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

SH Mshelia[1]*, UI Ibrahim[2], SU Hassan[3], KD Malgwi[1], S Jauro[4] & N Daniel[5]

[1]. *Veterinary Teaching Hospital, University of Maiduguri, Nigeria*
[2]. *Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria*
[3]. *Department of Veterinary Pathology, Faculty of Veterinary medicine, University of Maiduguri, Nigeria*
[4]. *Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria*
[5]. *Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria*

*Correspondence: Tel.: +234703819025; E-mail: sarayamshelia@gmail.com

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.

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 micro-organisms, 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 pelletized chick mash and layer mash (Vital Feed®) manufactured by Grand Cereals Limited, Jos, Plateau State at 1-28 and 29-60 days, respectively. Proximate analysis of the feed was carried out at the Feed Analysis Laboratory of the Department of Animal Science, the University of Maiduguri, to affirm the level of metabolizable energy, crude protein, crude fibre, moisture, ash content and dry matter. Feed and water were provided *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_{50}) of 10^9 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 0.1m 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 centrifuged. 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.

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:
$X_{geo} = antilog10 \{1/n (\Sigma filog10Xi)$
where fi=frequency and Xi=reciprocal of dilution and fi=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, in-coordination, 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

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

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

*Pi = Post-infection

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

Days	A ``(Uninfected) %	B (Infected) %
35	0 (0)	0 (0)
39	0 (0)	0 (0)
44	4 (0)	0 (0)
46	2 (10)	3 (15)
52	5 (26)	5(25)
58	6 (30)	5(25)
60	6 (30)	5(25)
TOTAL	23	18

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 diarrhoea. 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).
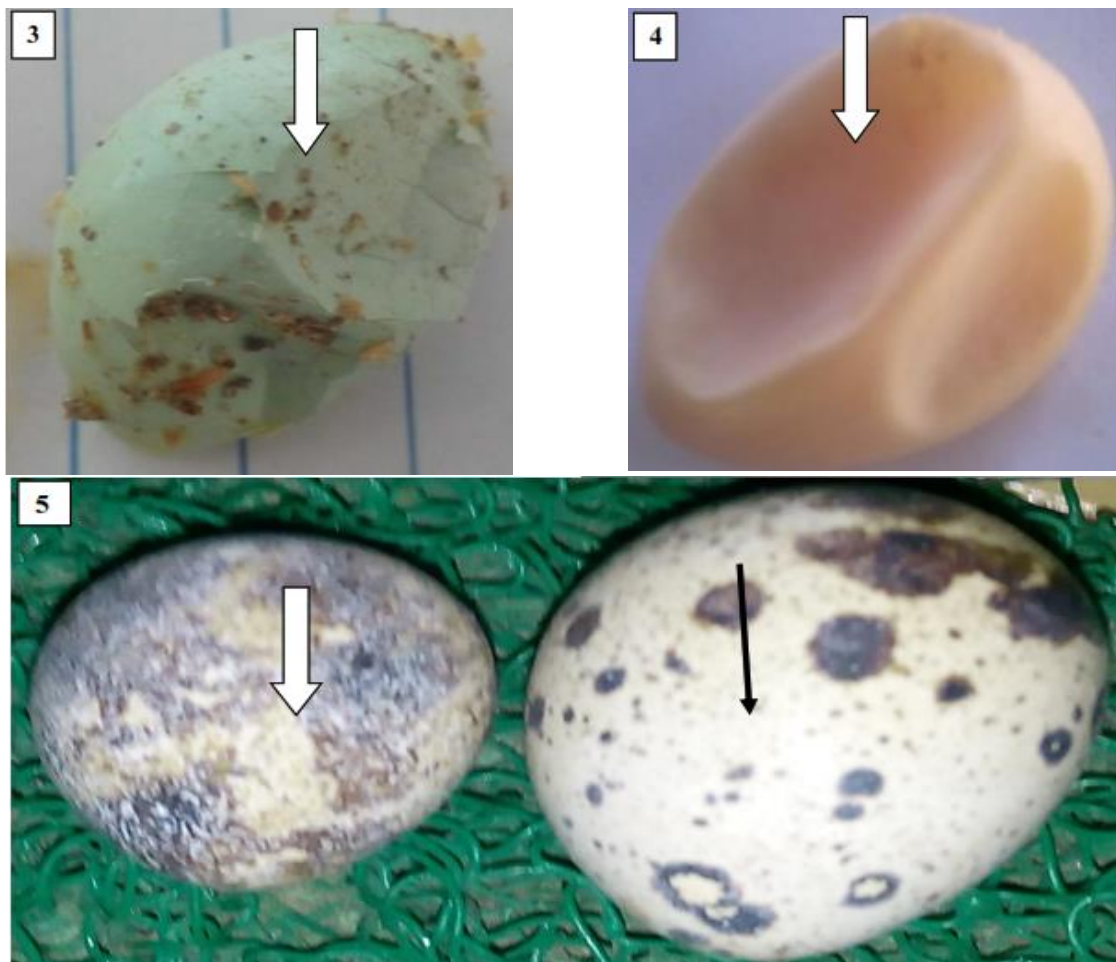

Plate I: Photograph of quail eggs in the infected group B and uninfected group A. Note the cracked and soft-shelled egg (3), misshapen, shelless and rubber-like egg (4), small size (thick arrow) (5) and a normal size (thin arrow) (5)
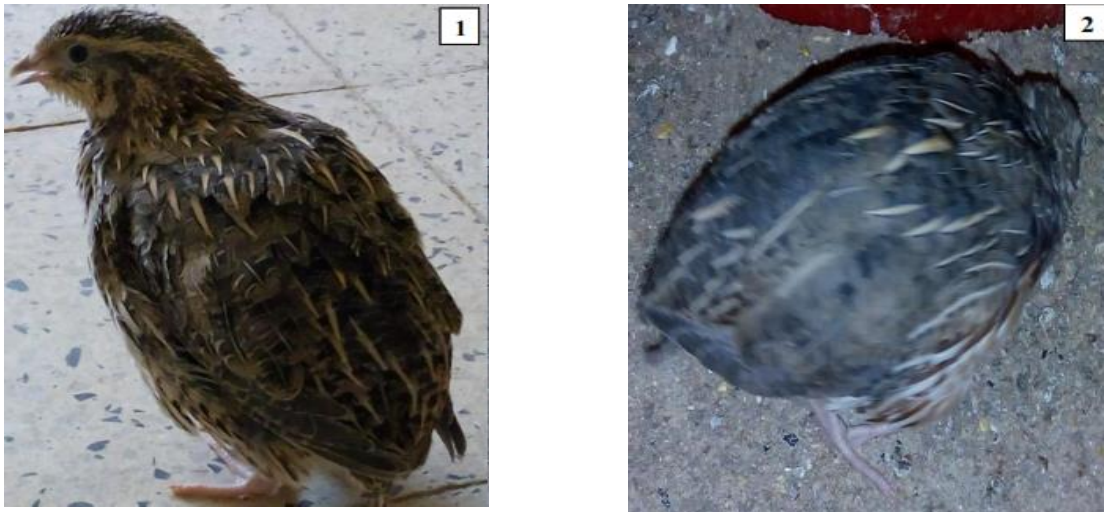
Plate II: Photograph of the infected quails in Group B
Note the manifestation of ruffle feathers, weakness (1), dropped wings, depression and torticollis (2)

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 tire between quails in groups

Plate III: Photograph of the in-contact chickens. Note the signs of weakness and ruffled feather (black arrow), nasal discharge and drooling saliva (yellow arrow), paralysis of the legs and wings (green and black arrow respectively)

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
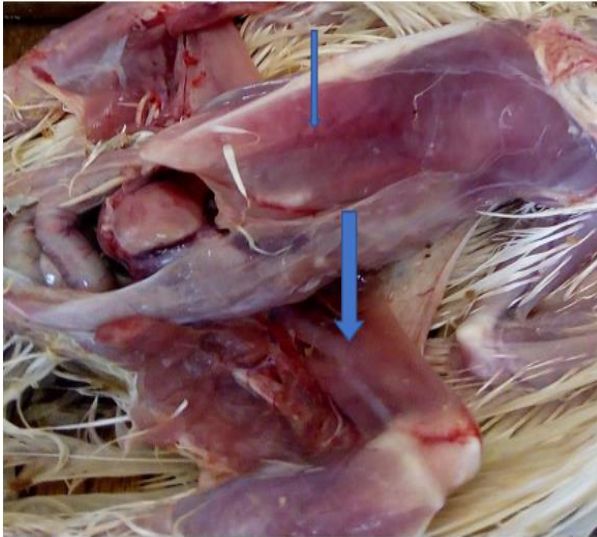
Plate IV: Breast and thigh muscle of a dead chicken in the In-contact group. Note the congestion and haemorrhages in the breast and thigh muscle (Thin and thick arrow)

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)
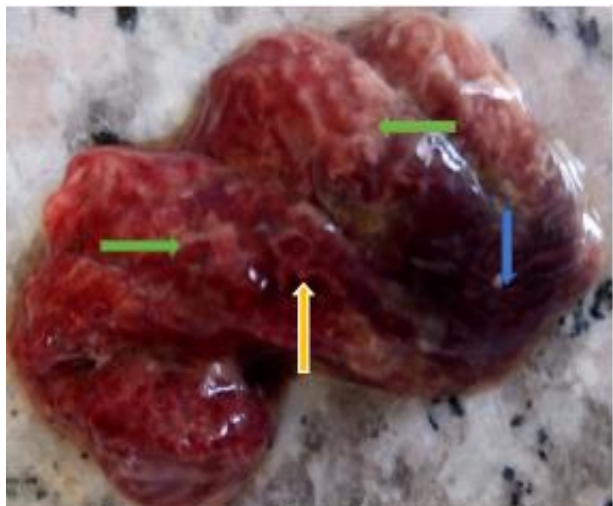

Plate VII: The lungs of a dead chicken from the in-contact group. Note the necrosis (blue arrow), inflammation (generalised) (green arrow) and haemorrhages (Orange arrow)

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

Groups/treatment regimen	Days of treatment				
	3	7	14	21	28
A: Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
B: Infected	0.0 ± 0.0	23.79 ± 0.0802*	24.25 ± 0.663*	25.40 ± 0.2123*	22.63 ± 0.1245*

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

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

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 direct contact (inhalation), which Lima *et al* (2004) considered an important carrier of ND virus. The clinical signs observed coupled with 100% mortality of the in-contact chickens were suggestive of NDV infection. This finding is in agreement with Lima *et al*. (2004), who demonstrated the ability of the Japanese quails to transmit NDV to specific pathogen-free (SPF) chickens. The in-contact chickens showed classical signs of NDV characterised by greenish diarrhoea, weakness, nasal discharges, drooling saliva, dyspnea
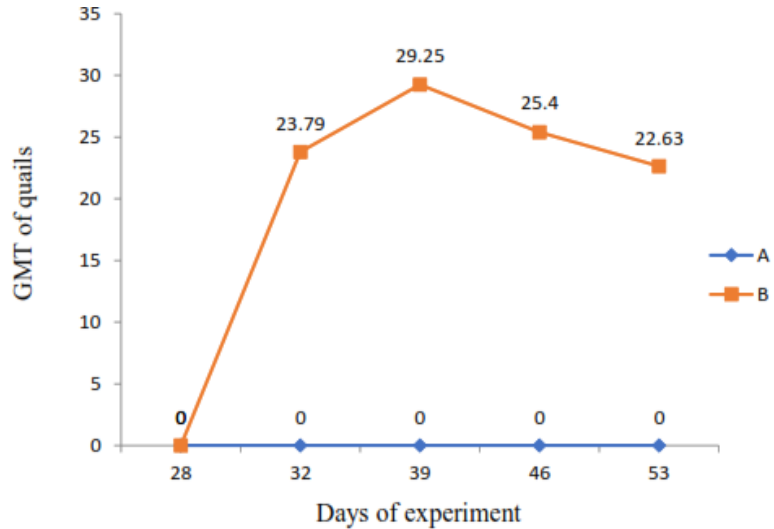
and nervous signs as reported by Chiville *et al.* (1972), Kowenhoven (1993) and Alexander (2003) but less frequently seen in other species (Haruna *et al.,* 1993). The diarrhoea observed in group B quails and in contact chickens could be associated with gastrointestinal lesions. NDV is said to have a predilection for intestinal epithelium, endothelium and lymphoid tissues, especially in the GIT (Hassan, 2007) and (Susta *et al.,* 2018). In this study, evidence of ND infection was observed in the quails by the rise of antibodies and demonstration of clinical signs. Serological evidence of increasing NDV antibodies after infection of the Japanese quails was also observed. It was concluded that Japanese quails are susceptible to NDV Kudu 113 strain. The ability of the quails to transmit NDV to in-contact chickens was demonstrated through clinical signs and pm lesions shown by the chickens. Quails should be routinely vaccinated against ND especially those kept close to chickens to avoid transmissions of the NDV.

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

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