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Histopathological Changes in Muscovy Ducks and Local ChickensInfected with Velogenic Viscerotropic Strain of Newcastle Disease Virus (XIVb)

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ABSTRACT

Newcastle disease is a severe disease of poultry with devastating consequences that is on the reportable diseases list 'A' of the Office of the International Epizootics (OIE). The study was carried out to determine the histopathological changes in local chickens and Muscovy ducks experimentally infected with a velogenic viscerotropic Newcastle disease virus (NDV) XlVb strain. Forty birds consisting of 20 local chicks and 20 ducklings were randomly divided into 4 groups of 10 birds each. The Groups were designated as group 1 (Infected chicks, IC), group 2 (control chicks, CC), group 3 (Infected ducklings, ID), group 4 (control ducklings, CD). Groups 1 and 3 (infected groups) were inoculated orally with $10^{7.8}$/0.1ml/bird as the embryo lethal dose (ELD_{50}) of the NDV (XIVb) strain. Histopathological changes in the IC thymus were interlobular congestion and haemorrhages with thymus lymphocytic depletion. We observed no lesions in the thymus of the ID. The IC liver was congested, while mild congestion and hepatocellular necrosis of the central veins occurred in the liver of ID. White pulps depletion with congested areas were observed in the spleen of the IC, while no lesions occurred in the spleen of ID. The ducklings and chicks infected with XIVb NDV strain, also showed mild necrosis with intestinal tract, visceral organs, and lymphoid of ID compared to severe necrosis observed in IC. The findings indicated that the Muscovy ducks are more resistant to XIVb strain of NDV than the chickens.

Keywords: Histopathology, Local chicks, Muscovy ducks, Newcastle disease,XIVb NDV strain

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INTRODUCTION

Newcastle disease (ND) is widely recognized as an endemic disease in Africa [1], and has therefore, been widely reported in many African countries and still remains the most important poultry disease in both commercial and rural poultry in Africa [2]. It has been ranked the fourth most important poultry disease in terms of the number of unit loss for poultry species after the highly pathogenic avian influenza, infectious bronchitis and low pathogenic avian influenza [2-4]. Histopathologically, ND is known to cause severe lymphoid depletion along with lymphocellular necrosis and apoptosis in the lymphoid organs such as the spleen, bursa of Fabricius and thymus, and is found in both chickens and SPF turkeys [5]. Atrophy of bursa of Fabricius, spleen, and thymus and necrosis and depletion of lymphocyte in those three organs along with severe haemorrhages in the proventiculus are most common pathologic lesions seen in susceptible host infected with velogenic viscerotropic NDV [6].

However, velogenic viscerotropic NDV is known to cause lymphocytic necrosis in spleen with fibrinous exudation macrophage proliferations, sinusoidal fibrin exudation in the liver, and macrophage proliferations within the heart, lung, caecal tonsils and thymus with necrosis of the bone marrow [6]. Tracheitis, conjunctivitis and necrosis of feather epithelial cells in specific pathogen free (SPF) chickens have also been reported [6-7]. Oedema and ulcerations have also been reported in intestinal mucosa and villi with increased ulcerative haemorrhages, congestion and hyperplasia of the goblet and crypt cells [7]. In the kidneys, peri-tubular congestion of blood vessels with cast and pyknosis of the glomeruli and renal tubules have also been reported [9-10-11-5]. Gliosis and perivascular cuffing within the cerebellum and brain stem have also been reported [9-10-11-5]. Epithelial cells and the secretory cells necrosis of the reproductive tract with mild inflammatory changes in the oviduct have been observed [12]. The aim of the study was to determine the histopathological changes in local chickens and Muscovy ducks experimentally infected with XlVb strain of Newcastle disease virus.

MATERIALS AND METHODS

Experimental Procedures

The Newcastle disease viral inoculum (XIVb NDV strain) was obtained from the National Veterinary Research Institute (NVRI), Vom, and used as the challenge virus. Forty (each of local chicken and Muscovy duck) eggs were obtained from Dan saa, Sogiji, Bomo, Sabonbirni, Basawa and Samaru villages, which are all within Zaria metropolis, Zaria, Kaduna State. Eggs were hatched from a locally fabricated commercial incubator. The hatching was tactfully synchronized (chicken eggs were introduced on day 18th after commencement of duck eggs hatching) so that the age of the chicks and the ducklings would be about the same time. Brooding of the chicks and the ducklings was done separately under the deep litre system of management in the Veterinary Teaching Hospital (VTH) poultry pens at the Ahmadu Bello University, Zaria. Twenty five voltage bulbs were lowered to a considerable level as a source of energy. The chicks and the ducklings were fed on chick mash starter, obtained from a commercial chick ration (Vital feed®) from day old (for one month) and then changed to grower's mash until the 10 weeks duration of the experiment. Water and feed were provided *ad libitum*. The chicks and the ducklings did not receive any vaccination or medication against NDV.

Experimental Birds

The birds were raised up to 5 weeks of age, then each of the chicks and the ducklings were randomly divided into 4 groups of 10 birds each. These four groups were designated as group 1 (Infected chicks, IC), group 2 (control chicks, CC), group 3 (Infected ducklings, ID), group 4 (control ducklings, CD).

Testing the Viability of the Viral Strain (XIVb)

The viability of the viral strain (XIVb of NDV) was tested in the Virology Laboratory of the NVRI, Vom as follows: 1ml of the test sample (along with some penicillin and gentamycin) was inoculated into 9-day old embryonated minimum pathogen free (MPF) chicken eggs obtained from the NVRI, Vom using a sterile 2ml-syringe and needle. The inoculation was done through the allantoic route, incubated at 37^{o}C and candled with a 25 voltage bulb twice daily for 4 days. Eggs which showed embryonic death at 24 hours appeared to contain viable virus, indicating the virus was alive and could be used for the research. The allantoic fluid from such eggs was harvested and a confirmatory test, using HI, was carried out. The inoculated titre as the embryo lethal dose ($ELD_{50/ml}$) was determined using HI with 1% washed chicken red blood cell to arrive at a titre of $10^{7.8}$/0.1 ml/bird, which serve as the inoculated embryo lethal dose as described by [13]. This was then kept in vials of 1mls at -20^{o}c and reconstituted at usage.

Reconstitution of the Strain XIVb

1ml vial containing the isolates was diluted with 9mls of phosphate buffered solution to make up to 10mls. Each 1ml now contains 10 doses of $10^{7.8}$ titre of the viral agent which was determined to be the embryo lethal dose ($10^{7.8}$/0.1 ml/ bird,ELD_{50}) as described by [13].

Duration of the Experiment

The experiment was for a 10-week period with the first 5 weeks being pre-inoculation, during which any maternal antibodies against ND would have fully waned, since maternal antibodies against ND is believed to be fully waned at about 20 days post-hatch, especially in broiler chickens [14-15].The remaining 5 weeks were for the experimental studies.

Innoculation of the Virus

At the commencement of the experiment (day 0), the chicks and ducklings in the control groups were inoculated each with 0.1 ml of normal saline orally. This was immediately, followed by inoculating the chicks and ducklings in the infected groups with the XlVb strain of NDV orally each with 0.1 ml (as the inoculum), using a special inoculation instrument (gavage and syringe) after the reconstitution as described by [20]

Histopathological findings

Tissues for histopathological studies were collected and fixed in 10% neutral buffered formalin until use. The sampled tissues from organs (bursa of Fabricius, proventriculus, spleen, thymus, small intestines, and large intestines) were processed and stained with haematoxylin and eosin as described by [16]. The result for different tissues were graded according to [17] and displayed in tabular form. The photomicrographs of the lesions were snapped using Amscope digital camera for microscope version 3.0 (China) as described by [18]. The subjective method of [17] was used for the scoring of observed histopathological changes, using the criteria: (0) for normal or absence of any changes, (1) for mild lesions, (2) for moderate lesions, and (3) for severe lesions.

Data Analysis

The obtained data (mean ± standard error of mean) were subjected to analysis with

GraphPad software programme (GraphPad Prism, version 6) for statistical significance ($p<0.05$) following a One-way ANOVA analysis.

RESULTS

Histopathological Changes in Chicks and Ducklings infected with XIVb NDV Strain

There were interlobular congestion, haemorrhages and depletion of the thymuses of the infected chicks (Plate 1). No lesions were observed in the control. There were no lesions observed in the thymuses of the infected and control ducklings. The liver of infected chicks was congested (Plate 2), while the control chicks' liver had normal hepatic architecture with absence of enlargement and hepatic congestion. On the other hand, there were mild hepatocellular necrosis and congestion of the central veins of the liver in the infected ducklings (Plate 3), while the liver of the control ducklings appears normal. Depletion of the white pulps with areas of congestion were observed in the spleen of the infected chicks (Plate 4), while no lesions were observed in the control chicks, infected and control ducklings. Desquamation of glandular epithelia with the deposition of necrotic debris of the proventricular glands were observed in the infected chicks (Plate 5), while the proventricular glands of the control chicks, infected and control ducklings were normal.

The results of lesions grading in different tissues of chicks and ducklings infected with infected with XIVb NDV strain and their control groups are presented in Table 1. The subjective method of [17] was used for the scoring of observed histopathological changes. The grading of lesions in various tissues indicated that infected chicks had significantly higher ($p< 0.05$) mean values compared to the infected ducklings during the period of study (Figure 1).

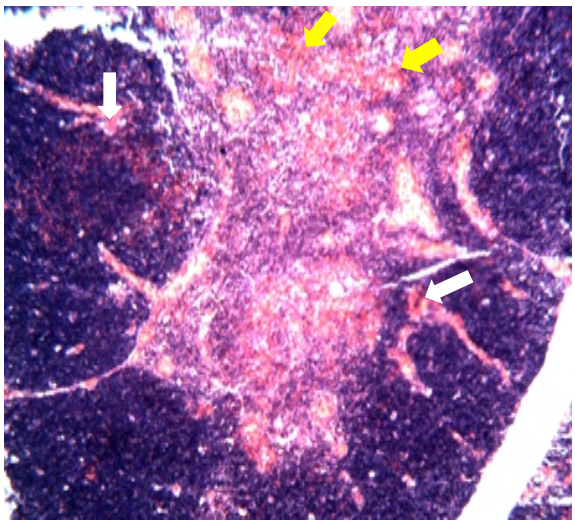

Plate 1: *Photomicrograph of a sectionof the thymus of an infected chick that died on day 7PI showing severe interlobular congestion (white arrows) and haemorrhages in the medulla (yellow arrows). H&E(x100).*

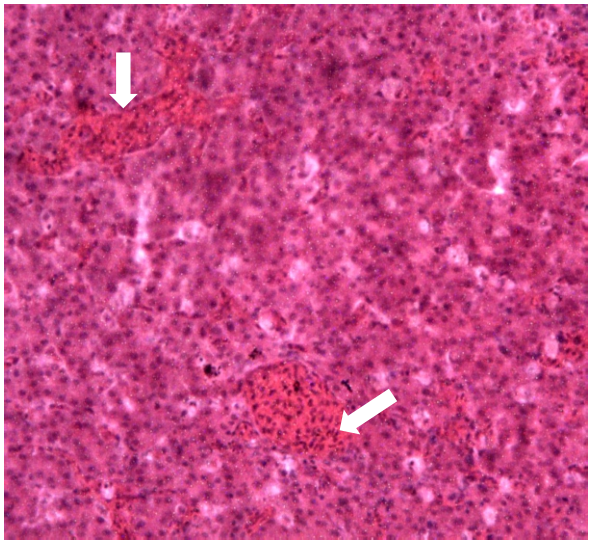

Plate 2: *Photomicrograph of a section of the liver of an infected chick that died on day7PI showing congested central veins (white arrows). H&E (x250)*

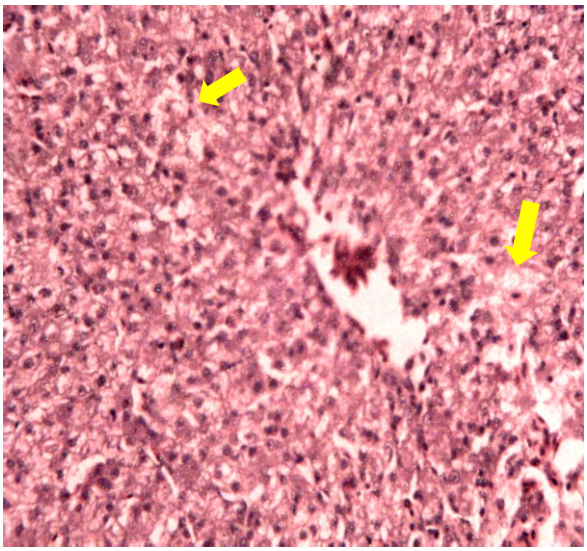

Plate 3: *Photomicrograph of a section of the liver of an infected duckling sacrificed on day 7PI showing mild hepatocellular necrosis (arrows) and congested central veins.H&E (x250)*

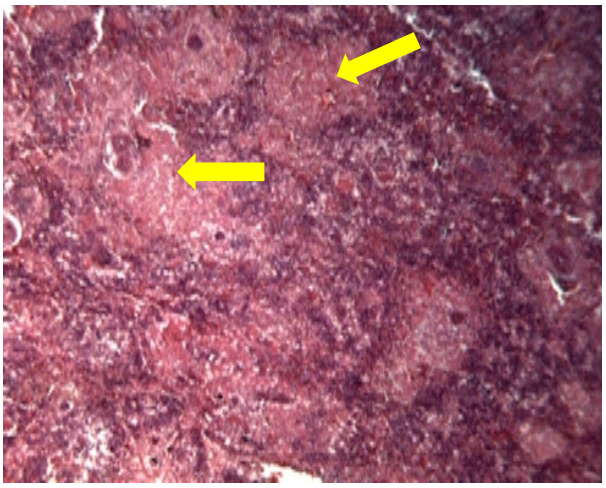

Plate 4: Photomicrograph of a section of the spleen of an infected chick that died on day 7PI, showing lymphoid depletion in white pulps (yellow arrows). H&E(x100).

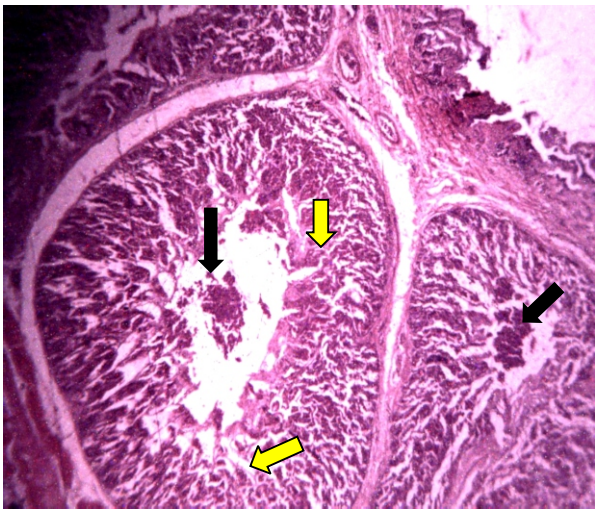

Plate 5: *Photomicrograph of a section of the proventriculus of an infected chick that died on day 7PI, showing desquamation of primary proventricular gland (yellow arrows) and deposition of necrotic debris in secondary proventricular gland (black arrows).H&E(x40).*

Table 1: Result of histopathological lesions grading of different groups of birds after challenge with XIVb strain at day 7 PI using the subjective method of [17]

Organ/ tissue of bird	Infected chicks	Control Chicks	Infected Ducklings	Control Ducklings
Bursa of Fabricius	2.60±0.25*	0.20±0.20*	0.20±0.37	0.00±0.00
Thymus	2.40±0.25*	0.20±0.20*	0.40±0.20	0.20±0.20
Spleen	2.80±0.20*	0.20±0.20*	1.00±0.32*	0.00±0.00*
Proventriculus	2.80±0.20*	0.40±0.25*	0.40±0.25*	0.20±0.20*
Intestines	2.60±0.25*	0.00±0.00*	0.40±0.37	0.00±0.00

* Mean values are significantly different ($p<0.05$) within the rows.

DISCUSSION

The interlobular congestion, haemorrhages and depletion of the thymuses observed in the infected chicks is almost in line with the finding of [20-6-5] who reported severe lymphoid depletion with lymphocellular necrosis and apoptosis in the lymphoid organs such as thymus, spleen and bursa of Fabricius. The congestion and necrosis observed in the liver of infected chicks agrees with the finding of [11-7] who reported similar finding in local chickens infected with NDV Kudu 113 strain. The observed desquamation of glandular epithelia and the deposition of necrotic debris of the proventricular glands slightly differed from the report of [6] that showed severe haemorrhages in the proventriculus.

The significantly higher microscopic subjective grade score of lesion severity (>2.0) in almost all the 5 organs graded in the infected chicks than the control chicks could have resulted from the general dehydration, necrosis and atrophy induced by the virus. Also, the results showed that chickens are more likely susceptible to theXIVb strain of NDV than the ducks as earlier reported by [19]. The infected ducklings also showed a higher score in all the organs than their control counterparts, with grade of less than 1.0 score (mild) in all the organs except in the spleen with 1.0 score. This showed significance difference only in the spleen as opposed to that of the infected chicks, which showed significance differences in all the 5 organs observed. This could also be as a result of the mild lesions (necrosis and atrophy) in the intestinal tract, visceral organs and lymphoid tissues observed in the infected ducklings compared to the severe lesions in infected chicks. This finding indicates that the Muscovy ducks are more resistant to XIVb strain of NDV than the local chickens as postulated by histopathologic findings reported by [20] on NDV Kudu 113.

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