The chicks sacrificed on day 6 of the infection showed hyperplasia of reticular cells of the ellipsoid sheaths, and repopulation of the lymphoid cells throughout the spleen. Caecal tonsils had marked lymphocytic depletion of lymphoid follicles, desquamation of the epithelial lining of the villi and fossulae on day 3 of the infection, while those from birds euthanized on day 6 of the infection showed repopulation of the lymphocytes in the lymphoid follicles. The thymus of the chicks that died on days 2 and 3 of the infection showed necrosis of the lymphocytes in the lobules, marked congestion of the blood vessels and haemorrhages throughout the parenchyma, while those from chicks euthanized on day 6 of the infection were atrophic. Lesions in the thigh and pectoral muscles were marked haemorrhages, inflammatory oedema, and congestion of the blood vessels, infiltrations of heterophils and necroses of the muscle fibres (Plate 3). The kidneys showed marked congestion of the renal blood vessels and oedema causing expansion of the

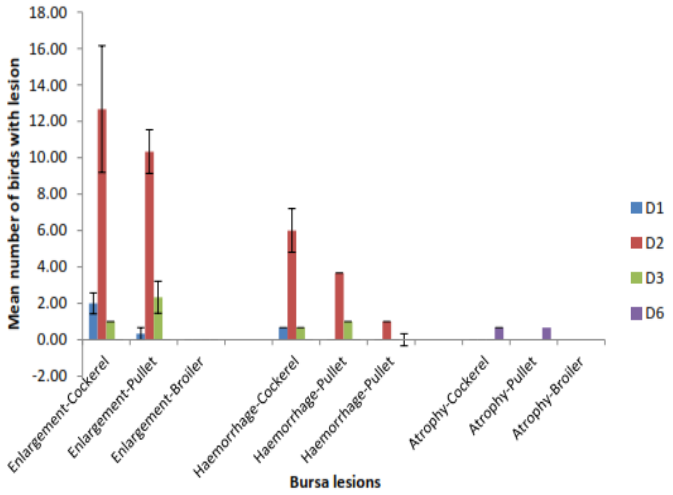

Figure 2: The daily mean number of birds with bursal lesions

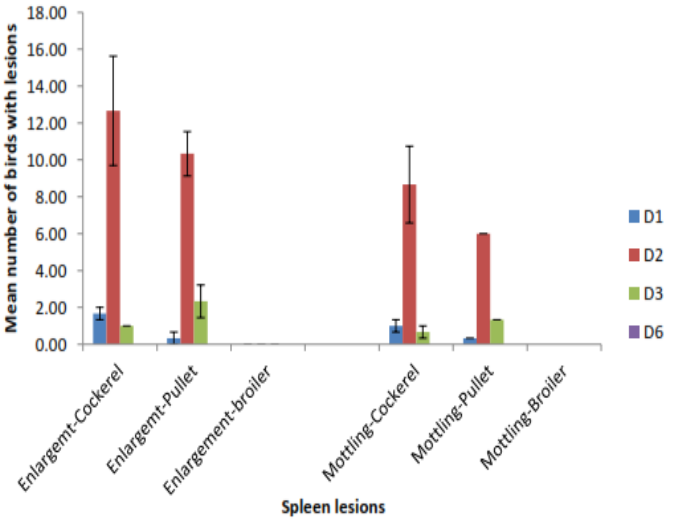

Figure 3: The daily mean number of birds with enlargement and mottling of the spleen

interstitial spaces (Plate 4). Most of the tubules and ducts contained eosinophilic casts and were detached from their basement membranes. The proventricular glands showed hyperaemia and necrotic glandular cells in the lamina. The histologic lesions in the broilers that died on day 2 of the infection showed similar marked histopathologic changes in the bursa as described for cockerels and pullets; however, marked haemorrhages and congestion of the blood vessels were not seen (Plate 5), while those from birds sacrificed on day 6 of the infection showed much folded plical epithelium, and different sizes of repopulating follicles (Plate 5). The thigh and pectoral muscles had mild oedema (Plate 5), while the kidney had only eosinophilic materials in the tubules and ducts (Plate 5). The spleen had only marked congestion of the sinuses, widespread abundance of heterophils, plasma cells and macrophages. Lymphocytic depletion of the follicles was not observed in the spleen (Plate 5), caecal tonsils and lobules of the thymus. No lesion was seen in the thymus and proventriculus-gizzard junction throughout the period of the study.

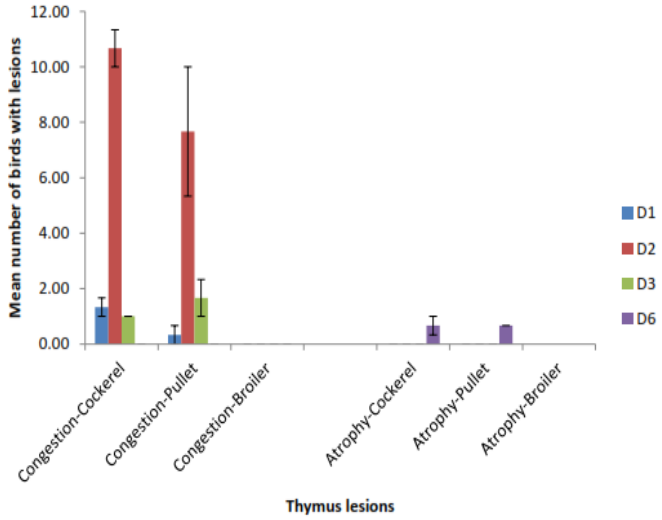

Figure 4: The daily mean number of birds with congestion and atrophy of the thymus

Table III: Distribution and frequency of gross lesions in the number of IBDV infected cockerels and pullets compared with those of the broilers in the gastrointestinal tract and kidney, Mean ± SEM

	Days after onset of the outbreak			
	D1	**D2**	**D3**	**D6**
Haemorrhage at the Proventricular-Gizzard juntion				
Cockerel	1.33 ± 0.33^{a}	10.00 ± 1.15^{a}	0.67 ± 0.33^{ab}	0.00
Pullet	0.33 ± 0.33^{b}	7.33 ± 3.38^{b}	1.67 ± 0.67^{a}	0.00
Broiler	0.33 ± 0.33^{b}	7.33 ± 3.38^{b}	0.00^{b}	0.00
Enteritis				
Cockerel	1.67 ± 0.33^{a}	12.67 ± 2.96^{a}	1.00 ± 0.00^{ab}	0.00
Pullet	0.33 ± 0.33^{b}	10.33 ± 1.20^{a}	2.33 ± 0.88^{a}	0.00
Broiler	0.00^{b}	1.00 ± 0.00^{b}	0.00^{b}	0.00
Caecal tonsil haemorrhage				
Cockerel	1.00 ± 0.00^{a}	8.33 ± 2.33^{a}	0.67 ± 0.33^{a}	0.00
Pullet	0.00^{b}	4.00 ±0.58^{ab}	1.00 ± 0.00^{a}	0.00
Broiler	0.00^{b}	0.00^{b}	0.00^{b}	0.00
Enlargement & Congestion of the Kidney				
Cockerel	1.67 ± 0.33^{a}	12.67 ± 2.96^{a}	1.00^{ab}	0.00
Pullet	0.33 ± 0.33^{b}	10.33 ± 1.20^{a}	2.33 ± 0.88^{a}	0.00
Broiler	0.00^{b}	0.67 ± 0.33^{b}	0.00^{b}	0.00

Different superscripts ^{abc} in a column indicate significant difference @ $P < 0.05$

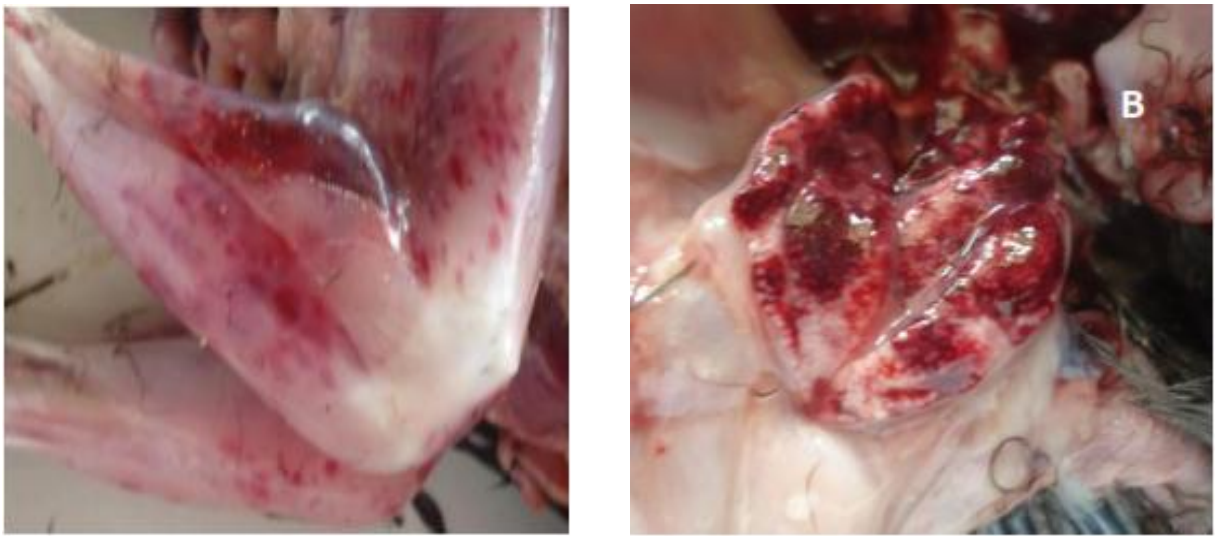

Plate 1: Gross lesions in 7-week-old cockerels (Ck) naturally infected with IBDV. Dead Ck-**A.** Paint-brush haemorrhages on the muscles of the thigh on day 2 of infection. Dead Ck -**B**, showing swollen and haemorrhagic bursa on day 2 of the infection

Infectious Bursal Disease Confirmation

All bursae of the infected birds were positive for IBDV antigen by AGID test showing precipitation lines within 36 - 48 hours post-incubation at 37^{o}C.

Bacteriological Examination

No bacterial growth was obtained from all the tissues cultured.

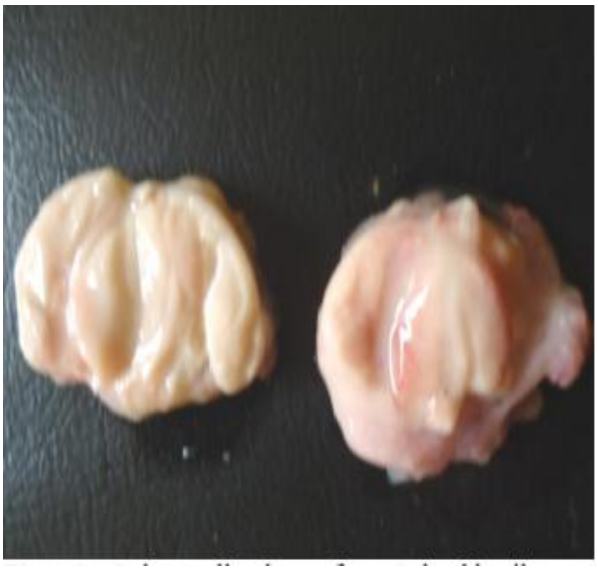

Plate 2: Only swollen bursa from 2 dead broilers on day 2 of the infection. Note loss of the plicae folds

DISCUSSION

The present study described comparatively the genetic influence on the susceptibility of exotic cockerels, pullets and broilers to natural IBDV chickens based on and as demonstrated by the clinical disease and pathological characteristics. This finding supports

previous observation that showed that exotic breeds of chickens are susceptible to IBDV (Okoye and Aba-Adulugba, 1998; Silva *et al*., 2016). A tentative diagnosis of clinical IBD was made based on the sudden onset, high morbidity and mortality curve which receded in a period of five to seven days; and was confirmed by identification of IBDV antigen using AGID test. Clinical signs in chicks infected with IBDV were most severe and lasted longer among the exotic Harco breeds than the White Marshall broiler breed while the pullet Harco breed had lesser clinical disease than the cockerel Harco breed in the present study. This observation suggests a breed-related variation in susceptibility to virulent IBD in these susceptible breeds of chickens and a probable genetic influence. Though the resistant attribute of broilers to disease caused by IBDV has been previously investigated by Okoye and Aba-Adulugba (1998) and Silva *et al*. (2016), it was however, not comparatively carried out

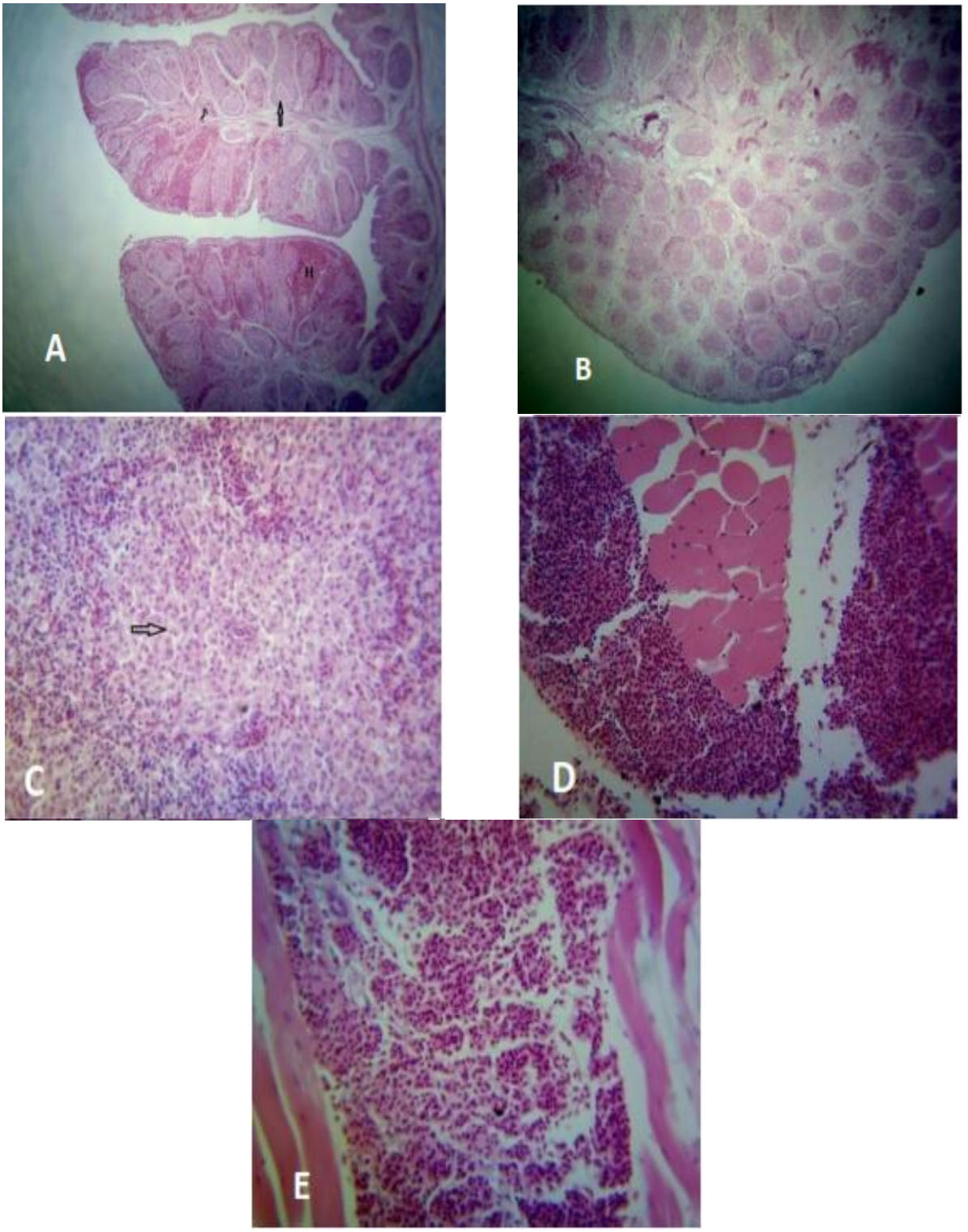

Plate 3: Histopathological findings in 7-week-old cockerels (Ck) and pullets (Pu) naturally infected with IBDV. Bursa, Dead Ck-A & Pu-B, showing marked haemorrhages, congestion of blood vessels **(B)**, oedema, lymphocytic depletion of the follicles and cystic cavities (arrow) on day 2 of the infection. H&E, X40. Spleen, Dead Ck- **C,** showing severe lymphocytic depletion (arrow) on day 2 of the infection. H &E, X400. Thigh muscles, Dead Ck- **D** & Pu- **E**, showing severe haemorrhages, oedema and necrosis of the muscle fibres on day 2 of the infection. H & E, X400

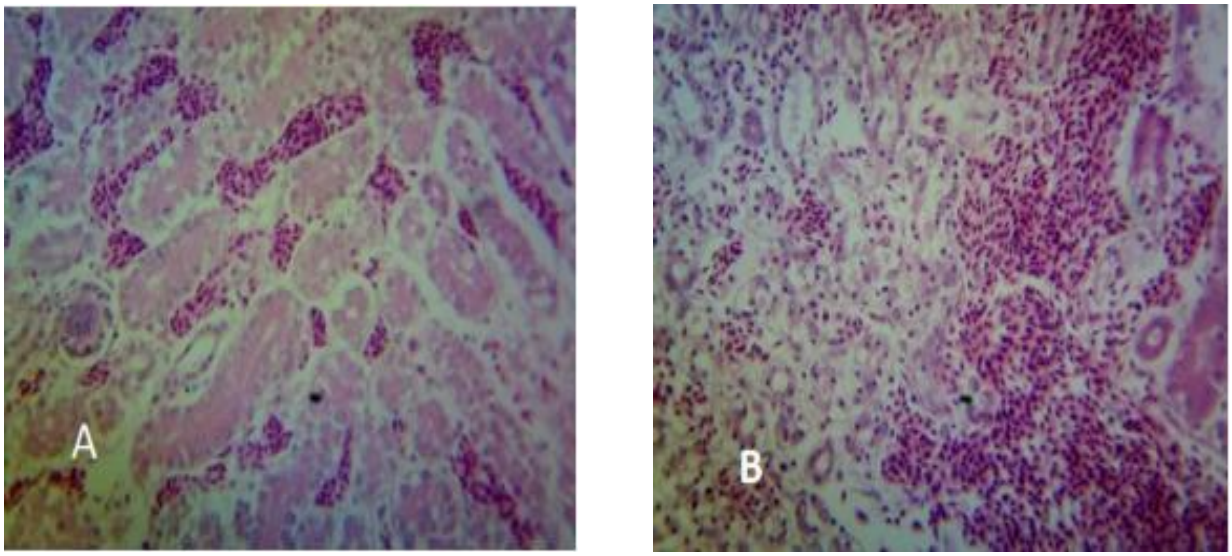

Plate 4: Kidney. Dead Ck-**A** & Pu-**B**, showing oedema, comgestion of renal blood vessels and eosinophilic materials in lumen of tubules and ducts on day 2 of the infection. H&E, X400

using cockerels and pullets. The higher resistance of commercial broilers compared to specific pathogen free pullets was also reported by Jackwood *et al*. (2009), however, the commercial birds used in the cited study originated from vaccinated flocks and had maternal antibodies against IBDV. Also, cockerels were not included in the cited study, so the results could not be compared to the current study using unvaccinated chicks. It can therefore be assumed that the differences observed reflect the possible differences in the genetic background of the chicks used in this study. The period of greatest susceptibility to severe clinical disease has been reported to be 3 to 6 weeks of age (Eterradossi and Saif, 2013; OIE, 2016). However, the marked clinical disease recorded in the present study in the three types of chickens at 7 weeks of age indicated that the virus involved in the current outbreak was a very virulent IBDV. The clinical signs are similar to those already described by other workers that reported natural infections (Chettle *et al*., 1989; Oluwayelu *et al*., 2002). However, it should be noted that these descriptions of natural infections in the literature were neither comparative nor chronological. Very virulent strains induce approximately 50 to 100% mortality and typical signs and lesions (Eterradossi and Saif, 2013). The present study showed that cockerels and pullets showed severe clinical signs and mortality rates than broilers. Although the disparity in morbidity and mortality rates recorded in the present study could be associated to breed. Light weight breeds of chickens have been reported to be more susceptible to virulent IBD than heavy weight breed of chickens in experimental infections (Okoye and Aba-Adulugba, 1998; Silva *et al*., 2016). It could also be that this virus lacks optimal adaptation to broilers because despite the 8% morbidity and 6% mortality recorded in the present study, there was no mortality in all the remaining broiler chicks from day 3 of the infection to the end of end of the study.

The cockerels and pullets had marked gross lesions similar to those already reported for very virulent IBDV in natural infections (Lukert and Saif, 2003) and comparative experimental infections in 3-week-old chickens (Silva *et al*., 2016). The broilers showed no paint-brush haemorrhages in the skeletal muscles, proventriculus-gizzard junction and caecal tonsils, and no multifocal necrotic foci in the spleen. The aforementioned lesions were highly prominent in cockerels and pullets. However, marked muscular lesions in

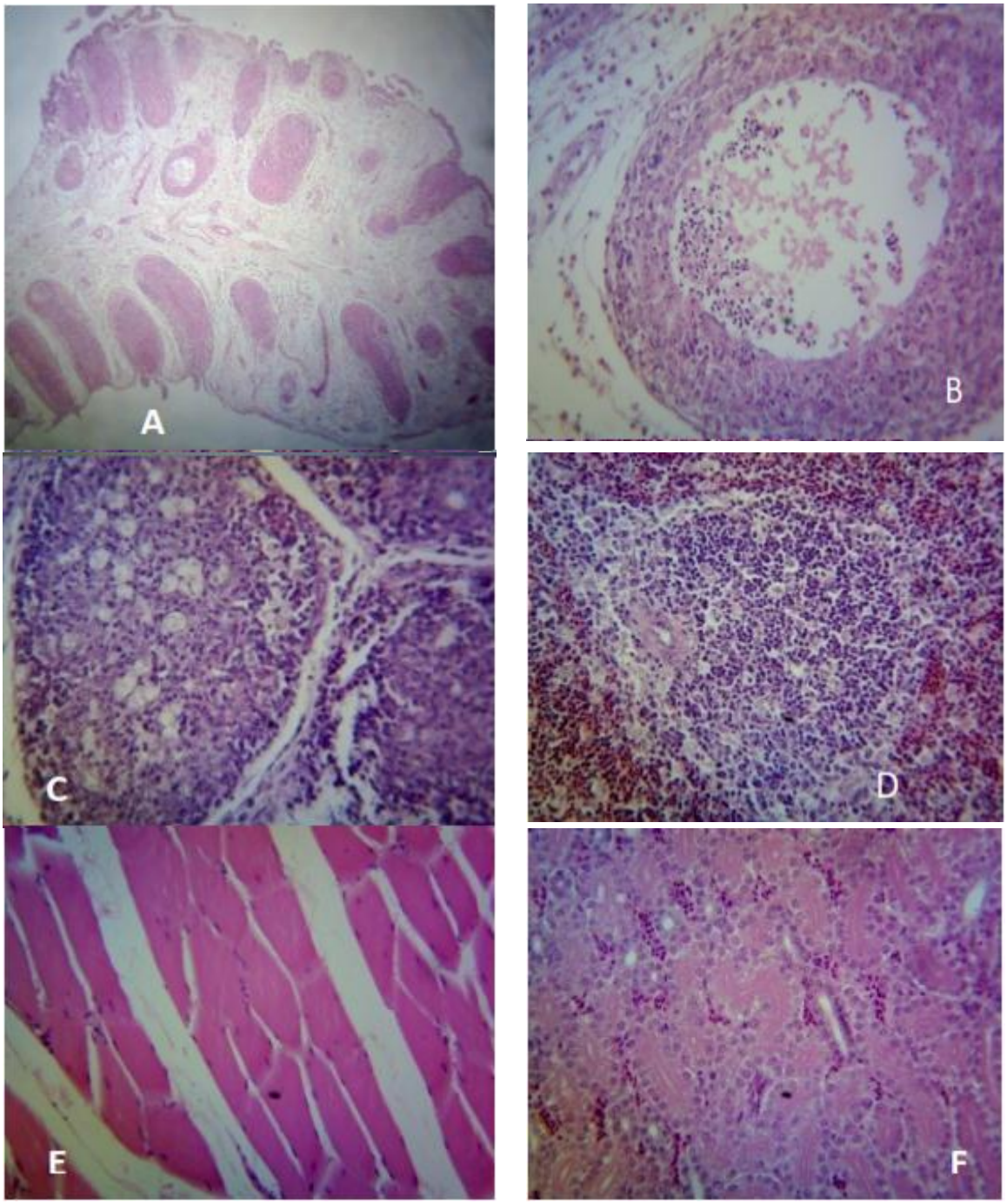

Plate 5: Histopathological findings in 7-week old broilers naturally infected with IBDV. Bursa. Dead Br. **A,** showing desquamated and convoluted bursal epithelium, severe oedema, fibroplasias, lymphcytic depletion of follicles and cystic cavities in medulla (H & E, X40**)**; **B** showing hyperplasia of corticomedullary layer and cystic cavity in the medulla filled with necrotic debris on day 2 of the infection. H&E, X400. Note absence of haemorrhages and congestion of blood vessels. **C,** showing repopulating follicles from a sacrificed broiler on day 6 of the infection. **D** Spleen. No apparent necrosis in the lymphoid follicles and diffused lymphoid tissues on day 2 of the infection. H&E, X400**. E** Thigh muscle**,** showing only oedema on day 2 of the infection. H&E, X400. **F** Kidney showing eosinophilic materials in the lumen of renal tubules and ducts and mild congestion of renal blood vessels on day 2 of the infection. H&E, X400

pullets and cockerels and enlargement of bursa seen in broilers were not reported by (Silva *et al*., 2016), but it concurs with a previous experimental study with a Nigerian IBDV strain in 6-week-old chickens (Okoye and Aba-Adulugba, 1998). The results of the present study showed that IBDV affected mainly the bursa, and mildly the general health, of broilers even though 6 % mortality was recorded.

Comparing the severity of the microscopic lesions, the results of the present study showed that the virulent IBDV caused marked lesions in the non-lymphoid and lymphoid organs of cockerels and pullets than those of the broilers but the bursal lesions in the three types of chickens were similar. This indicated that breed or genetic lineage is not a determinant factor in severity of bursa lesions of broilers to natural IBDV infection. This is contrary to the reports of Silva *et al*. (2016) that bursal lesions in broilers were less severe than those in pullets. Interestingly, all the three types of chickens affected during the natural infection were of the same age and in the same poultry pen and were above the 3 to 6 weeks associated with severe acute clinical disease and lesions (Okoye and Aba-Adulugba, 1998; OIE, 2016). These results suggest a potential virus virulence-age-related susceptibility factor in cockerels, pullets and broilers that call for further experimental study. It also indicated that bursal lesions could be more appropriate for the assessment of IBD viral infectivity in chickens. In the avian immune system, the bursa serves as one of the primary organs of lymphopoiesis (Taylor and McCorkle, 2009). In susceptible chickens, IBDV initially replicates primarily in proliferating B lymphocytes of the bursa of Fabricius (Kaufer and Weiss, 1980; Eterradossi and Saif, 2013). The microscopic lesions observed in the bursa of all the affected dead chickens were marked and diagnostic and coincided with the occurrence of severe clinical disease and gross lesions in the present study. Because this organ and cells are important constituents of the immune system, IBDV infection will alter these cells and influence the function of the immune system. The resultant effect would lead to decreased immunoprotective efficacies to vaccines and increased susceptibility to secondary infections in survivors (Okoye *et al*., 1991; Eterradossi and Saif 2013).

In addition, the results of the present study highlight the significance of vaccination in an endemic area and showed that unvaccinated chickens are highly susceptible to virulent field IBDV infection. This is contrary to the reports of Tong *et al*. (1993) that IBD was more likely to occur in vaccinated chickens than in non-vaccinated chickens, but in agreement with the reports of Tsukamoto *et al*. (1995) and Mundt *et al*. (2003) that unvaccinated chickens suffer severe form of highly virulent IBDV. The severe morbidity, mortality, gross and microscopic lesions of the present study, therefore, emphasized the importance of vaccination which will protect chickens against these effects from challenge with the highly virulent IBDV in an endemic area. Besides strict hygiene management of poultry farms, vaccination is the most important measure to control IBDV in the field (Mundt *et al*., 2003; Müller *et al*., 2012). This is because studies have shown that IBDV is resistant to many chemical agents and to adverse weather conditions and last for a long time in infected litter and premises (Eterradossi and Saif, 2013; Aliyu *et al*., 2016).

CONCLUSION

The White Marshal broiler chickens were more resistant to clinical disease and pathologic effects of clinical IBD than the Black Harco breed. Also within the Black Harco breed, the pullets were more resistant than cockerels. Based on these results, it is suggested that the observed differences between cockerels, pullets and broilers might be attributed to breed and genetic

differences and should be utilized in selective breeding. These differences on the severity of clinical disease and lesions of IBD are also worthy of note to field veterinarians, pathologists, researchers and respective authorities that design diagnostic, prevention and control measures of the disease.

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