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Pathogenesis of Newcastle Disease Virus Kudu 113 Strain in Relation to Neuraminidase Production in Chickens

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Abstract: Twenty-five Shaver Brown chickens were inoculated intramuscularly at 4 weeks with Newcastle Disease Virus (NDV) Kudu 113 strain. Another 25 chickens served as controls. Values of neuramindase activity (NA), free serum sialic acid (FSSA), erythocytes surface sialic acid (ESSA), packed cell volume (PCV) and haemagglutination inhibition (HI) antibody titres to NDV were determined for each chicken. The infected chickens had clinical signs due to newcastle disease (ND) by day three post-infection (pi). Mortality and morbidity were 52 and 100%, respectively. The major gross lesions observed in the infected chickens were haemorrhages in the proventricular mucosa, the caecal tonsils and the mucosal layer of the intestine. There was a significant decrease in daily mean values of PCV from days 3-7 pi in the infected chickens. This period coincided with the time of elevated values of HI anitibody titre, NA, FSSA and a decrease in ESSA.

Key words: Pathogenesis, newcastle disease virus, neuraminidase, chicken

INTRODUCTION

Newcastle disease constitutes one of the major disease problems of poultry in many parts of the world. The disease has a high morbidity and mortality in both domestic and wild birds (Alexander, 1997; Okoye *et al*., 2000; Oladele *et al*., 2003).

Newcastle disease virus Kudu 113 strain was isolated from ducks in Nigeria. This isolate has been classified as a velogenic NDV strain and it is believed to be one of the virulent NDVs, causing ND in chickens in Nigeria (Echeonwu *et al*., 1993).

It is established that the surface structure of erythrocytes from several animal species, including poultry contain sialic acids (Herrler *et al*., 1987; Schauer *et al*., 1995). The enzymatic removal of sialic acids from carbohydrate-containing molecules is believed to play an important role in pathogenicity of diseases (Corfield, 1992; Lichtensteiger and Virm, 2003). In order to aid digestion of cell surface glycoconjugates, several microorganisms produce neuraminidases, which inturn cleave sialic acids from sugar residues and glycoproteins, thus facilitating infections (Schauer *et al*., 1995).

It has been shown that NDV Kudu 113 strain produced neuraminidase which cleaved sialic acid from fetuin (substrate) *in vitro* (Oladele *et al*., 2002). It is possible that this same mechanism is operative in NDV infected chickens *in vivo*. It is therefore, imperative to study the pathogenesis of NDV Kudu 113 strain in relation to the production of neuraminidase, an enzyme believed to play a crucial role in the pathogenicity of diseases (McNulty *et al*., 1975; Corfield, 1992; Lichtensteiger and Virm, 2003). This is because complete understanding of the pathogenesis of ND is essential for the control and possible eradication of the disease. Therefore, if the role of neuraminidase is known in the pathogenesis of ND, it will become easier to develop vaccine(s) or drug(s) against its activity during NDV infection.

Although, NDV Kudu 113 strain has been isolated and characterized (Echeonwu *et al*., 1992), there is no information on neuraminidase production by this NDV strain *in vivo*. This study therefore, describes for the first time the pathogenesis of ND in relation to neuraminidase production in Shaver Brown chickens infected with NDV Kudu 113 strain.

The objective of this study, was to sequentially determine the levels of neuraminidase during the course of ND in chickens experimentally infected with NDV Kudu 113 strain. Results of this study may give further insight into the prevention and control of ND in poultry.

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MATERIALS AND METHODS

Chickens and management: A total of 50, one-day-old Shaver Brown pullet chicks were obtained. They were immediately randomly allocated into 2 groups of 25 chicks each. The 2 groups were housed separately in enclosed building protected from pathogens and were not vaccinated with any NDV vaccines. The chicks were kept in a deep litter system with water and feed supplied *ad libitum*.

The experiment commenced at 4 weeks of age because it is generally believed that at about 4 weeks, the maternal antibodies in the chicks would have been lost or reduced to a level that may not interfere with challenge virus (Beard, 1989; Alexander, 1997; Okoye *et al*., 2000).

The values of NA, FSSA, ESSA, PCV and HI were determined in the 2 groups of chickens 3 days (days 3 to 1; that is, pre-infection days) before the chickens in the infected group were inoculated with NDV Kudu 113 strain. This was done to establish the values of these parameters before the experiment commenced at four weeks of age.

Newcastle disease virus inoculum and challenge of chickens: One vial of the lyophilized NDV Kudu 113 strain was diluted with 2 mL of sterile phosphate buffered saline (pH 7.2). Each chicken in the infected group was inoculated intramuscularly (i.m) with 0.2 mL of the virus at the breast muscle at 4 weeks of age. Chickens in the control group were not inoculated with the virus.

Clinical and pathological examinations: Both groups of chickens were observed on a daily basis for any clinical signs. Dead chickens were examined for gross lesions. Samples of the spleen, liver, brain, lung, caecum, intestine and kidney were fixed in 10% buffered formalin, processed, embedded in paraffin wax and sectioned. They were stained with haematoxylin and eosin (H and E) and examined under light microscope at the magnification of ×200. The remaining chickens were sacrifised at the end the experiment.

Blood sampling: Blood sampling started at 4 weeks of age through wing venepuncture, using 23 gauge sterile hypodermic needles and syringes. About 0.5-1 mL of blood were collected on each day of the experiment from the chickens in the 2 groups.

Blood for haematological values were collected into Bijou bottles, containing ethylene diamine tetra acetic acid (2 mg mL^{-1} of blood) as anticoagulant. Serum samples for biochemical and HI analyses were taken without anticoagulant. The serum samples were separated by centrifugation at 1,000 g for 10 min and frozen in plastic vials until the HI titres were determined. Values of PCV were determined by microhaematocrit method.

Determination of haemagglutination inhibition antibody titres: Quantification of the HI titre was done using the haemagglutination and HI procedures of Beard (1989). The NDV La Sota strain obtained from NVRI, Vom, Plateau State, Nigeria served as antigen for the HI test.

Preparation of haemoglobin-free erythrocyte ghosts: Haemoglobini-free erythrocyte ghosts were prepared using the standard methods described by Harris (1971).

Assay for neuraminidase activities, free serum sialic acid and erythrocytes surface sialic acid concentrations: Neuraminidase activities, free serum sialic acid and erythrocytes surface sialic acid concentrations were assayed by the standard procedures described by Reuter and Schauer (1994).

Statistical analysis: The data obtained were analysed using Student's t-test analysis. Values were expressed as means±standard deviation. The values of ($p<0.05$) were considered significant. The analysed data were used to draw bar charts.

RESULTS AND DISCUSSION

All the infected chickens developed clinical signs of ND. The prepatent period following i.m injection with the virus varied between three to 4 days. Clinical signs were progressive and in the following general order of development: fever, anorexia, anaemia, lethargy, diarrhoea, dehydration, clonic convulsions, paralysis of legs and wings, prostration and death. Mortality was first observed on day four pi (5 chickens). Peak mortality occurred on day seven pi (8 chickens). The total mortality was 52%. The control chickens had no clinical signs and mortality.

At necropsy, the dead chickens had dehydration, weight loss and congestion of the muscles of the breast, thighs and legs (Fig. 1). There were haemorrhages in the wall of the intestine and the mucosa of the proventriculus. The lung, spleen and liver were congested (Fig. 2). The control chickens showed no gross lesions when all the organs were examined after sacrifising them.

In general, the histopathological lesions were focal or diffuse necrosis and mononuclear cells infiltration of the tissues of the spleen, liver, kidney, lung, intestine, caecum, proventriculus and the brain. However, some specific lesions were found in some organs. For example, in the spleen, there were focal necrosis and depletion of lymphocytes (Fig. 3). There were neuronal degeneration and encephalitis in the brain (Fig. 4).

Fig. 1: Gross pathological changes in Shaver Brown Chicken which died of acute. Newcastle disease caused by NDV Kudu 113 strain infection. Note the emaciation and congestion of the muscles of the breast, thighs and legs on the carcass

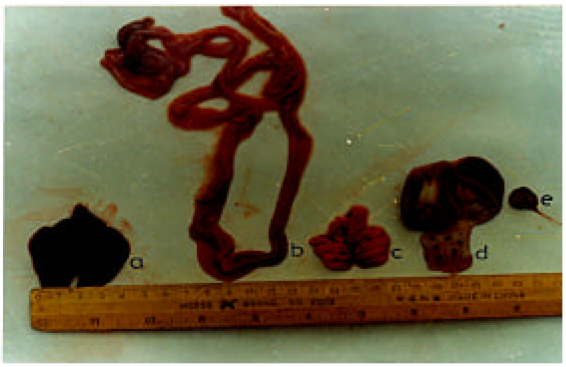

Fig. 2: Gross pathological changes in Shaver Brown chicken which died of acute. Newcastle disease caused by NDV Kudu 113 strain infection. Note the congestion and slightly darkened liver (a), haemorrhages in the wall of intestine (b), congested lung (c), haemorrhages in the mucosa of the proventriculus (d) and congested spleen (e)

The results of this study indicate that chickens infected with NDV Kudu 113 strain developed severe ND with clinical signs and gross lesions similar to those reported by other workers (Alexander, 1997; Okoye *et al*., 2000).

The histopathological lesions observed in the brain tissue of the infected chickens in this study were significant findings. This is because most workers could not demonstrate any histopathological lesions in the brain of chickens infected with NDV (Alexander, 1991; Hamind *et al*., 1991; Spradbrow, 1992).

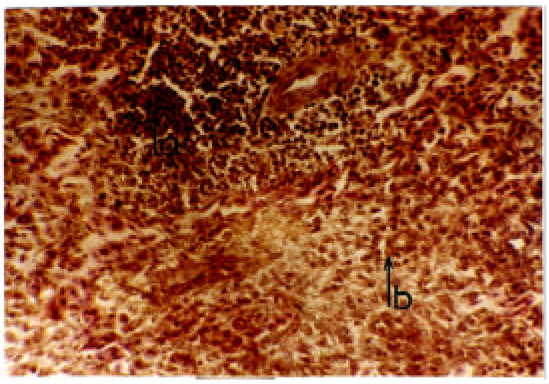

Fig. 3: A section from the spleen of chicken infected with NDV Kudu 113 strain. Note focal area of necrosis (a) and depleted lymphocytes (b). H and E stain ×200

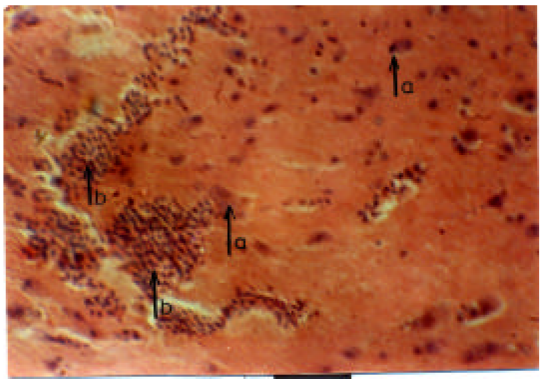

Fig. 4: A section from the brain (cerebrum) of chicken infected with NDV Kudu 113 strain. Note the neuronal degeneration (arrow heads a) and mononuclear cells infiltration (arrow heads b) (encephalitis). H and E stain ×200

The mortality rate of 52% recorded in this study is lower than the value of 60-100% reported for NDV Hert's 33/56 strain (Shamaki *et al*., 1989) and the Indonesian strain of NDV (Hamid *et al*., 1991). The lower mortality obtained in this study may be due to variation in a number of factors, such as the maternal antibodies of the chickens, passage history of the virus, laboratory storage conditions of the virus, virulence of infecting strain of virus and possibly the environmental stress, prevailing during infection (Alexander, 1997; Okoye *et al*., 2000).

The development of anaemia as determined by PCV values started by day 2 pi. The lowest mean PCV value of 20.39±5.28% was obtained on day 5 pi in the infected chickens. The PCV values for the control chickens remained relatively constant during the experiment (Fig. 5).

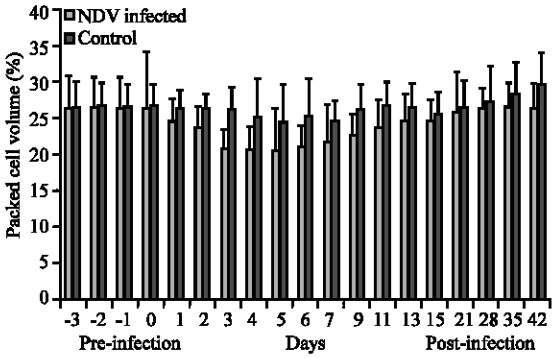

Fig. 5: Mean (±SD) changes in packed cell volume of NDV Kudu 113 infected and control chickens

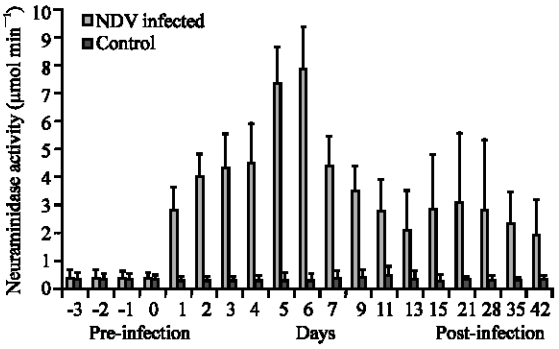

Fig. 7: Mean (±SD) changes in neuraminidase activities of NDV Kudu 113 infected and control chickens

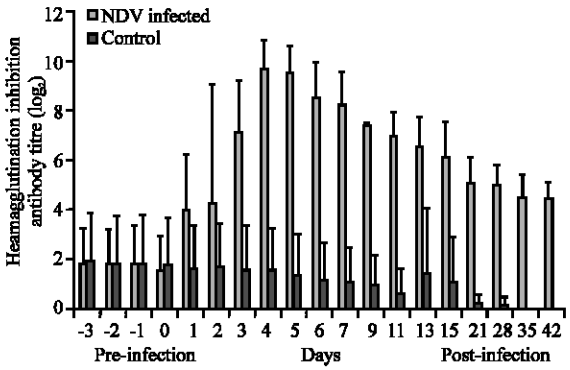

Fig. 6: Mean (±SD) changes in haemagglutination inhibition antibody titre of NDV Kudu 113 infected and control chickens

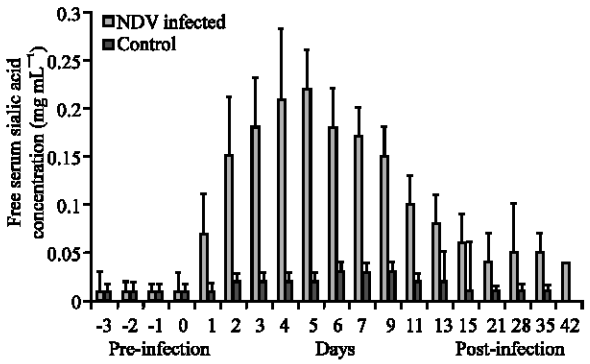

Fig 8: Mean (±SD) changes in free serum sialic acid concentrations of NDV Kudu 113 infected and control chickens

Following infection, there was an increase in HI antibody titres, reaching the maximum daily mean HI antibody titre of $\log_2$ 9.67±1.15 by day 4 pi. The HI antibody titres for the control chickens declined as the experiment progressed (Fig. 6).

The daily mean NA in the sera of infected chickens increased from day 1 pi, with the value of 2.78±0.82 µmoL min^{-1} by day 6 pi. The daily mean NA obtained in the control chickens were low and relatively constant during the experiment (Fig. 7).

There was a rise in daily mean FSSA concentration from 0.07±0.02 mg mL^{-1} in the sera of the infected chickens, beginning from day one pi and it continued until it reached the peak value of 0.22±0.07 mg mL^{-1} by day 5 pi. Low and relatively constant values of FSSA were obtained in the control chickens during the experiment (Fig. 8).

In the infected chickens, there was a decline in the daily mean ESSA concentration, beginning from day 1 pi. The lowest daily mean ESSA concentration of 0.02±0.001 mg mL^{-1} was obtained by day six pi. Relatively constant values of ESSA were recorded for the control chickens throughout the experimental period (Fig. 9).

A significant increase in FSSA occurred during the time of high NA and HI antibody titres to NDV, therefore, it is likely that the period of high NA and FSSA concentrations in the infected chickens was related to the time of high levels of circulating NDV, which inturn, produced neuraminidase which hydrolysed the sialic acid. It has been shown that NDV Kudu 113 strain produced neuraminidase *in vitro* and that this enzyme cleaved off sialic acid from fetuin (Oladele *et al*., 2002). The finding that a reduced ESSA concentration and high NA and HI occurring concurrently pi, is an evidence that NDV Kudu 113 strain also produced neuraminidase *in vivo*, which cleaved off sialic acid from erythrocytes surface.

The reduction of ESSA concentrations at a period of significant drop in the PCV values is probably a mechanism of RBCs destruction and could be important in senescence of erythrocytes. This might be due to the effect of neuraminidases which cleave off the surface sialic acids from erythrocytes and rendered RBCs prone

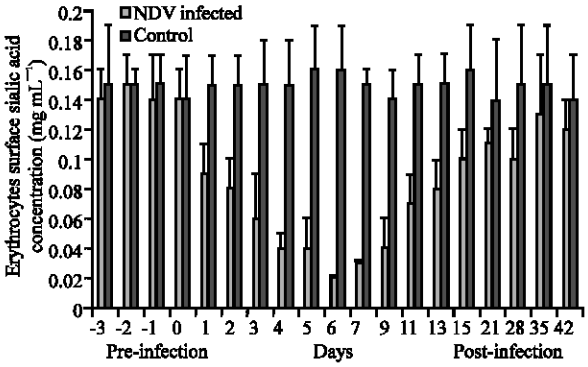

Fig. 9: Mean (±SD) changes in erythrocytes surface sialic acid concentrations of NDV Kudu 113 infected and control chickens

to erythrophagocytosis and consequently, resulting in anaemia (Durocher *et al*., 1975; Schauer *et al*., 1995). Therefore, the acute anaemia observed in the infected chickens may be attributed, at least in part, to the activities of the circulating NDV Kudu 113 strain neuraminidase, cleaving off RBCs surface sialic acid, increasing the FSSA concentrations in the plasma and the removal of such desialytated RBCs from circulation by macrophages, with an attendant reduction in PCV. This is probably responsible for the phenomenon of erythrophagocytosis observed in the infected chickens in this study.

It was reported that frequent anaemia in NDV infection was due, at least in part, to replication of the virus, lysis of erythrocytes and haemorrhages in the wall of the intestine and proventricular mucosa (Cheville and Beard, 1972; Cheville *et al*., 1972). However, the *in vivo* removal of ESSA by neuraminidase of NDV Kudu 113 strain in this study, with subsequent reduction in the PCV values has added another mechanism to the pathogenesis of NDV. Since, neuraminidase of NDV Kudu 113 strain seems to play an important role in the pathogenesis of ND in this study, any mechanism that could reduce its activity in NDV infection may probably be of use in the prevention of ND, such that in the nearest future, vaccines or drugs may be developed to inactivate or block neuraminidase's target site during NDV infection.

In the infected chickens, NDV Kudu 113 strain induced necrosis and depletion of reticulo-endothelial cells, especially the lymphocytes of the spleen (Fig. 3) and other interstitial lymphoid tissues. These findings are in line with the results of Cheville *et al.* (1972) and Lam (1996). The exact mechanism of lymphoid depletion in NDV infection is still unknown. However, Cheville and Beard (1972) postulated that NDV could be lymphocidal and that after NDV infection, the lymphocyte surface, receptors could be altered and their migration patterns changed, so as to cause seeding of lymphocytes in the lymphoid organs. It is therefore, reasonable to assume that the neuraminidase production by NDV Kudu 113 strain as reported in this study, might be cleaving sialic acid off lymphocytes too, thus altering their surface receptors and migrating patterns.

CONCLUSION

In conclusion, the removal of ESSA from RBCs surface structure by the activities of neuraminidase of NDV Kudu 113 strain, with the attendant reduction in PCV values which occurred concurrently, may have contributed, at least in part, to the acute anaemia observed in the infected chickens.

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