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Clinical and gross-pathological changes in Muscovy ducks and Nigerian local chickens infected with Newcastle disease virus (XIVb strain)

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Abstract

Newcastle disease (ND) is an acute highly contagious viral disease, spreading rapidly within flocks and affecting birds of all ages. Muscovy ducks, geese and other anseriforms have been tested against different strains of Newcastle disease virus (NDV) and are found to be potential reservoirs showing mild or no clinical signs when infected experimentally with strains that are virulent to chickens. The aim of this work was to compare the clinical and gross pathological changes in Nigerian local chickens and Muscovy ducks experimentally infected with XlVb strain of Newcastle disease virus. Forty birds consisting of 20 chicks and 20 ducklings were randomly selected and 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 were inoculated orally with 107.8/ 0.1ml /bird as the embryo lethal dose (ELD50/ml) of the NDV (XIVb) strain. Clinical signs observed were inappetance, sitting on hock, diarrhoea, depression and death which were severe in infected chicks but mild in infected ducklings. Gross pathological findings were severe congestions and haemorrhages in most of the lymphoid and other visceral organs of the IC compared to the ID. Muscovy ducks could serve as a source of the ND caused by strain XIVb to the local chickens while suffering mild form of the disease. It is recommended that mixing of Muscovy ducks and domestic chickens in the villages by poultry farmers should be discouraged.

Keywords: Clinical signs, local chicks, Muscovy ducklings, Newcastle disease, NDV XIVb strain

Introduction

Newcastle disease (ND) is a highly infectious and contagious avian disease that spreads rapidly, affecting large number of avian species of all ages (Alexander, 2000; Abdu *et al.,* 2004; Haque, 2010). It is also thought to be one of the greatest problems of poultry production in rural areas and small communities across the developing countries of Africa (Abdu *et al.,* 1992; Sa'idu *et al.,* 2006. It is one of Nigeria's most economically important avian diseases affecting both local and commercial poultry (Adu *et al.,* 1986). It has been reported that ND outbreaks in Nigeria reaches 200 to 250 cases per annum for the different avian species (Adu *et al*., 1986; Okeke *et al.,* 1988; Haruna *et al.,* 1993).

The Newcastle disease virus (NDV) belongs to the order *Mononegavirales* in the family *Paramyxoviridae*, and genus Avulavirus with a wide host range and genetic diversity, exhibiting varying virulence within the genotypes (Amarasinghe *et al*., 2018; Dimitrov *et al*., 2019). NDV isolates have been classified into 2 broad classes (I and II), with class I comprising only a single genotype (class I, genotype I) and with class -II containing up to 18 genotypes (class II, genotypes I–XVIII) (Susta *et al*., 2015). Class-I basically contains avirulent NDV strains; with, APMV-1/chicken/Ireland 48/90 that is found mainly in waterfowls, the only exception (Susta *et al*., 2015). While class-II of the NDV contains the virulent and avirulent strains (Susta *et al*., 2015).

Clinical signs of ND could range from acute disease with almost 100% mortality to subclinical disease with no lesion (Cattoli *et al*., 2011). It may be characterized by a sudden onset of clinical signs such as inappetance, hoarse chirps (in chicks), nasal discharges, diarrhoea, droopy wings and tail feathers, dullness, dyspnoea or gasping and facial swelling after an incubation period of 2-15 days (Sa'idu *et al*., 2006; Shankar, 2008).

Ducks as part of poultry production have gained good levels of recognition in the past few decades and will surely continue to play an increasingly important role in the world with respect to food security and production (Huang *et al*., 2012). Among duck population, the Muscovy duck (*Cairina moschata*) which was said to have originated from Central and South America has the largest share (74%) of duck farming in Nigeria (Ikani, 2001). The Nigerian Muscovy duck with soft meat resembling beef, hence adding to its taste and attraction to many people is also reported to have higher dressing quality than the exotic breeds of Reuen and Pekin ducks (Huang *et al*., 2012; Yakubu, 2013).

Newcastle disease is more likely to occur when farmers keep mixed species of poultry including exotic birds (Abdu *et al*., 2005; Sa'idu *et al*., 2006). The system governing the production of indigenous poultry in Nigeria is basically under the typical extensive management where they roam and scavenge naturally for most of their food without receiving any vaccine or prophylactic treatment and therefore serve to transmit diseases to the exotic birds (Abdu *et al*., 2006). Muscovy ducks are mostly kept together in villages by local farmers (Sa'idu *et al*., 2006) and there is a high chance of disease transmission between these species (Abdu *et al*., 2005).

The aim of this work was to compare the clinical and gross pathological changes in Nigerian 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 strain of NDV) was obtained from the National Veterinary Research Institute (NVRI), Vom, and was used as the challenge virus. A total of 40 eggs from apparently healthy Nigerian local chickens and forty from apparently healthy Muscovy ducks were obtained from some villages around Samaru in Zaria, Kaduna State. The eggs were hatched from a locally fabricated commercial incubator. The incubation was tactfully synchronized, (chicken eggs were introduced on day 18 th after commencement of duck eggs hatching) so that the age of the chicks and the ducklings would be about the same time. The hatchability rates for chicken eggs were 17, 7 and 9 on days 21, 22 and 23 with 82.5% while for duck eggs were 8, 10 and 9 on days 38, 39 and 40 with 67.5% for the total number of 40 eggs each for chicks and ducks used for incubation. 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 (ABU), Zaria, with strict brooding conditions, temperature, biosecurity, ventilation and draft control as recommended by ABUVTH poultry experts. Electric bulbs were lowered to conducive limits (1-2m high depending on the environmental conditions) as source of light and energy. The chicks and the ducklings were fed on Vital feed chick mash (Grand Cereal Ltd, Jos, Nigeria) from day old up to one month) and then changed to grower's mash from same company until the end of the experiment. Water and feed were provided ad libitum. The chicks and the ducklings did not receive any vaccination against NDV.

Ethical clearance

Ethical clearance for this study was obtained from the Animal Welfare Committee of ABU with the following approval number; ABU/CAUC/2018/072.

Experimental birds

The birds were raised up to 5 weeks to allow for the decay of maternally derived antibodies (MDA), hence 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 Newcastle disease virus strain (XIVb)

The viability of the NDV strain was tested in the Virology Laboratory at NVRI, Vom as follows: 1ml of the test sample mixed with some amount of antibiotics (penicillin and gentamycin) was inoculated into 9-day old embryonated minimum pathogen free (MPF) chicken eggs obtained from NVRI, Vom using a sterile 2ml-syringe and needle. The inoculation was done through the allantoic route and incubated at 37℃ and candled with a 25-voltage bulb twice daily for 4 days. Eggs which showed embryonic death within or in less than 24 hours appeared to contain viable virus. The allantoic fluid from such eggs was harvested and confirmatory test using Haemagglutination Inhibition (HI) was carried out as stated by pathogenicity test. The titre to be inoculated into each subject (embryo lethal dose: ELD50/ml) was also determined using HI test with 1% washed chicken red blood cell and arrived at a titre of 107.8/0.1 ml/bird as the embryo lethal dose (ELD50/ml) to be inoculated orally. It was then kept in vials of 1ml at -20℃ only to be 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 contains 10 doses of 107.8 titre of the viral agent and each dose was determined to be the embryo lethal dose (107.8/0.1 ml/ bird, ELD_{50}) as described by Shittu *et al*. (2016).

Duration of the experiment

The experiment lasted for 10 weeks, with the first 5 weeks being pre-inoculation, during which any MDA against ND would have fully waned since MDA against ND is believed to be fully waned at about 20 days post-hatch (Erganis & Ucan, 2003; Sa'ad & Kamal, 2013). The remaining 5 weeks were for the experimental studies.

Inoculation of the virus

At day 0 post inoculation (D0 PI), 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.

Pathology procedures

Clinical signs: All the birds in all the four groups were observed three times (visual examination) daily (in the morning, afternoon and evening) for clinical signs of ND. Presence of clinical signs, such as depression, sitting on hock, whitish or greenish diarrhoea, inappetence, torticollis, leg/wing paralysis, oculo-nasal discharge, facial swelling, coughing/ sneezing, dullness/somnolence, muscular tremor, dropped wings, ruffled feathers, soiled vent and death were all carefully looked for and those found or seen were recorded in tabular form according to the method of Sa'idu *et al*. (2006), Shankar (2008) and Dai *et al*. (2014).

Organo-somatic indices of the birds

The live body weight of the birds were measured using digital weighing balance on the commencement day of the experiment (day 0) and carcass body weight (CBW) of the birds were also measured (in grammes) immediately after death or sacrifice and the organs were carefully removed and measured (in grammes) using the digital weighing balance as described by Latif *et al*. (2014). The organo-somatic index (OSI) of the birds was given by the formula: weight of organ / weight of bird x100 and the results were expressed for each of the experimental groups as the arithmetic mean and standard error of the mean in tabular form as described by Latif *et al*. (2014).

Gross pathological findings

Organs such as spleen, thymus, proventriculus, small intestine/large intestine, trachea, lung, heart, liver, caecal tonsils, bursa of Fabricius and the kidneys were all grossly examined for pathological changes and the findings recorded.

Data analysis

Data obtained were subjected to statistical analysis using Graph-pad prism-6.0 of the computer software (One-way ANOVA) and the level of significance was determined and accepted at $p < 0.05$ for all the results. The mean ± standard deviation (SD) of the results obtained in the experiment was calculated and presented in tables.

Results

In the IC group, clinical signs started to manifest on day 4 post inoculation (PI) as a slight depression and reduced feed intake in 3 chicks. By day 5 PI, depression and huddling set in and were severe in 5 of the chicks (Plate I) followed by the death of 3 chicks late in the afternoon of day 5. Two dead chicks were picked in the early morning hours of day 7 PI. Other clinical signs, such as sitting on hock, greenish-yellow diarrhoea with soiled vent, ruffled feathers and droopy wings were observed subsequently (Table 1). Two chicks died on day 9 PI, 1 on day 10 PI and another 1 chick on day 14 PI; leaving a single survivor

which recovered but showed clinical sign of torticollis from day 18 PI (Plate II).

Likewise, in the infected group of ducklings, the clinical signs manifested include slight depression, reduced feed intake and slight diarrhoea from day 5 PI to day 9 PI. The depression persisted to day 14 PI in only one duckling (Plate III).

The result of the organ-body weight ratio of the birds showed that the infected chicks had significantly lower mean values ($p<0.05$) in the pancreas and trachea than their control group. The infected ducklings had lower values of organ-body weight ratio than their control in all the organs examined (Table 2).

Table 1: Clinical signs of Newcastle disease in chicks and ducklings infected with XIVb strain (days 5 -14 PI)

Clinical signs of birds	Infected Chicks (%)	Control Chicks (%)	Infected ducklings (%)	Control ducklings (%)
Depression	100	0	100	0
Sitting on hock	40	0	0	0
Diarrhoea	60	0	40	0
Inappetence	100	0	70	0
Torticollis	10	0	0	0
Facial swelling	40	0	0	0
Dullness/ somnolence	90	0	0	0
Muscular tremor	50	0	0	0
Dropped wings	80	0	10	0
Ruffled feathers	70	0	0	0
Death	90	0	0	0

Plate I: Infected chicks showing depression and huddling together on day 5PI

Plate II: The only survivor of the infected chicks showing torticollis on day 18 PI

Table 2: Mean organ-body weight ratio (g) of infected and control chicks and ducklings

Days post inoculation	Infected chick	Control chick	Infected duckling	Control duckling
Bursa	0.91±0.20	0.52±0.04	0.23±0.03	0.24±0.05
Provent./Gizzard	3.17±0.31	5.47±0.13	6.06±0.07	7.65±1.12
Heart	0.48±0.11	0.99±0.12	0.81±0.09	3.43±1..07
Intestine	5.74±0.21	7.82±0.08	6.24±0.50	7.45±0.05
Kidneys	0.65±0.05	1.06±0.08	1.17±0.07	1.47±0.12
Liver	3.46±0.06	4.25±0.07	4.35±0.10	4.58±0.16
Lungs	0.74±0.05	1.45±0.06	1.06±0.08	1.99±0.12
Pancreas	0.37±0.05*	0.64±0.06*	0.48±0.12	0.47±0.12
Spleen	0.38±0.04	0.34±0.06	0.26±0.03	0.46±0.12
Thymus	0.93±0.04	1.08±0.06	0.54±0.05	0.64±0.04
Trachea	0.32±0.04*	0.65±0.05*	0.32±0.04	0.43±0.05

*Means values along the rows are significantly different at $p < 0.05$

Plate III: An infected duckling showing depression on day 14 PI (arrow)

Plate IV: Carcass of an infected chick (IC) that died on day 7PI showing congested visceral organs and thigh/ breast muscles as compared to its control (CC)

Table 3: Post mortem findings in different groups of birds after the challenge with XIVb strain of NDV at days 5 and 7 PI

Clinical signs in birds	Infected chicks (%)	Control chicks (%)	Infected ducklings (%)	Control ducklings (%)
Congested liver	80	0	0	0
Congested/ atrophic thymus	100	0	20	0
Congested thigh/breast muscles	100	0	0	0
Enlarged/ congested kidneys	40	0	0	0
Haemorrhagic/ congested intestines	100	0	0	0
Congested lungs	40	0	0	0
Haemorrhagic/ congested caecal tonsils	100	0	0	0
Haemorrhages in the proventriculus	80	0	0	0
Atrophic and congested bursa	100	0	0	0
Urates in the ureters	80	0	0	0
Swollen or atrophic spleen	60	0	20	0

Two chicks died on day 5 PI and three on day 7 PI from the infected group of chicks (IC), and the corresponding numbers of birds (two on day 5 PI and three on day 7 PI) were euthanized from each of the other 3 groups in accordance with the method of Sparrey *et al*. (2014). The gross lesions observed at postmortem of chicks and ducklings during the experiment are as presented in Table 3. Among infected chicks, there were congestion of most of the visceral organs and the thigh and breast muscles (Plate IV), marked atrophy and congestion of the bursa of Fabricius (Plate V) and thymuses (Plate VI), severe haemorrhages and congestion of the proventriculus and gizzards (Plate VII), haemorrhages in the caecal tonsils (Plate VIII) and presence of urates in the ureters (Plate IX). On the other hand, congestion was observed in one lobe of the thymus in one duckling (Plate X).

Discussion

The average body weight was 234.74±5.20 gm and clinical signs observed in infected chicks in this study were signs seen in velogenic viscerotropic NDV infection with high mortality with few or no neurological signs, which are consistent with the findings in previous reports (Echeonwu *et al*., 1993; Susta *et al*., 2015: Shittu *et al*., 2016).

The absence of mortality in the infected ducklings against 90% in the infected chicks during this study is clear evidence indicating the lesser susceptibility of

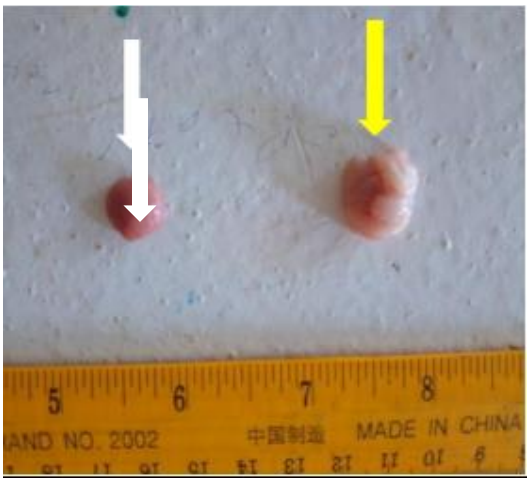

Plate V: The bursa of Fabricius of an infected chick that died on day 7PI showing atrophy of the bursa of Fabricius and slight congestion (white arrow), when compared with the control group (yellow arrow)

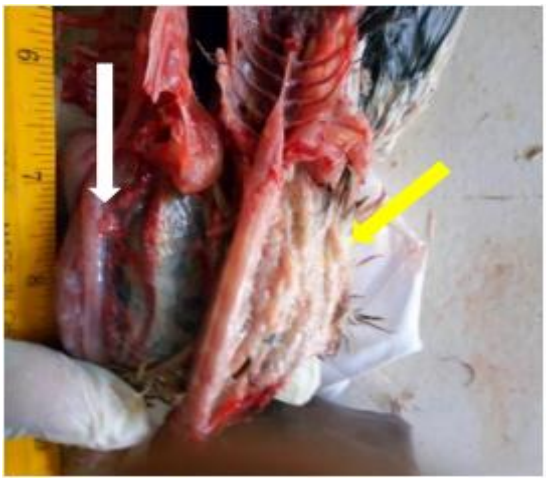

Plate VI: Thymus of an infected chick that died on day 7PI showing severe atrophy and congestion of the thymus (white arrow) against its control which showed apparently normal thymus (yellow arrow)

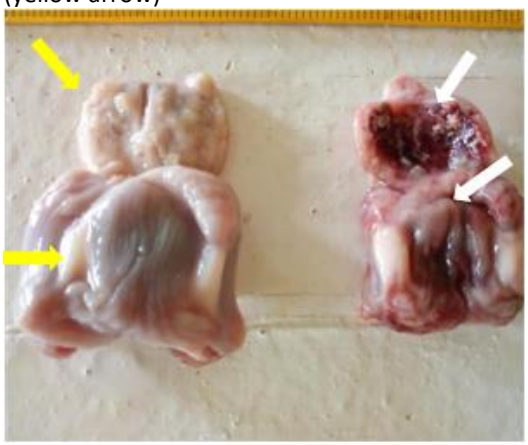

Plate VII: Proventriculus and gizzard of an infected chick that died on day 7 PI showing severe haemorrhages in the proventriculus and gizzard (White arrows) against its control which was looking apparently normal (Yellow arrow)

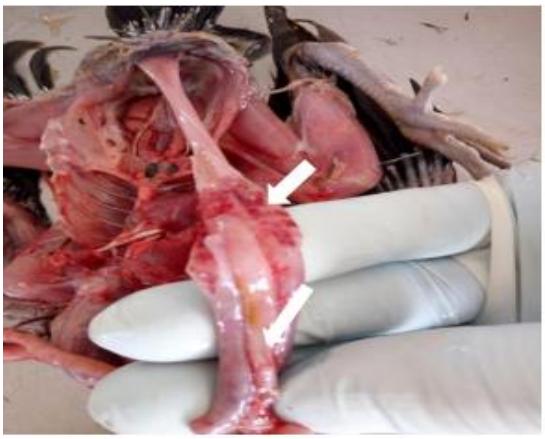

Plate VIII: Large intestine of an infected chick that died on day 5PI showing haemorrhages in the caecal tonsil and the caecae (arrows)

the ducks to XIVb strain of NDV than the chickens. This finding may be lower than the findings of Eze *et al*. (2014) who recorded 25% mortality with KUDU 113 which is also a velogenic viscerotropic NDV strain (that is similar to strain XIVb) in the infected ducklings against the infected chicks with 58.3% mortality, but still indicated that the ducks were also less susceptible to NDV KUDU113 strain as they were to XIVb strain of NDV in this study compared to the domestic chicks. The variability in the findings could be as a result of the age of the subjects, individual genetic makeup, or differences in resistance shown by the subjects to the strain. The result in this study for chicks is in line with findings of Sa'idu *et al*. (2006) who reported 92% mortality and a morbidity of 100% in local chickens infected with NDV KUDU 113 strain. The lower organ-body weight ratio obtained in the infected chicks and ducklings than their control counterparts could be due to the viral tropism in many organs and tissues of the birds which destroys the tissues or set them to atrophy. This finding is in agreement with that of Dortmans *et al*. (2011) who reported viral tropism and atrophy in many organs of chickens experimentally infected with Pigeon paramyxovirus - 1. It is also in line with the findings of Igwe *et al*. (2014) who reported atrophy in lymphoid organs of guinea fowls and chickens experimentally infected with Kudu- 113. This could also be attributed

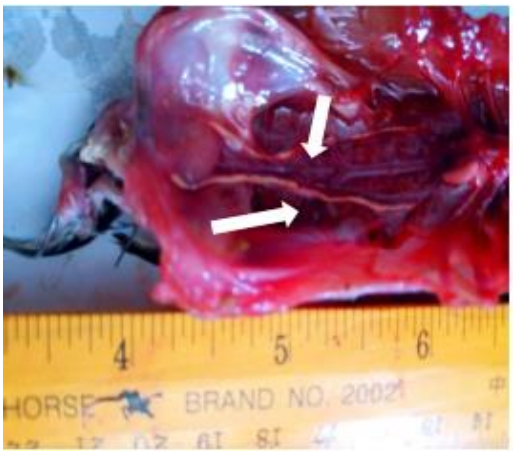

Plate IX: Ureters of an infected chick that died on day 5PI showing the presence of urate in the ureters (arrows)

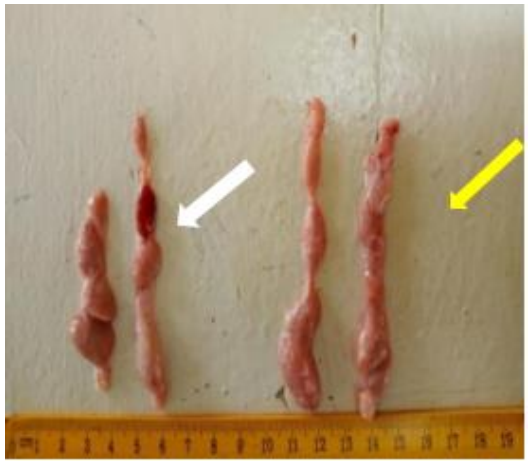

Plate X: Thymus of an infected duckling sacrificed on day 7PI showing congestion in only one lobe of thymus (white arrow) against its control showing apparently normal thymus (yellow arrow)

to the general decrease in the body weight due to reduction in feed intake, dehydration and emaciation associated with the XIVb strain of Newcastle disease virus infection in this study. This finding is also in line with the results of Wakamatsu *et al*. (2006), Igwe *et al*. (2014) and Eze *et al*. (2014) which showed severe atrophy in most of the lymphoid organs of chicks but mild in the ducklings infected with NDV. The increase in the weight of the bursa of Fabricius of the chicks could be due to edema which was quite observed in some of the bursae of the infected chicks.

However, the reduction in the weight of the pancreas and trachea of the infected chicks against their control groups is significant and could have been due to emaciation, resulting from severely reduced feed and water in-take or due to necrosis induced by the virus in those organs.

The postmortem finding was mostly severe in the infected chicks than in the infected duckling group. The restriction of the lesions particularly to the lymphoid organs and other visceral organs in this study also supports the findings of Pandaranga *et al*. (2016) when chickens were infected with NDV Kvuzat/13 and Karachi/07 strains; of Oladele (2008) and Eze *et al*. (2014) when chickens were infected with NDV Kudu 113 strain. The result of this study also agrees with the findings of Susta *et al*. (2015) and Shittu *et al*. (2016) who reported that XIVb strain is a velogenic viscerotropic NDV pathotype.

The milder manifestation of the postmortem lesions observed in the infected ducklings in this study is in line with the findings of Eze *et al*. (2014) who reported mild gross changes in ducks and which was attributed to their ability to neutralize the virus earlier and avoid the serious damage to various organs in the body.

In conclusion, the NDV strain of XIVb has caused severe clinical signs and pathological changes in chicks, but mild ones in ducklings. Hence the mild effect of XIVb strain of NDV in Muscovy ducks, and therefore ducks could serve as source of ND caused by this strain of NDV to local chickens reared together in the villages. It is therefore recommended that mixing of Muscovy ducks and domestic chickens on the free range by the local farmers in the villages should be discouraged as the Muscovy ducks can be sources of infection of strain XIVb of ND to the domestic fowls in the villages

Conflicts of Interest

The authors declare no conflict of interest.

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