Susceptibility of Japanese quails (*Cortunix cortunix japonica*) to experimental infection with Newcastle disease virus, Kudu 113 strain

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Abstract

This study was carried out to determine the response of Japanese quails experimentally infected with Newcastle disease virus (NDV) kudu 113 strain using a haemagglutination inhibition test and the ability of the species to transmit the infection to chickens. The administration of kudu 113 strain of Newcastle disease virus (10^8.5 /ml) orally at 0.1ml/quail in the infected group (group B) resulted in an antibody response with a geometric mean titre of 23.79 on day 32 when compared to non-infected quails (group A) which did not show (p>0.05) evidence of Newcastle disease antibodies throughout the experiment and also differed significantly (p<0.05) from group B, indicating that oral inoculation of the virus was successful and the birds were infected. Clinical signs of ND were first observed in the quails 7 days post-infection (pi) with effects on egg production and egg quality. The transmission of the velogenic NDV from the quails (group B) to the sentinel chickens was clinically evident 4 days after they were placed in close contact with the infected quails. There was 100% mortality in the sentinel chickens between 4 to 7 days post contact. Thus, quails could serve as a potential source of ND for chickens.

Keywords: Antibodies, Japanese quails, Newcastle disease, Newcastle disease virus, In-contact chickens

Introduction

Newcastle disease (ND) occurs in avian species and can have a devastating economic effect on domestic poultry production globally (Brown & Bevins, 2017). Most commonly affected species include chickens, turkeys, ducks, pigeons (Zhang *et al*., 2011), guinea fowl, Japanese quail and many wild birds of all ages (Nanthakumar *et al*., 2000). It is caused by virulent strains of Newcastle disease virus (NDV) which is an
enveloped virus within the avula virus. It is synonymous with avian paramyxovirus type 1 (APMV-1) but due to changes in taxonomy is now referred to as avian avulavirus (Amarasinghe et al; 2017). Virulent strains of NDV, typically mesogenic and velogenic, are endemic in most parts of Asia, Africa, and the Middle East as well as Central and South America in domestic poultry species (CFSHP, 2016). Newcastle disease virus is primarily transmitted via inhalation or ingestion of the virus shed in faeces and respiratory secretions by infected birds for a period of time (Leighton & Heckert 2007; CFSHP, 2016). Clinical signs depend on factors such as the virus strain, host species, host age, co-infection with other microorganisms, environmental stress, and immune status (Al-Habeeb et al., 2013). This work was designed to determine the response of quails to experimental infections with NDV through the clinical manifestation of the disease, their ability to infect other species of birds and changes in the haemagglutination inhibition (HI) antibody titres.

**Materials and Methods**

The study was performed at the Poultry Research Unit of the Department of Veterinary Medicine, University of Maiduguri. Forty-day-old Japanese quail chicks of both sexes were obtained from the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria.

**Housing**

Mesh cages (tagged A and B) of 120 × 140 × 120 cm in size in different rooms of an enclosed house were used to house the quails. The litter was changed once a week throughout the period of the experiment. The quails of each group were attended by a different attendant to prevent transmission of disease.

**Feeds, feeding and feeds analysis**

The quails were fed with a pelleted chick mash and layer mash (Vital Feed®) manufactured by Grand Cereals Limited, Jos, Plateau State at ad libitum.

**Challenge virus**

The virus used for the experimental infection was a velogenic local field isolate of the ND virus (Kudu-113 strain) and was characterized biologically by Echeonwu et al. (1993). The inoculum had a median embryo lethal dose (ELD<sub>50</sub>) of 10<sup>9</sup> per ml. The virus was considered velogenic because of the following pathogenicity indices: intracerebral pathogenicity index, 1.56; mean lethal dose, log 8.00; mean death time, 49.60 hours; intravenous pathogenicity index, 2.18; embryo infective dose 50% endpoint per ml, 8.46. The vial contains 1ml of the viral inoculum. Challenge birds (group B) were inoculated with 0.1 ml per os according to the manufacturer’s recommendation.

Ten healthy cockerels aged 5 weeks old were raised in isolation under standard management with no history of vaccination and treatment. Five birds were placed in contact with the challenged quails and the remaining five birds were kept as control. The five in-contact cockerels were checked for the manifestation of clinical signs of ND and a post-mortem was conducted on the dead carcasses. They were not subjected to HI test.

**Sample collection**

About 2 ml of blood were collected from experimental quails at 28, 35, 42, 49 and 53 days of age via the wing vein for determination of antibody titre levels to Newcastle disease (ND). Following disinfection, blood was collected using a 5 mL syringe and a 23-gauge needle. The collected blood was poured into a screw-capped plain sample bottle without anticoagulant, kept on a table at room temperature for sixty minutes and centrifugated. The serum was carefully removed using a Pasteur pipette and transferred into a 2ml plain serum bottle made of plastic and stored in a freezer at -20ºC until used.

**Chicken erythrocyte suspension**

Blood from adult susceptible chicken (9 ml collected at an interval of 12 hours) was collected through the wing vein puncture on 4% sodium citrate (1 ml of anticoagulant + 9 ml blood). An equal amount of 25 µl buffered physiological saline was added to the suspension and the cells were subjected to 3 cycles of washing and centrifugation in physiological saline at 800 rpm for 15 minutes. The packed red cells were then diluted in saline to make 1% suspension for haemagglutination (HA) and haemagglutination inhibition (HI) tests.

**Preparation of a 4 HA (haemagglutination) antigen**

This was done according to the protocols of Allan & Gough (1974) as modified by Baba et al. (1999). A freeze-dried ND Lasota vaccine obtained from National Veterinary Research Institute, Vom, Nigeria, was used as the antigen. It was dissolved in 2ml sterile NSS with a sterile syringe and needle for the haemagglutination test. A twofold serial dilution of
the antigen was carried out in a U-shaped microtitre plate. About 25µl of the antigen was added to the first well on the row containing 0.025ml of normal saline. The mixture was looped out to well 2 through well 11 while well 12 served as control using a multi-channel pipette. Additional 0.025ml of normal saline was added to all the wells. Also, 0.05ml of 0.9% chicken red blood cells (RBC) were added to all the wells. The microtitre plate was incubated at room temperature for 45 minutes. Positive wells were identified by the formation of a uniform carpet of RBCs, that forms a button at the bottom of the wells. The last well that showed haemagglutination was considered to contain a haemagglutination unit, to arrive at a working dilution. The titre of such wells was divided by 4.

Haemagglutination inhibition (HI) test
On days 28, 35, 42, 49 and 53 HI tests were performed on 4 birds of each group as described by Grimes (2002) using the Beta procedure. The steps were as follows: an equal volume of physiological saline (25 microliters µl) was added throughout the wells (1-12). A test serum was added to well 1 then 2-fold diluted up to well 11. About 50 (µl) of 4 haemagglutinations (4HA) units of NDV antigen were added up to well 11 and incubated at room temperature for 30-40 minutes. Thereafter, a 1% chicken RBC suspension was added to all the wells (1-12) and incubated for another 40 minutes. All samples were tested in duplicate. The wells were read. Positive and negative serum controls were included on each plate. The HI titre was determined as the highest dilution of serum causing complete inhibition of haemagglutination.

Table 1: Clinical signs of Newcastle disease observed in experimentally infected quails and in contact chickens

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Groups</th>
<th>A</th>
<th>B (4 pi) (%)</th>
<th>In contact chickens (3 and 7 pi) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia</td>
<td>-</td>
<td>11 (55%)</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Depression, weakness, Somnolence</td>
<td>-</td>
<td>17 (85%)</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Rales</td>
<td>-</td>
<td>5 (25%)</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Ruffled feathers</td>
<td>-</td>
<td>17 (85%)</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Coughing &amp; sneezing</td>
<td>-</td>
<td>10 (50%)</td>
<td>4 (80%)</td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>-</td>
<td>6 (30%)</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Recumbency</td>
<td>-</td>
<td>10 (50%)</td>
<td>4 (80%)</td>
<td></td>
</tr>
<tr>
<td>Dropped wing and leg</td>
<td>-</td>
<td>4 (20%)</td>
<td>3 (60%)</td>
<td></td>
</tr>
<tr>
<td>Torticollis</td>
<td>-</td>
<td>4 (20%)</td>
<td>2 (40%)</td>
<td></td>
</tr>
<tr>
<td>Ataxia</td>
<td>-</td>
<td>4 (20%)</td>
<td>2 (40%)</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>-</td>
<td>1 (5%)</td>
<td>5 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

*Pi = Post-infection

Clinical parameters
Clinical signs, morbidity, mortality, post-mortem lesions and performance in terms of egg production were observed daily and recorded in all the experimental quails following inoculation with kudu 113 ND virus.

Statistical analysis
The data obtained were subjected to descriptive statistics. Data obtained from HI test were converted to geometric mean titre (GMT) values using the formula:

\[ X_{geo} = \text{antilog} 10 \left\{ \frac{1}{n} \left( \sum f \log_{10} X_i \right) \right\} \]

where \( f_i = \text{frequency} \) and \( X_i = \text{reciprocal of dilution} \) and \( f_i = \text{frequency} \). They were subjected to repeated measure one-way analysis of variance (ANOVA) to determine the difference in the parameters between the experimental groups. This was followed by Tukey’s post-hoc multiple comparison tests using Graph-pad prism version 4.0 for windows. Values of \( P<0.05 \) were considered significant. Some results were expressed in percentages (%).

Results
Quails in group A (negative control) showed no clinical signs of ND with normal feeding and physical activities. Quails in group B (infected), developed clinical signs which started manifesting on day 7 (Table 1). The first signs were decreased activity and decreased feed intake in all quails. Other signs observed were dullness, depression, huddling, incoordination, somnolence and ruffled feathers in 85% of the quails on day 10 pi. Mortality recorded was only one. At necropsy, the carcass of the infected quail was dehydrated and the muscle of the thigh and legs were congested. Inflammatory and haemorrhagic lesions were seen in the respiratory system. The air sacs were cloudy and the lungs oedematous. The liver, spleen, kidneys and heart showed haemorrhagic spots on the serosal surface. Multifocal pinpoint haemorrhages were seen on the proventriculus and the intestines were congested. Egg
production was also observed and recorded. Generally, egg production started on day 44 with 4 eggs per day in group A which increased steadily to a peak of 23 eggs per day by day 60. Whereas, in the infected group B, there was a delay in egg production. The quails started laying at day 46 with 3 eggs per day which reached a peak of 18 eggs by day 60 (Table 2).

**Table 2: Effects of Newcastle disease on egg production in quails**

<table>
<thead>
<tr>
<th>Days</th>
<th>A (Uninfected) %</th>
<th>B (Infected) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>39</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>44</td>
<td>4 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>46</td>
<td>2 (10)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>52</td>
<td>5 (26)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>58</td>
<td>6 (30)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>60</td>
<td>6 (30)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>23</td>
<td>18</td>
</tr>
</tbody>
</table>

The eggs of group B birds appeared misshapen, smaller in size, cracked and shell-less (Plate I). The clinical signs observed in the in-contact chickens (5 cockerels) included loss of appetite, dullness, weakness, somnolence, ruffled feathers, huddling, torticollis (Plate II) and death of 3 (60 %) chickens on day 4. Other signs observed were respiratory signs, including sneezing, coughing, swollen face, and drooling salivation. Nervous signs with loss of balance, torticollis, convulsive spasms, twisted neck, wings and leg (Plate III) were also observed. The digestive signs included diarrhea. Mortality was recorded in three out of the five birds on day 4 of coming into contact with the infected quails and the remaining 2 died on day 7 post-exposure. At necropsy, there were congestion on the breast and thigh muscles (Plate IV) ecchymoses and haemorrhages in the caecal tonsils (Plate V) and the proventriculus has multifocal pinpoint haemorrhages (Plate VI). The lungs were haemorrhagic, congested, and oedematous with areas of necrosis (Plate VII).
The geometric mean titre (GMT) of ND antibodies of different experimental groups is as presented in Figure 1. On day 28 (at 4 weeks of age) no antibody titre was detected in both experimental groups. Quails in group A (negative control) revealed no noticeable antibodies against NDV on days 28, 32, 39, 46 and 53. Quails in group B revealed 0.00, 23.79, 29.25, 25.4, and 22.63 % seropositivity in GMT of HI antibodies at 28, 32, 39, 46, and 53 days of treatment, respectively (Table 3).

**Discussion**

The administration of NDV per os (po) in group B quails resulted in an obvious antibody response with a mean titre of (29.25) on days 39 (11 days post-infection) (pi) when compared to uninfected birds in group A (0.00). The findings of this study showed a significant difference (P<0.05) in ND antibody titre between quails in groups A and B which demonstrated that inoculation of the virus was successful in the infected quails. After day 11 pi, there was an insignificant rise in antibody
Plate IV: Breast and thigh muscle of a dead chicken in the In-contact group. Note the congestion and haemorrhages in the breast and thigh muscle (Thin and thick arrow).

Plate V: Caecal tonsil of a dead chicken from the In-contact group. Note the haemorrhage of the caecal tonsil (Yellow arrow).

Plate VI: The proventriculus of dead chicken from the in-contact group. Note the multi-focal pinpoint haemorrhages (Black arrow).

Plate VII: The lungs of a dead chicken from the in-contact group. Note the necrosis (blue arrow), inflammation (generalised) (green arrow) and haemorrhages (Orange arrow).

Table 3: Mean ND antibody titres of experimental group A quails as compared with infected group B

<table>
<thead>
<tr>
<th>Groups/treatment regimen</th>
<th>Days of treatment</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Control</td>
<td></td>
<td>0.0</td>
<td>± 0.0</td>
<td>± 0.0</td>
<td>± 0.0</td>
<td>± 0.0</td>
</tr>
<tr>
<td>B: Infected</td>
<td></td>
<td>± 0.0</td>
<td>± 0.0802*</td>
<td>± 0.663*</td>
<td>± 0.2123*</td>
<td>± 0.1245*</td>
</tr>
</tbody>
</table>

titre observed in group B which could be due to slow immune response of the quails as previously reported by (Mishra et al., 2001; Barbezange & Jestin, 2003). This may also be attributed to the resistant nature of the quails to NDV. The clinical signs observed in the infected group B on day 7 Pi were consistent with the findings of Atikah et al. (2015) who reported clinical signs of depression, anorexia, torticollis, coughing...
and sneezing, diarrhoea, recumbency, wing and leg paralysis, and ataxia from days 6 to 9. This study demonstrated the ability of quails to transmit ND to other species of birds by the in-contact group where all the 5 in-contact chickens got infected and manifested classical clinical signs of NDV. Therefore, frequent contact between quails and chickens should be avoided even after vaccination. The incubation period for NDV in this study is much longer than the 2 to 3 days normally observed in chicken and guinea fowl (Alexander, 2000). Only 1 mortality was recorded in the infected quails. These differences observed, could be due to host species differences in response to NDV and the virulence of NDV strain, as previously reported by Alexander (2000) and Maw et al. (2003). Quails in group B showed some clinical signs indicating pathogenicity, which started on day 7 with decreased activity, dullness and drop in feed intake, somnolence, torticollis, ataxia, and death. These findings agree with a previous report by Igwe et al. (2014) who detected clinical signs such as ruffled feathers, depression, reduction in feed intake by day 2 PI, and that of Usman & Diarra (2008) who reported that the major clinical signs in quails infected with NDV were dullness, ruffled feathers, respiratory and neurological signs. However, the findings by Lima et al. (2004) contradict the findings in this study by reporting that they did not observe any clinical signs of NDV in quails. The low production with poor quality of eggs seen in the infected group is one of the important effects of ND. This finding agrees with those of Okoye et al. (2000) and Abdu et al. (2004) who reported a marked decrease in egg production both in size and quantity in laying hens infected with ND. The ability of the quails to transmit NDV to in-contact chickens was demonstrated through clinical signs and post-mortem lesions shown by the chickens. Quails should be routinely vaccinated against ND especially those kept close to chickens to avoid transmissions of the NDV.

Figure 1: Geometric Mean Titre (GMT) of Quails in Groups A (controlled) and B (infected)

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Conflict of Interest
The authors declare that there is no conflict of interest.

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