Research Article

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Comparative Pathological Changes Induced by Very Virulent Infectious Bursal Disease Virus Infection in Inoculated, Sentinel Pigeons and Chickens

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Abstract: In this study, the authors determined whether vvIBDV could be transmitted from chickens to pigeons and vice versa, and the relative severity of the lesions in the two species. Thirty 3-to 6-week-old pigeons and thirty 3-week-old chickens were grouped as follows: A (10 uninoculated pigeons), B (10 inoculated pigeons+10 sentinel chickens), C (10 inoculated chickens+10 sentinel pigeons) and D (10 uninoculated chickens). Inoculated birds were administered 0.20 mL of vvIBDV (titre of $10^{9.76}$ CID/mL) followed by introduction of their respective sentinels post-inoculation. Post-inoculation/exposure (pi/pe), dead birds were necropsied, organs grossly examined, weighed, and sections processed for histopathology. Results revealed mild, gross and histopathological lesions in pigeons at 7 and 14 dpi/dpe. In chickens, gross and histopathological lesions were severe at 3 and 4 dpi/dpe, moderate at 7 dpi/dpe and mild at 14 dpi/dpe. Carcass weight showed no statistical difference ($P > 0.05$) in all pigeons, but was statistically higher in uninoculated compared to inoculated and sentinel chickens. Relative weight (RW) of the liver was significantly lower at 14 dpi/dpe in pigeons. In chickens, RW of the bursa of Fabricius

(BF) was significantly higher in inoculated and sentinel at 3 and 4 dpi/dpe. In conclusion, there was transmission of vvIBDV from pigeons to chickens and pathological changes due to vvIBDV infection were less severe in pigeons than in chickens.

Keywords: vvIBDV, pigeons, chickens

1 Introduction

Infectious bursal disease (IBD) or Gumboro disease (GD) is an acute, highly contagious viral disease of young chickens caused by the infectious bursal disease virus (IBDV). The disease is characterized by an increased immunosuppression and mortality in 3- to 6-week-old chickens [1-3]. Two serotypes (1 and 2) of IBDV have been identified, with serotype 1 considered to be virulent while serotype 2 is avirulent [4, 5]. Faeco-oral route constitutes the predominant mode of transmission of IBD. The IBDV is extremely lymphocidal and shows selective tropism for the bursa of Fabricius (BF) where it attacks immature B lymphocytes thereby inducing bursal lesions [2, 6]. However, other lymphoid organs such as the thymus, spleen, caecal tonsils, Peyer's patches, Harderian gland and bone marrow have been reported to be affected by IBDV [3, 7, 8].

Chickens and turkeys constitute the natural host of IBDV but other species have been reported to present serological evidence of the disease with no clinical disease [2, 9, 10]. In free-living wild birds, IBDV has been documented to cause sub-clinical infection characterized by lymphoid depletion in the BF [11]. In pigeons and guinea fowls, the genome of IBDV has been detected in the cloaca [12].

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There is an increase in pigeon production as pigeons are being bred as source of food for humans [13, 14]. Pigeons have also been found in close association with local chickens in live bird markets and also interact with commercial poultry on farms [14, 15]. They have been suggested to play a role in spreading some zoonoses as well as being reservoirs of many diseases of poultry [16, 17]. Thus, they could become infected with IBDV during the process of interaction and migration as showed in some serological studies [18]. However, there are no reports on the transmission of very virulent IBDV (vvIBDV) between pigeons and chickens, and on severity of pathological changes due to vvIBDV infection in pigeons. Hence in this study, the authors determined whether vvIBDV could be transmitted from chickens to pigeons and vice versa, and the relative severity of the lesions in the two species.

2 Materials and Methods

2.1 Experimental birds

Thirty 3-week-old ISA white cockerels and thirty 3- to 6-week-old domestic pigeons acquired from reputable breeders were screened and confirmed seronegative for IBDV antibody using an agar gel precipitation test (AGPT) and enzyme linked immunosorbent assay (ELISA).

Ethical approval: The research related to animals' use has been complied with all the relevant national regulations and was approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with the approval number ABUCAUC/2021/002.

2.2 Experimental design

Both pigeons and chickens were grouped as follows: A (10 uninoculated pigeons only), B (10 inoculated pigeons + 10 sentinel chickens), C (10 inoculated chickens + 10 sentinel pigeons), and D (10 uninoculated chickens only). Uninoculated pigeons and chickens were kept in separate pens. Inoculated pigeons and chickens were each administered 0.20 mL of inoculum containing vvIBDV (Nigerian strain) with a titre of $10^{9.76}$ CID/mL. Both sentinel chickens and pigeons were introduced into and kept together with inoculated pigeons and chickens, respectively, in separate pens post-inoculation. All birds were provided with feed and water *ad libitum*.

2.3 Experimental groupings

Group	Bird composition
A	10 uninoculated pigeons
B	10 inoculated pigeons + 10 sentinel (contact) chickens
C	10 inoculated chickens + 10 sentinel (contact) pigeons
D	10 uninoculated chickens

2.4 Confirmation of IBDV infection

The presence of vvIBDV was confirmed in the cloacal swabs of all inoculated and sentinel birds using RT-PCR.

2.5 Gross examination

Following inoculation and/or exposure, chickens that died from the disease at 3 and 4 days post-inoculation and/or exposure (dpi/dpe) were examined for the presence of gross lesions. Also, at 7 and 14 dpi/dpe, 3 live chickens and 5 live pigeons from each group were humanely euthanized and examined for gross lesions. The organs (including BF, thymus, spleen and liver) of each bird were examined, lesions scored and graded, based on modification of the criteria described by Ward and Thoolen [19]. The lesion scores were graded as follows: 0 = no lesion, 1 = mild, 2 = moderate, ≥ 3 = severe.

2.6 Carcass weight and relative weight of organs

The carcass weight of each bird and the organs (BF, thymus, spleen and liver) were determined. The relative weights of these organs were calculated for each bird using the formula by Lucio and Hitchner [20].

2.7 Histopathological examination

The BF, thymus, spleen and liver were processed for histopathological examination using standard histology technique [21]. The histopathological lesions were scored and graded using described principles with modifications [22, 23].

2.8 Data Analyses

Data was presented using tables, photographs and photomicrographs. The values obtained from gross histopathological lesion scores, weights and relative weights were expressed as mean $\pm$ SEM and subjected to a Two-way analysis of variance (Two-way ANOVA). Graphpad prism version 5.0 (San Diego California, USA) was used for these analyses. Values of $p \leq 0.05$ were considered significant.

3 Results

3.1 Clinical manifestations

At 2 dpi/dpe, clinical signs were observed in pigeons and chickens (incubation period of 2 days). There was anorexia and reduced activity in inoculated pigeons. In chickens, the clinical signs presented were reduced appetite and activity, ruffled feathers, huddling, somnolence, watery diarrhoea, prostration and death.

3.2 Gross pathology findings

There was no gross lesion (score of 0) in uninoculated pigeons and chickens (Table 1). Gross lesions in inoculated and sentinel pigeons at 7 and 14 dpi/dpe were mild (score of 1) and included congested liver and enlarged spleen.

In inoculated and sentinel chickens gross lesions were severe (score of 3) at 3 and 4 dpi/dpe, moderate (score of 2) at 7 dpi/dpe and mild (score of 1) at 14 dpi/dpe (Table 1). At 3 and 4 dpi/dpe, the gross lesions observed were dehydration and emaciation, haemorrhages in the pectoral, thigh and leg muscles, haemorrhagic thymic lobes (Figure 1), congested liver, enlarged spleen, enlarged bursae with haemorrhages on the serosa layer and plicae (Figure 2). At 7 dpi/dpe congested liver, thigh and leg muscles and slight haemorrhage on the bursal plicae were observed. There was congested liver at 14 dpi/dpe.

3.3 Changes in carcass weight and relative weight of organs

Carcass weights showed no statistical difference ($p > 0.05$) in all groups of pigeons at 7 dpi/dpe and 14 dpi/dpe (Table 2). The relative weights (RW) of BF, thymus and spleen showed no statistical difference ($p > 0.05$) at 7 and

14 dpi/dpe in all pigeons. The relative weights of the liver was significantly higher ($p < 0.05$) at 7 and 14 dpi/dpe in inoculated compared to uninoculated and sentinel pigeons (Table 2).

In inoculated and sentinel chickens carcass weight was significantly higher ($p < 0.05$) in uninoculated chickens at 7 dpi/dpe (Table 3). At 14 dpi/dpe, there was no statistical difference ($p > 0.05$) in carcass weights of inoculated and sentinel chickens but these were lower significantly ($p < 0.05$) compared to uninoculated chickens (Table 3). The relative weight of BF was significantly higher ($p < 0.05$) at 3 and 4 dpi/dpe in inoculated and sentinel. At 7 and 14 dpi/dpe, the RWBF showed no significant difference ($p > 0.05$) in uninoculated, inoculated and sentinel chickens (Table 3). There was no significant difference ($p > 0.05$) in the RW of liver at 7 and 14 dpi/dpe in uninoculated, inoculated and sentinel chickens (Table 3). There was no statistical difference ($p > 0.05$) in the relative weights of the thymus and spleen in all groups of chickens at 3, 4, 7 and 14 dpi/dpe (Table 3).

3.4 Histopathological changes

Histopathological changes were absent (score of 0) in uninoculated pigeons and chickens (Table 4). In inoculated and sentinel pigeons, histopathological changes were mild (score of 1) at 7 and 14 dpi/dpe (Table 4). The changes observed were vacuolations in the follicular cortices of the BF, slight depletion of the thymic medulla and splenic white pulp and congestion in the liver (Figures 3-5).

In inoculated and sentinel chickens, histopathological changes were severe (score of 3) at 3 and 4 dpi/dpe. At 7 and 14 dpi/dpe, histopathological changes were moderate (score of 2) and mild (score of 1), respectively (Table 4). At 3 and 4 dpi/dpe, the histopathologic changes in the BF were follicular haemorrhages and oedema, thickened interfollicular spaces, depletions of follicular cortices and medullae, heterophilic infiltration of follicular cortices, vacuolations and cystic formations. There was haemorrhage and depletion of medulla in the thymus, haemorrhages and depletion of white pulps of the spleen, congestion in the liver, and haemorrhages in the kidney and thigh muscle. At 7 dpi/dpe, there were thickened interfollicular spaces, depletion of follicular cortex and medulla in the BF, haemorrhage and depletion of white pulps in the spleen and congestion in the liver. There was repopulation of the follicles in the BF at 14 dpi/dpe (Figures 6-7).

Table 1: Gross lesion score in pigeons and chickens following inoculation with and/or exposure to a very virulent infectious bursal disease virus

	Uninoculated pigeons	Inoculated pigeons	Sentinel pigeons	Uninoculated chickens	Inoculated chickens	Sentinel chickens
Days post-inoculation and/or exposure	Gross lesion score (approximate value)					
3	-	-	-	0	2.69 ± 0.47 (3)	2.59 ± 0.12 (3)
4	-	-	-	0	2.63 ± 0.71 (3)	2.54 ± 0.70 (3)
7	0	1.00 ± 0.32 (1)	1.00 ± 0.32 (1)	0	1.54 ± 0.05 (2)	1.52 ± 0.14 (2)
14	0	0.80 ± 0.32 (1)	0.80 ± 0.32 (1)	0	1.00 ± 0.00 (1)	1.00 ± 0.00 (1)

0 = No lesion, 1 = Mild, 2 = Moderate, ≥3 = Severe; lesions were graded from scoring of the bursa of Fabricius, thymus and spleen.

Table 2: Carcass weights and relative weights of some organs of pigeons following inoculation and/or exposure to a very virulent infectious bursal disease virus

Days post-inoculation and/or exposure	Group	Carcass weight (g)	Bursa of Fabricius	Thymus	Spleen	Liver
			Relative weights			
7	Uninoculated pigeons	227 ± 10 ^a	0.19 ± 0.01	0.30 ± 0.03	0.26 ± 0.02	3.33 ± 0.24 ^a
	Inoculated pigeons	201 ± 9.5 ^a	0.22 ± 0.02	0.28 ± 0.04	0.31 ± 0.02	4.17 ± 0.24 ^b
	Sentinel pigeons	214 ± 10 ^a	0.20 ± 0.02	0.26 ± 0.01	0.27 ± 0.03	3.87 ± 0.51 ^b
14	Uninoculated pigeons	276 ± 7.5 ^b	0.17 ± 0.01	0.29 ± 0.02	0.21 ± 0.02	2.31 ± 0.19 ^c
	Inoculated pigeons	246 ± 13 ^b	0.20 ± 0.02	0.22 ± 0.02	0.24 ± 0.04	2.83 ± 0.06 ^d
	Sentinel pigeons	261 ± 8.2 ^b	0.18 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	2.49 ± 0.08 ^{cd}

Values with different superscript alphabets along the same column differ significantly at $P < 0.05$.

Table 3: Carcass weights and relative weights of some organs of chickens following inoculation and/or exposure to a very virulent infectious bursal disease virus

Days post-inoculation and/or exposure	Group	Carcass weight (g)	Bursa of Fabricius	Thymus	Spleen	Liver
			Relative weights			
3	Uninoculated chickens	-	-	-	-	-
	Inoculated chickens	193 ± 12	0.74 ± 0.12	0.55 ± 0.06	0.37 ± 0.04	4.52 ± 0.28
	Sentinel chickens	205 ± 11	0.64 ± 0.01	0.51 ± 0.00	0.25 ± 0.04	3.97 ± 0.35
4	Uninoculated chickens	-	-	-	-	-
	Inoculated chickens	197 ± 7.5	0.53 ± 0.05	0.56 ± 0.06	0.27 ± 0.00	3.95 ± 0.35
	Sentinel chickens	209 ± 13	0.48 ± 0.04	0.42 ± 0.02	0.26 ± 0.01	3.94 ± 0.12
7	Uninoculated chickens	251 ± 15 ^a	0.20 ± 0.04	0.38 ± 0.04	0.24 ± 0.01	3.42 ± 0.10
	Inoculated chickens	213 ± 7.3 ^b	0.24 ± 0.04	0.51 ± 0.05	0.24 ± 0.02	3.63 ± 0.50
	Sentinel chickens	220 ± 8.8 ^b	0.23 ± 0.03	0.38 ± 0.05	0.22 ± 0.02	3.66 ± 0.04
14	Uninoculated chickens	289 ± 8.2 ^a	0.19 ± 0.02 ^a	0.33 ± 0.04	0.26 ± 0.01	2.98 ± 0.20
	Inoculated chickens	237 ± 8.3 ^b	0.09 ± 0.01 ^b	0.33 ± 0.02	0.26 ± 0.02	2.98 ± 0.13
	Sentinel chickens	251 ± 13 ^b	0.11 ± 0.02 ^b	0.35 ± 0.03	0.21 ± 0.05	2.77 ± 0.11

Values with different superscript alphabets along the same column in the same day differ significantly at $P < 0.05$.

Table 4: Histopathological score in pigeons and chickens following inoculation with and/or exposure to a very virulent infectious bursal disease virus

	Uninoculated pigeons	Inoculated pigeons	Sentinel pigeons	Uninoculated chickens	Inoculated chickens	Sentinel chickens
Days post-inoculation and/or exposure	Histopathological score (approximate value)					
3	-	-	-	0	2.9 ± 0.06 (3)	2.97 ± 0.22 (3)
4	-	-	-	0	2.97 ± 0.11 (3)	3.00 ± 0.06 (3)
7	0	1.00 ± 0.00 (1)	1.00 ± 0.00 (1)	0	2.10 ± 0.10 (2)	1.93 ± 0.29 (2)
14	0	1.00 ± 0.20 (1)	1.00 ± 0.20 (1)	0	1.00 ± 0.00 (1)	1.00 ± 0.00 (1)

0 = No lesion, 1 = Mild, 2 = Moderate, ≥3 = Severe; lesions were graded from scoring of the bursa of Fabricius, thymus and spleen.

Figure 1: Photograph of thymus of chickens post-inoculation and/or exposure to a very virulent infectious bursal disease virus. Note intact thymic lobes in group A (a), congested, haemorrhagic thymic lobes (arrows) in group B (b) and C (c) chickens.

Figure 2: Photograph of bursal plicae of chickens post-inoculation and/or exposure to a very virulent infectious bursal disease virus. Note intact plicae in group A (a), congested plicae (arrows) in group B (b), haemorrhagic plicae (arrows) in group C (c) chickens.

Figure 3: Photomicrograph of section of bursae of pigeons post-inoculation and/or exposure to a very virulent infectious bursal disease virus. Note intact follicles (F) in group A (a); vacuolations (arrows) in groups B (b) and C (c) pigeons. H & E x 200

Figure 4: Photomicrograph of section of thymus of pigeons post-inoculation and/or exposure to a very virulent infectious bursal disease virus. Note intact cortex (c) and medulla (m) in uninoculated pigeon (a), slight depletion of medulla (arrows) in inoculated (b) and sentinel (c) pigeons. H & E x 200

Figure 5: Photomicrograph of section of spleen of pigeons post-inoculation and/or exposure to a very virulent infectious bursal disease virus. Note intact white (w) and red (r) pulps in uninoculated pigeon (a), slight depletion of white pulp (arrows) in inoculated (b) and sentinel (c) pigeons. H & E x 200

Figure 6: Photomicrograph of section of bursae of chickens post-inoculation and/or exposure to a very virulent infectious bursal disease virus. Note intact follicles (f) in uninoculated chicken (a); vacuolations (v), depletion of follicular cortices and medullae (arrows), haemorrhage (arrow heads) and thickened interfollicular spaces (s) in inoculated (b) and sentinel (c) chickens. H & E x 200

Figure 7: Photomicrograph of section of spleen of chickens following inoculation and/or exposure to a very virulent infectious bursal disease virus. Note intact white (W) and red (R) pulps in uninoculated chicken (a), depletion of white pulp (arrows) and haemorrhage (arrow heads) in inoculated (b) and sentinel (c) chickens. H & E x 200

4 Discussion

In study, there were mild, gross and histopathological changes due to vvIBDV infection in pigeons and also successful transmission of vvIBDV from pigeons to chickens, and vice versa. Until now, there were no previous reports on the gross and histopathological lesions in pigeons following inoculation/exposure to a vvIBDV. However, the lesions observed in both inoculated and sentinel chickens are consistent with the reports of several authors [7, 24-27]. In chickens, the lesions were severe at 3 and 4 dpi/dpe, thus, resulting in the deaths at these days.

The dehydration and emaciation due to IBD in chicken in this study could be associated with loss of fluid from

watery diarrhoea and decreased feed and water intake due to anorexia. Watery diarrhoea in IBDV infection might have resulted from inflammatory processes triggered by the virus in the gut-associated lymphoid tissues leading to intestinal secretion imbalance and/or microflora disruption [28]. Haemorrhages in the pectoral, thigh and leg muscles and BF in IBD might be associated with virus-induced deficiency in the coagulation cascade resulting from disseminated intravascular coagulopathy, endothelial cells damage, prolonged whole blood recalcification time (WBRT), prothrombin time (PT), activated partial thromboplastin time (APTT) or a combination of these changes [26, 29]. The enlargement and congestion of liver and spleen in this study might be due to direct viral injury as IBDV has been demonstrated

in these organs of chickens [2, 30]. Since IBDV primarily targets the IgM+ B-cells of the BF, the gross lesions observed in the BF in this study were associated with direct viral injury of the organ leading to inflammatory reactions accompanied by release of cytokines (IFN- γ and IL-6) and nitric oxide (NO) [31, 32]. The study of Cardoso et al. [30] has demonstrated the presence of vvIBDV in the thymus of chickens, hence, the gross lesions observed in this study might be due to direct viral injury to the thymus and/or indirectly via virus-induced inflammatory responses.

The significantly decreased carcass weights in inoculated and sentinel birds might be due to the decreased feed intake with subsequent emaciation resulting from anorexia induced by the vvIBDV infection [33, 34]. The relative weights (RW) of BF, thymus and spleen showed no difference in uninoculated, inoculated and sentinel pigeons. This might be indicative of minimal bursal response to IBDV infection in pigeons. In chickens, RWBF was significantly higher at 3 and 4 dpi/dpe in inoculated and sentinel chickens, then decreased at 7 and 14 dpi/dpe while RW of thymus and spleen showed no differences. Relative bursal weight (RBW) of 0.30 was reported as the proposed reference standard for birds with no specification to age and breed [35]. Studies by Cazaban et al. [36] have documented standard RBW of 0.11-0.13 for male Cobb 500 commercial broilers. The initial increase in RWBF was associated with enlargement, congestion and haemorrhage due to the IBDV infection. In chronic or sub-acute IBD, RBW was reported to significantly decrease in size [37]. The subsequent decrease in RWBF in chickens in this study might be due to bursal atrophy following recovery from extensive damage by the IBDV infection.

The decrease in RW of liver with no significant changes in RW of spleen with age in this study was consistent with the findings of other researchers [38-40]. The higher liver RW in the groups of pigeons might be due to the congestion and/or corticosterone-induced hepatic lipogenesis and concomitant lipid accumulation in liver tissues resulting from stress of small relative space [41-43]. The disparity in responses of organs RW between pigeons and chickens in this study might be due to a possible variability factors such as species, breed, age and genetics.

On histopathology, the vacuolations in the BF of pigeons was not consistent with the gross observation in which no lesions were seen. In chickens, the observed histopathological changes due to vvIBDV infection were similar to those documented in previous reports by several researchers [23, 24, 27, 44, 45]. The histopathological changes in the BF, thymus, spleen, liver and muscle of

chickens in this study were in the consonance with the various gross observations in the respective organs. The presence of bursal lesions was suggestive of IBDV migration to the BF and subsequent replication as the BF is the primary target for the virus [46]. In the BF, vvIBDV attacks IgM+ B-cells and other lymphoid cells resulting in lymphocytic necrosis and subsequent follicular depletion, vacuolations and cystic formation [46]. This also could be reason for the depletion of thymic medulla and splenic white pulps. The destructive effects of the virus in the BF produced tissue debris thus leading to the observed heterophilic infiltrations as heterophils have been reported to play critical role in phagocytosis of tissue debris [47]. The haemorrhages in the BF, thymus, spleen and muscle, and congestion in the liver might be associated with direct viral injury to these organs [2, 30]. The presentation of pathological and organ relative weight changes in inoculated pigeons suggested that they were susceptible to vvIBDV infection. Also, pathological and organ relative weight changes in sentinel birds suggested successful shedding of infectious IBDV into the environment by the inoculated birds and subsequent ingestion by the sentinel birds.

5 Conclusions

The pathological changes induced by vvIBDV were mild in pigeons but severe, moderate and mild in chickens. There was transmission of vvIBDV from pigeons to chickens and pathological changes due to vvIBDV infection were less severe in pigeons than in chickens.

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