Experimental Vologenic Newcastle Disease Can Be Very Severe and Viscerotrophic in Chickens but Moderate and Neurotrophic in Guinea Fowls

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Abstract: Information about the pathogenesis of Newcastle disease (ND) is still limited in many avian species including some poultry birds. Four weeks old cockerels and guinea fowls were inoculated with a local Nigerian velogenic ND (VND) Virus (VNDV) strain, Kudu-113, intramuscularly. The main clinical signs in chickens were severe depression and diarrhea while the guinea fowls showed mainly leg paralysis. Weight loss was significant in the infected birds in both species from days 3-21 post inoculation (PI). The total mortalities in the guinea fowls and chickens were 22.2 and 94.6%, respectively. The guinea fowls showed no proventricular haemorrhage, intestinal ulcers, haemorrhages and swelling of the caecal tonsil which were all prominent in the cockerels. Gross congestion of the brain was observed in the guinea fowls only. But lesions in the lymphoid organs and microscopic changes in the brain were similar in both species. The antibody response to the viral inoculation was higher in the chickens than the guinea fowls. The above observations confirm experimentally that chickens are more susceptible to VND than guinea fowls. Furthermore, the Kudu-113 infection was viscerotropic in chickens but neurotropic in guinea fowls.

Key words: Guinea fowl, chickens, pathogenesis, velogenic ND virus

INTRODUCTION

Newcastle disease (ND) is a major disease problem of poultry world-wide (Alexander, 2001; Ezema et al., 2009). The velogenic ND (VND) is the most severe form of the disease causes fulminating outbreaks in Africa and Asia where the aetiological pathotype is enzootic (Matheran et al., 2004; Nakamura et al., 2004; Oladele et al., 2008). VND in Europe and America where it is exotic is a grade A reportable disease. Control of outbreaks in these countries is very expensive because it is by eradication or stamping out policy which involves destruction of large populations of birds, quarantine, surveillance and payment of compensation. Outbreaks also lead to loss of export trade in poultry and poultry products. Generally the prevention of ND is by vaccination and biosecurity (Baba et al., 2006; Ezema et al., 2008; Van Boven et al., 2008). However, many out breaks of ND still occur in vaccinated birds (Saidu et al., 2004; Alexander and Senne, 2008). This could be due to the existence of highly virulent field strains of the virus which can break down vaccine immunity, use of expired and impotent vaccines and wrong vaccination procedures. Furthermore vaccination protects against clinical signs but not against lesions and some multiplication and shedding of the pathogenic field virus (Kapczynski and King, 2005; Ezema et al., 2009). All the ND viruses are serologically homologous but minor antigenic variations have been reported (Lim et al., 2003; Ibu et al., 2008).

One of the major problems in the control and epizootiology of ND is that the virus has a very wide host range, infecting over 250 avian species including poultry, cage and wild birds (Saidu et al., 2004; Oladele et al., 2008; Ibu et al., 2009). The severity of the infection depends on the species, host resistance and age, virulence and dose of the virus, route of infection and presence of concurrent infections (Alexander and Senne, 2008; Ezema et al., 2009). This makes the manifestations of the disease often unpredictable and difficult to recognize in the field. In many host species the infection is sub clinical but these birds serve as reservoirs of infections for susceptible birds (Kommers, 2001; Saidu et al., 2004; Ibu et al., 2009). No pathognomonie lesion has been identified for ND (Hamid et al., 1991; Brown et al., 1999; Okoye et al., 2000) and this makes rapid diagnosis and early control measures difficult. The clinical signs, lesions and pathogenesis of ND have been described by several authors in the domestic chickens (Brown et al., 1999; Okoye et al., 2000) which are the most susceptible species to ND. Information on the other poultry species is limited. In this project we did a comparative study of experimental VND in chickens and Guinea fowls using a local Nigerian strain of the virus.

MATERIALS AND METHODS

This study was scrutinized and approved by the University Committee on Medical and Scientific Research Ethics, University of Nigeria, Nsukka.

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Flock history: The chickens used were cockerels which were obtained at day old from a local hatchery. But the guinea fowl keets were obtained from the Poultry Research Department of the National Veterinary Research Institute (NVRI) Vom, Nigeria. Both species were hatched the same day. The parent stocks of the cockerels were vaccinated against ND while those of the guinea fowls were not. Both groups were brooded on deep litter; feed and water were supplied ad libitum. The birds were not vaccinated against any disease. The principles of humane laboratory animal care were followed throughout the study.

VND virus inoculum: The virus used was VND virus (VNDV) strain, known as Kuru duck-113 (Kudu-113). The virus was isolated in Kuru Plateau State of Nigeria from an apparently healthy duck and characterized biologically by Echeonwu et al. (1993). The inoculum had a median embryo lethal dose (ELD50) of 10^-1.00 per ml.

NDV challenge: At 4 weeks of age the two species of birds were each randomly divided into two groups. Group 1 cockerels were 60 birds while Group 2 contained 30. Group 1 guinea fowls contained 40 birds while Group 2 contained 35. At six weeks of age each of the cockerels and guinea fowls in groups 1 was inoculated intramuscularly (IM) with 0.2 ml of the inoculum (infected groups). The birds in groups 2 each had 0.2 ml of phosphate buffered saline (PBS) IM (uninfected groups). The four groups were reared separately in isolated pens.

Clinical signs and pathological examinations: The birds were observed twice daily for clinical signs from days 0 to 21 post inoculations (PI). Ten birds in each group were randomly selected and weighed on days 0, 3, 6, 10, 15, 21 PI. Three birds in each group were sacrificed on days 3, 6, 10, 15, 21 PI. In the infected groups the sacrificed birds were those showing clinical signs. The sacrificed and dead birds were necropsied. Samples of the spleen, bursa of Fabricius, thymus, kidney, liver, intestines and brain were collected and fixed in 10% formal saline for not less than 24 h. The tissues were processed and embedded in paraffin wax. Sections of 5 mm thick were cut and stained with haematoxylin and eosin and examined under the light microscope.

Serology: Blood samples were collected from ten birds in each group on days 0, 10, 14 and 21 PI. Serum samples were harvested and stored at -20°C until assayed. To remove non-specific inhibitors from Guinea fowl sera, the samples were adsorbed to chicken red blood cells (RBC). This was done by adding 0.025 ml of packed chicken RBC to 0.5 ml of guinea fowl sera, shaking gently and leaving for at least 30 min. The RBCs were then pelleted by centifugation at 800 g for 5 min. The serum samples were inactivated by heating at 56°C for 30 min in water bath. The haemagglutination (HA) and haemagglutination inhibition (HI) tests were done using the microtitre methods of Beard (1989). The geometric mean titres (GMT) of the samples were calculated using the Tube Number (modified log) and Tables provided by Villages and Purchase (1989). The antigen used for the HI test was a PBS suspension of LaSota vaccine which had 10HA unit.

Statistical analysis: Mean values and significance of differences between the mean body weights were analyzed using student t-test within groups. Significant means were separated using t-test for Equality of means and Leveque's test for Equality of variances (Chatfield, 1983). All tests were performed with a 5% level of significance.

RESULTS

Clinical signs: Clinical signs were first observed in 13% of the 90 infected chickens on day 2PI and these included ruffled feathers, depression, reduction in feed and water consumption. By day 3PI, 90% of the infected chickens were severely depressed and lethargic. Some were prostrate. There was whitish and greenish diarrhea. Some of the birds tucked their heads under droopy wings, huddled together and had hunched posture. On day 4PI, 100% of the birds were depressed. Coughing and noisy respiration with serous ocular discharges was observed in four birds. Jerking of the head was observed in one chicken while paralysis was evident in 3. By day 6PI, head tremors, wing and leg paralysis were observed in two chickens. On day 7PI head tremors and torticollis occurred in 4 chickens, paralysis in 5 and droopy wings in 5. There was full recovery by day 11PI.

The guinea fowls showed depression in 10% of the 40 infected on day 2PI. By day 4PI, 52% of them had paralysis of the legs. Some also showed jerking of the head, ataxia, recumbency and torticollis. The feathers were ruffled. There was drop in feed and water consumption and some had whitish-green diarrhea. On day 5PI the number of paralysed birds increased. Droopy wings occurred in 7 birds. Morbidity increased to 91% by day 6PI. Torticollis was seen in 9 birds. Nervous signs were prominent up to day 11PI when improvement started. Full recovery was observed on day 15PI. Weight loss was highly significant in infected chickens and guinea fowls from days 3 through to 21PI (p<0.05) (Table 1).

Mortality was first observed in chickens on day 4PI when 11 birds died. Peak mortality occurred on days 5 and 6PI involving 22 and 16 birds, respectively. Only 1 chicken died on day 8PI which was the last day mortality occurred. The first mortality in the guinea fowls was recorded on day 6PI when 4 birds died and the last was
Table 1: Mean body weights of birds (g±SEM)

<table>
<thead>
<tr>
<th>DPI (Group)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>10</th>
<th>15</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHC</td>
<td>455±13.84</td>
<td>548±13.87</td>
<td>645±20.09</td>
<td>688±19.38</td>
<td>790±23.33</td>
<td>888±31.14</td>
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<tr>
<td>CHI</td>
<td>475±14.43</td>
<td>398±8.17*</td>
<td>356±25.00*</td>
<td>375±28.97*</td>
<td>417±36.32*</td>
<td>483±22.09*</td>
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<tr>
<td>GFC</td>
<td>446±7.27</td>
<td>500±11.16</td>
<td>590±10.41</td>
<td>630±10.54</td>
<td>680±11.46</td>
<td>730±10.97</td>
</tr>
<tr>
<td>GFI</td>
<td>470±13.84</td>
<td>400±9.13*</td>
<td>360±12.47*</td>
<td>335±15.00*</td>
<td>403±17.66*</td>
<td>483±23.88*</td>
</tr>
</tbody>
</table>

Means with asterisks are highly significantly lower than their controls (p<0.05)

CHC: Chicken control
GFC: Guinea fowl control
GFI: Guinea fowl infected
SEM: Standard error means the mean values of control and infected birds

Table 2: Morbidity and Mortality in the infected groups of chickens and guinea fowl (%)

<table>
<thead>
<tr>
<th>Days PI</th>
<th>Chickens</th>
<th>Mortality</th>
<th>Depression</th>
<th>Mortality</th>
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</thead>
<tbody>
<tr>
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<td>0/160(0)</td>
<td>0</td>
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<tr>
<td>2</td>
<td>8/80(13.33)</td>
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<td>4/40(10)</td>
<td>0</td>
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<tr>
<td>3</td>
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<td>0</td>
<td>1/5(0)</td>
<td>0</td>
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<tr>
<td>4</td>
<td>5/65(7.59)</td>
<td>2/11.96(19.64)</td>
<td>1/6(52)</td>
<td>0</td>
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<tr>
<td>5</td>
<td>4/45(9.89)</td>
<td>2/4(4.44)</td>
<td>2/3(6.67)</td>
<td>0</td>
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<tr>
<td>6</td>
<td>2/23(01)</td>
<td>1/6(0.66)</td>
<td>2/03(001)</td>
<td>4 (11.11)</td>
</tr>
<tr>
<td>7</td>
<td>3/42(0.69)</td>
<td>2/03(001)</td>
<td>2/03(001)</td>
<td>4 (12.50)</td>
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<tr>
<td>8</td>
<td>1/4(25)</td>
<td>1/25</td>
<td>1/24(12)</td>
<td>0</td>
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<tr>
<td>9</td>
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<td>1/12(45)</td>
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<td>21</td>
<td>0/0(0)</td>
<td>0</td>
<td>0/12(45)</td>
<td>0</td>
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</table>

Total: 50/100 (50) 53/90 (60.0) 28/91 (29.9) 8 (22.2)

*: Number positive for depression
+: Total number of birds per group. It is also the number of birds remaining in a group when first and subsequent mortalities were observed

4 birds on day 7PI. The total mortalities were 22.2 and 94.6% in guinea fowls and chickens respectively excluding the sacrificed birds (Table 2) the uninfected groups showed no clinical signs.

Gross lesions: Infected chickens showed congestion of the muscles of breast, thigh and legs. Haemorrhages on the mucosa of proventriculus were prominent. In some cases the haemorrhages were linear at the proventriculus-oesophagus junction (Fig. 1). Sharply demarcated ulcers were observed in the ileum (Fig. 2) while the upper parts of the intestines had haemorrhagic or catarrhal enteritis. The ulcers were evident from the mucosal and serosal surfaces. The caecal tonsils were swollen, haemorrhagic and often contained cheesy necrotic materials. Samples of the spleen were initially enlarged, mottled with dark spots on the serosal surface and later atrophic (Fig. 3). The organ later regained its normal size. The thymus was atrophic (Fig. 4) and later could no longer be detected but gradually started regaining its normal size. The bursa was initially enlarged followed by atrophy (Fig. 5). The kidneys were enlarged, congested and haemorrhagic while the liver showed pale or parboiled colour. The distribution and frequency of the lesions are shown in Table 3.

Infected guinea fowls showed congestion of the muscles of the breast, thigh and legs. The spleen was initially swollen mottled and later atrophic. The thymus and bursa were atrophic (Fig. 6). Grossly there was marked congestion of the blood vessels of the brain.

Fig. 1: Haemorrhages on the proventricular mucosa of infected chickens that died on day 4PI. Note the linear lesion at the proventriculus-oesophagus junction (X)

Fig. 2: Haemorrhagic ulcers (U) on the mucosa of the ileum in infected chickens that died on day 4PI
The gastrointestinal tract was empty in most of the infected guinea fowls with evidence of catarrhal enteritis. Both the uninfected chickens and guinea fowls showed no lesion. The distribution and persistence of the lesions are shown on Table 4.

**Histopathology:** The microscopic lesions in the chickens and guinea fowls were similar but the lesions were more severe in the chickens. Thymus showed hyperaemia, severe necrosis and depletion of lymphocytes. Similar lesions were observed in the spleen. In addition there was deposition of fibrin around the sheathed arterioles. By day 15PI repopulation of the spleen with lymphocytes was almost complete and there was marked increase in the number of lymphoid follicles. The bursa also had severe necrosis and depletion of the lymphocytes. Follicular epithelium was hyperplastic, hyperaemia, balloonming degeneration (Fig. 7), intra and inter follicular oedema and cystic cavities were observed. By day 7PI, the follicles were atrophic in one chicken and two guinea fowls. Sections of the brain had severe hyperaemia, degeneration and necrosis of neurons, neuronophagia, gliosis, demyelination and endotheliosis (Fig. 8, 9). There was severe hyperaemia of the peritubular blood vessels in the kidney with degeneration and necrosis of the tubules. The liver in guinea fowls only showed hyperaemia of the hepatic vessels and necrosis of the

![Fig. 3: Atrophy of the spleen of infected chickens that died on day 4PI](image-url)
Table 6: Hemagglutination Inhibition antibody titers in infected chickens and infected guinea fowls

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<tr>
<th>DPI</th>
<th>Infected groups</th>
<th>Uninfected groups</th>
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<td>10</td>
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<td></td>
<td>Ch</td>
<td>GF</td>
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GMT: Geometric mean titer

CH: Chickens
GF: Guinea fowls

Infected Control

Fig. 4: Atrophy of the thymus of infected chickens that died on day 4PI

Infected Control

Fig. 5: Atrophy of the bursa of infected chickens that died on day 6PI

Serology: Infection with the virus lead to seroconversion on days 10-21PI (Table 5).

DISCUSSION

Based on the severity of the clinical signs, lesions and mortalities observed, the above results confirm the general belief that chickens are more susceptible and suffer more severe form of VND than guinea fowls (Kawamura et al., 1987; Alexander and Senne, 2003) with the virus used in the present study. To the best of our knowledge this is the first comparative study of VND in chickens and guinea fowls and should provide accurate information on the susceptibility or resistance of the two avian species to VND. The IM route of inoculation was used to ensure that the full dose of the inoculum got into the system of each bird infected. The main clinical sign of VND in guinea fowls was paralysis of the legs which was not common in chickens. The chickens showed mainly severe depression and diarrhoea. Paralysis has also been described as the main clinical manifestation of ND in double-crested cormorants (Kuiken et al., 1999), ostriches (Allwright, 1996; Huchzermeyer, 1996), turkeys (Piacenti et al., 2006), Japanese quails (Olaode et al., 2008) and pheasants (Muller et al., 1999). The lesions in the two species were also different in some organs. The guinea fowls showed no proventricular haemorrhage, intestinal ulcers, enlargement, necrosis and haemorrhages in the caecal tonsils which were very prominent in the...
Infected Control

Fig. 6: Severe atrophy of the thymus of infected guinea fowls that died on day 4PI

Fig. 7: Bursa of infected chicken sacrificed on day 3PI showing interfollicular oedema (O), lymphocytic depletion, necrosis, ballooning degeneration (B) hyperaemia (H) in the follicle on day 3PI. H and E X 400

Fig. 8: Hyperaemia (H) and demyelination (D) of the cerebellum of infected guinea fowl on day 6PI. H and E X 400

Fig. 9: Endotheliosis (E) in the cerebellum of infected guinea fowl on day 15PI. H and E X 400

earlier workers on VND (Hamid et al., 1991; Brown et al., 1999; Okoye et al., 2000; Ezema et al., 2008). Natural outbreaks of ND have been reported in guinea fowls by some workers (Crawford, 1931; Moine, 1950; Balarini, 1984; Durojaye and Adene, 1988; Okaeme et al., 1988; Haruna et al., 1993). Agoha et al. (1992) studied experimental ND in guinea fowls using chicken strains of NDV while Mishra et al. (2001) studied the pathogenicity of NDV in guinea fowls using chicken and guinea fowl isolates of the virus. They reported incubation period of 5 days, depression and leg paralysis. The clinical signs seen in the guinea fowls in this study are similar to those described by Okaeme (1988), Agoha et al. (1992) and Mishra et al. (2001). While total mortality in this study for guinea fowls was 22.2%, Agoha et al. (1992) observed a mortality of 100%, Haruna et al. (1993) 21.3% while Mishra et al.
(2001) recorded 8 and 52% with guinea fowl and chicken isolates respectively. Our mortality and that of Mishra et al. (2001) did not include sacrificed birds. In this study the incubation period of VND in guinea fowls was 2 days PI, mortalities occurred on days 6 and 7 PI only. By day 16 PI depression was no longer observed. But Mishra et al. (2001) reported peak mortalities on days 9 and 11 PI in infected guinea fowls. Recovery from depression occurred on day 14 PI. The incubation period for chickens in our study was 2 days PI. This is in agreement with the observations of Paredes and Young (1960), Ojok and Brown (1996), Galindo-Muniz et al. (2001) and Kommer et al. (2003) in infected chickens. In this study, mortalities started in chickens on day 4 PI, peaked on days 5 and 6 PI. Recovery occurred on day 9 PI. But Mishra et al. (2001) observed that mortalities in chickens started on day 5 PI, peaked on day 12 PI and recovery from clinical signs and death occurred on day 14 PI. Okoye et al. (2000) also reported that mortalities started in chickens on day 5 PI, peaked on days 7 and 8 PI and by day 18 PI, only torticollis persisted in 3 surviving birds. The differences between our observations and those of some other workers could be due to the variation in the dose and virulence of the inoculum and the breeds of birds used.

The microscopic lesions observed in the bursa and spleen in this study are similar to those described by Mishra et al. (2001), but the gross haemorrhage they reported in the proventriculus and caecal tonsil of infected guinea fowls were not seen in this study and that of Agohia et al. (1992). Ezerna et al. (2009) described ballooning degeneration in the bursa of chickens infected with VNDV strain VGF-1 and proposed that the lesion might be pathognomonic for VND. The lesion was also seen in this study in both chickens and guinea fowls. Similar lesions were shown in geese by Wan et al. (2004). The microscopic lesion observed in the brain in this study were also described by Agohia et al. (1992) and Mishra et al. (2001) in guinea fowls, Kuiken et al. (1999) in double-crested cormorants, Wan et al. (2004) in geese and Okoye et al. (2000) in chickens. The gross and microscopic lesions of VND in the bursa, thymus, spleen, proventriculus and caecal tonsil closely resemble those of infectious bursal disease (IBD) of chickens (Okoye and Uzosukwu, 1990) and highly pathogenic avian influenza (H5N1). They show the highly lymphocoidal nature of the three diseases. But while the VND often produces longitudinal or linear haemorrhage on the mucosa of the proventriculus-oesophagus junction, IBD often produces similar lesion at the proventriculus-izzard junction.

The inoculation of the chickens and guinea fowls with NDV KUDU-113 strain lead to sero conversion in chickens and the guinea fowls from days 10-21 PI. The HI antibody titres in chickens were higher than those of the guinea fowls because the disease was more severe in chickens which had four times more mortalities. Saidu et al. (2004) in a field survey of semi-domesticated birds in Zaria area of Nigeria reported 92% positive for ND HI antibody with mean titre of 7.3 log2; in chickens while 76% was positive in guinea fowls with mean titre of 4.1 log2. In a similar survey in Jos area of Nigeria, Mai et al. (2004) reported 6.7% positive for ND HI antibodies in ducks and 13.6% positive in guinea fowls. The difference between the susceptibility or resistance of guinea fowls and chickens to VND could be genetic. Hassan et al. (2004) observed variation in the genetic resistance of four breeds of native Egyptian chickens to VND and IBD. Variation in the genetic susceptibility to important viral pathogens of poultry including Marek’s disease virus, leucovirus, IBD virus and infectious bronchitis virus has been reported (Bumstead, 1998).

REFERENCES


