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Changes in Histopathology, Haematocrit, Haemoglobin, Haemagglutination Inhibition Antibody Titre and Total Protein of Japanese Quails (*Coturnix coturnix japonica*) Administered Different Doses of Newcastle Disease Virus

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Abstract: Experiments were performed to determine changes in Histopathology (HP), Haematocrit (HT), Haemoglobin (HB), Haemagglutination Inhibition (HI) antibody titre and total protein (TP) of Japanese quails (*Coturnix coturnix japonica*) administered different doses of Newcastle disease virus (NDV) Kudu 113 strain through intramuscular (im) route or per os (po). The values of HT,HB,HI and TP were determined by standard laboratory procedures. The results showed that some of the infected quails developed Newcastle disease (ND) with classical clinical signs and gross lesions of the disease. Histopathological lesions observed include focal necrosis, mononuclear cells infiltration and depletion of lymphoid tissues. There was a reduction in values of HT and HB in the infected quails, when compared with the corresponding mean values obtained in the control group. Following infection of quails with NDV Kudu 113 strain, there was a rise in HI antibody titre from zero to maximum mean antibody titres of $\log_2 10.56 \pm 0.29$ and $\log_2 9.89 \pm 0.48$ in quails administered 0.3 mL of the virus im and po, respectively on day 7 post infection (pi). There was a slight reduction in TP concentration in all the infected groups, irrespective of the route of administration, when compared with the mean value obtained in the control group. It was concluded that vital body parameters were altered in quails infected with NDV Kudu 113 strain.

Key words: Haemagglutination, histopathology, haematocrit, protien, newcastle disease virus

INTRODUCTION

Quail production is sharply on the increase throughout the world, as it is appreciated as a good supplier of eggs and meat. Studies have shown that quails can easily adapt to commercial management conditions with good performance in terms of meat and egg production. This is as a result of improvement in the genetic quality of the bird, which led to better productivity (Haruna *et al*., 1997 a, b; Lombin, 2007).

Newcastle disease (ND) which is caused by Newcastle disease virus (NDV) is a major disease problem of poultry in many countries of the world, especially in Africa and Asia (Spradbrow, 1992; Awan *et al*., 1994; Oladele *et al*., 2005).

Since, 1953 when ND was first documented in Nigeria (Hill *et al*., 1953), the disease has become a threat to poultry industry in Nigeria, where it causes havoc to different species of birds, all the year round.

The present interest in ND in what appears to be an existing problem follows the report that Japanese quails (*Coturnix coturnix japonica*) could harbour NDV in their systems without showing any clinical signs of ND (Lima *et al*., 2004). It therefore means that vital body parameters of the quails could also be altered unnoticed, when NDV circulates in the systems of the quails.

Changes in values of vital body parameters, such as the haematocrit, haemoglobin, haemagglutination inhibition antibody titres and total protein concentrations, during infection of susceptible birds with pathogenic agents, like NDV could be a reflection of pathological changes, in the body of the bird, which could be useful in assessing the health status of he bird. For example, haematocrit value is a good indicator of the bird's haemogram, because it is an index of the number of circulating red blood cells. It is also an indirect index of circulating haemoglobin. Reduced total protein concentration is frequently observed in hepatic or renal diseases and malnutrition (Campbell and Coles, 1986), while the haemagglutination inhibition antibody titres to specific agents can be used to assess the immune status of the bird (Beard, 1989) whenever it is required.

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Therefore, sequential determination of the changes which take place in these vital body parameters during NDV infection could offer valuable information for the assessment of health status of birds.

Since, it is generally believed that quails can harbour NDV in their systems without showing any clinical signs of ND, information on the changes in some vital body parameters of quail during NDV infection is very scarce. Therefore, this study was designed to determine changes in histopathology, haematocrit, haemoglobin, haemagglutination inhibition antibody titre and total protein of quails infected with NDV Kudu 113 strain. To our knowledge, this is the first report on these parameters in quails infected with this NDV strain.

MATERIALS AND METHODS

Site of experiment: The experiment was performed in the Faculty of Veterinary Medicine, Ahmadu Bello University, Samaru, located within Zaria metropolis (11°10' to 07°38'E), in the Northern Guinea Savannah zone of Nigeria.

Experimental birds: A total of 119 Japanese quails (*Coturnix coturnix japonica*) were obtained at 6 weeks old from National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The quails were randomly selected into seven groups of 17 quails per group. Quails in each group were kept in different cages. Water and feed were supplied *ad libitum*.

Newcastle disease virus: Newcastle disease virus Kudu 113 strain was obtained from NVRI. The virus has been classified as velogenic NDV by Echeonwu *et al*. (1993).

Challenge of the birds: At 6 weeks of age, the quails were administered different doses of NDV Kudu 113 strain through intramuscular route (im) or per os (po) as follows:

- Group 1 was administered 0.1 mL of the virus po.
- Group 2 was administered 0.2 mL of the virus po.
- Group 3 was administered 0.3 mL of the virus po.
- Group 4 was administered 0.1 mL of the virus im.
- Group 5 was administered 0.2 mL of the virus im.
- Group 6 was administered 0.3 mL of the virus im.
- Group 7 was the control group. No virus was administered to quails in this group.

None of the quails both in the infected or control groups had previously received any NDV vaccines.

Clinical and pathological examinations: Quails in all the experimental groups were observed daily for clinical signs. Dead quails were examined for gross lesions. Samples of the spleen, liver, heart, kidney, brain, lungs, intestine and proventriculus 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 the light microscope for any histopathological lesions.

Blood sampling: Blood sampling was through wing venepuncture, using 25 gauge sterile hypodermic needles and syringes. Blood samples were collected on each day of the experiment from quails in the seven groups.

Blood for haematological values were collected into labeled Bijou bottles, containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Serum samples for HI analysis were taken without anticoagulant. The serum samples were separated by centrifugation at 1,000 g for 10 min and stored frozen in plastic vials until laboratory determinations were made.

Haematocrit, haemoglobin and total protein determination: Haematocrit and haemoglobin concentrations were determined by the method of Benjamin (1985): Blood samples, containing EDTA were aspirated into a set of plain capillary tubes. The tubes were sealed and then placed on Hittich centrifuge and spun at 5,000 g for 5 min. After 5 min, the tubes were removed and the haematocrit values were read as a percentage directly from graphic reader. Total protein concentrations were also determined by refractometric methods of Benjamin (1985).

Serology: Newcastle disease virus haemagglutination inhibition antibody quantification was done using haemagglutination and HI procedures of Beard (1989). Two-fold dilutions of 50 μL of the sera were made in phosphate buffered saline (pH 7.2) and 4 haemagglutination (4HA) units of NDV La Sota as antigen in 50 μL were added. Thereafter, 50 μL of 1% erythrocytes suspension was added to each well of the microtitre plate and left for 45 min at room temperature (26-30°C). The HI titre for each serum sample was determined at the highest dilution of serum which causes complete inhibition of 4HA units.

Statistical analysis: All the data obtained were subjected to statistical analysis using Student's t-test analysis. Values of ($p<0.05$) were considered significant.

RESULTS

Table 1 shows that there was a gradual reduction in haematocrit values as the days of infection progresses, in both the quails that were administered NDV Kudu 113 strain either im or po, when compared with the mean

Table 1: Changes in haematocrit values of quails administered different doses of NDV Kudu 113 strain through different routes (Mean±SEM)

	Route of administration	
Dose of NDV (mL)	IM	PO
	4 Days post infection	
0.1	38.89±1.35[a] (n = 17)	39.67±1.79[a] (n = 17)
0.2	38.11±1.47[a] (n = 17)	38.33±1.50[a] (n = 17)
0.3	35.33±2.47[a] (n = 17)	35.89±2.62[a] (n = 17)
	7 days post infection	
0.1	38.55±1.90[a] (n = 17)	38.88±2.08[a] (n = 17)
0.2	38.56±1.45[a] (n = 17)	37.56±0.71[a] (n = 17)
0.3	35.78±3.48[a] (n = 17)	36.89±1.65[a] (n = 17)
	14 days post infection	
0.1	34.33±4.56[a] (n = 17)	37.22±3.17[a] (n = 17)
0.2	33.77±4.94[a] (n = 17)	35.11±3.68[a] (n = 17)
0.3	30.11±3.25[b] (n = 17)	33.00±3.88[b] (n = 17)
	21 days post infection	
0.1	35.33±2.25[a] (n = 17)	32.11±2.34[a] (n = 17)
0.2	32.33±2.93[a] (n = 17)	37.11±3.66[b] (n = 17)
0.3	30.22±1.74[b] (n = 17)	31.33±3.60[a] (n = 17)
	28 days post infection	
0.1	33.00±2.93[a] (n = 17)	33.66±0.52[a] (n = 17)
0.2	32.00±2.83[a] (n = 17)	32.33±3.34[a] (n = 17)
0.3	30.33±1.01[a] (n = 17)	31.55±3.10[a] (n = 17)
	Control group	
Mean value during experiment = 39.80±0.91%		

a,b = In each group above, data along the same column with different superscript alphabets are statistically significant ($p<0.05$), while data with the same superscript alphabets are not statistically significant ($p>0.05$)

Table 2: Changes in haemoglobin concentration of quails administered different doses of NDV Kudu 113 strain through different routes (Mean±SEM)

	Route of administration	
Dose of NDV (mL)	IM	PO
	4 days post infection	
0.1	12.93±0.45[a] (n = 17)	12.05±1.21[a] (n = 17)
0.2	12.68±0.49[a] (n = 17)	12.08±0.49[a] (n = 17)
0.3	11.74±0.82[a] (n = 17)	10.93±0.87[a] (n = 17)
	7 days post infection	
0.1	12.82±0.65[a] (n = 17)	12.92±0.22[a] (n = 17)
0.2	13.25±0.39[a] (n = 17)	13.22±0.27[a] (n = 17)
0.3	11.78±1.16[a] (n = 17)	12.27±0.55[a] (n = 17)
	14 days post infection	
0.1	11.53±1.51[a] (n = 17)	11.63±1.05[a] (n = 17)
0.2	11.74±3.64[a] (n = 17)	12.26±1.21[a] (n = 17)
0.3	11.33±1.09[a] (n = 17)	11.03±1.29[a] (n = 17)
	21 days post infection	
0.1	12.50±1.07[a] (n = 17)	12.90±0.71[a] (n = 17)
0.2	11.64±0.94[a] (n = 17)	11.21±1.19[a] (n = 17)
0.3	9.70±0.59[a] (n = 17)	9.73±1.20[a] (n = 17)
	28 days post infection	
0.1	11.63±0.97[a] (n = 17)	11.85±0.17[a] (n = 17)
0.2	7.63±0.96[b] (n = 17)	10.73±1.11[a] (n = 17)
0.3	6.73±0.34[a] (n = 17)	7.47±1.01[b] (n = 17)
	Control group	
Mean value during experiment = 13.22±0.30g%		

a,b = In each group above, data along the same column with different superscript alphabets are statistically significant ($p<0.05$), while data with the same superscript alphabets are not statistically significant ($p>0.05$)

haematocrit value of 39.80±0.91% obtained in the control group. Route of administration seems not to have effect on the haematocrit values. On the other hand, the dose of the virus has negative effect on the haematocrit value, as observed during the experimental period, where the haematocrit values of the quails administered 0.3 mL of the virus either im or po were lower (though not significant, except on days 14 and 21 pi) than the corresponding values obtained from quails administered 0.1 or 0.2 mL either im or po, on each day of the experiment.

Soon after the establishment of patent infection, evidence of reduction in haemoglobin concentration was observed from day 4 to day 28 pi in both groups that were administered NDV Kudu 113 strain im or po, when compared with the mean value of 13.22±0.30 g% obtained in the control group. In general, quails that were administered 0.3 mL of the virus either im or po had lower haemoglobin concentration (though not significant, except on day 28 pi) than their counterparts which received 0.1 or 0.2 mL of the virus either im or po on each day of the experiment (Table 2).

Following infection, there was an increase in HI antibodies to NDV Kudu 113 strain, reaching the maximum mean HI antibody titres of $\log_2$ 10.56±0.29 and $\log_2$ 9.89±0.48 in quails that were administered the virus im and po, respectively on day seven pi. Thereafter, the HI antibody titre decreased from days 14 to 21 pi. However, by day 28 pi there was a rise in HI antibody titre. Quails which received various doses of the virus im tend to have higher HI antibody titres to NDV Kudu 113 strain than their counterparts which were administered the virus po, except on day 28 pi, where the HI antibody titres to the virus were higher in quails that received the virus po than those that were inoculated with the virus im (Table 3).

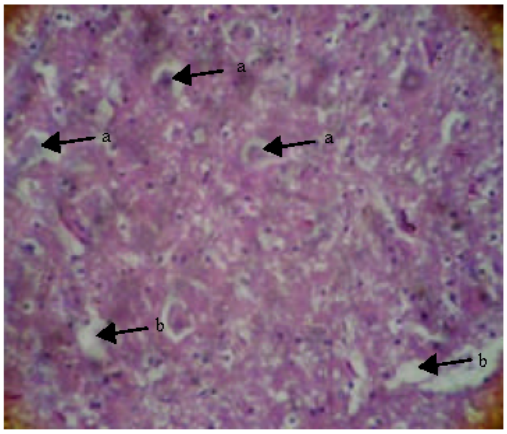

Fig. 1: A section from the brain (cerebrum) of quail infected with NDV Kudu 113 strain. Note the neuronal degeneration (arrow heads a) and vacuolation (arrow heads b). H and E stain×400

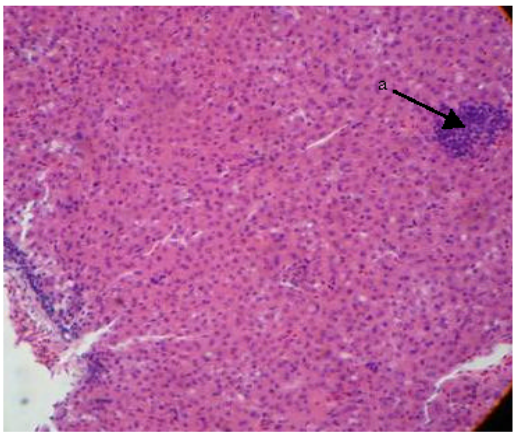

Fig. 2: A section from the liver of quail infected with NDV Kudu 113 strain. Note the focal area of coagulation necrosis with inflammatory cells (arrow head a). H and E stain×400

Table 3: Changes in haemagglutination inhibition antibody titre of quails administered different doses of NDV Kudu 113 strain through different routes (Mean±SEM)

	Route of administration	
Dose of NDV (mL)	IM	PO
	4 days post infection	
0.1	8.67±0.40[a] (n = 17)	7.44±0.24[a] (n = 17)
0.2	8.67±0.33[a] (n = 17)	6.89±0.26[a] (n = 17)
0.3	9.56±0.53[a] (n = 17)	6.56±0.47[a] (n = 17)
	7 days post infection	
0.1	10.22±0.27[a] (n = 17)	9.78±0.46[a] (n = 17)
0.2	10.44±0.24[a] (n = 17)	9.78±0.49[a] (n = 17)
0.3	10.56±0.29[a] (n = 17)	9.89±0.48[a] (n = 17)
	14 days post infection	
0.1	6.89±0.35[a] (n = 17)	6.22±0.40[a] (n = 17)
0.2	8.00±0.57[a] (n = 17)	6.89±0.56[a] (n = 17)
0.3	8.44±0.44[a] (n = 17)	6.56±0.33[a] (n = 17)
	21 days post infection	
0.1	6.67±0.23[a] (n = 17)	5.67±0.50[a] (n = 17)
0.2	7.11±0.53[a] (n = 17)	6.22±0.32[a] (n = 17)
0.3	7.67±0.33[a] (n = 17)	6.33±0.44[a] (n = 17)
	28 days post infection	
0.1	9.33±0.60[a] (n = 17)	10.33±0.33[a] (n = 17)
0.2	8.89±0.35[a] (n = 17)	10.56±0.17[a] (n = 17)
0.3	9.33±0.83[a] (n = 17)	9.89±0.35[a] (n = 17)
	Control group	
No HI antibody titre to NDV was detected in the control quails		

a,b = In each group above, data along the same column with different superscript alphabets are statistically significant ($p<0.05$), while data with the same superscript alphabets are not statistically significant ($p>0.05$)

Table 4: Changes in total protein concentration of quails administered different doses of NDV Kudu 113 strain through different routes (Mean±SEM)

	Route of administration	
Dose of NDV (mL)	IM	PO
	4 days post infection	
0.1	6.15±0.23[a] (n = 17)	5.87±0.56[a] (n = 17)
0.2	5.97±0.35[a] (n = 17)	6.24±0.24[a] (n = 17)
0.3	5.64±0.54[a] (n = 17)	6.30±0.47[a] (n = 17)
	7 days post infection	
0.1	4.73±0.34[a] (n = 17)	5.13±0.22[a] (n = 17)
0.2	5.20±0.17[a] (n = 17)	5.53±0.24[a] (n = 17)
0.3	5.95±0.44[a] (n = 17)	6.01±0.37[a] (n = 17)
	14 days post infection	
0.1	4.97±0.27[a] (n = 17)	6.31±0.29[a] (n = 17)
0.2	4.80±0.35[a] (n = 17)	5.52±0.36[a] (n = 17)
0.3	5.67±0.52[a] (n = 17)	5.67±0.30[a] (n = 17)
	21 days post infection	
0.1	5.68±0.44[a] (n = 17)	6.24±0.25[a] (n = 17)
0.2	5.73±0.27[a] (n = 17)	5.72±0.31[a] (n = 17)
0.3	5.28±0.24[a] (n = 17)	5.90±0.26[a] (n = 17)
	28 days post infection	
0.1	4.83±0.83[a] (n = 17)	6.38±0.35[a] (n = 17)
0.2	5.10±0.30[a] (n = 17)	5.83±0.36[a] (n = 17)
0.3	5.33±0.21[a] (n = 17)	5.90±0.33[a] (n = 17)
	Control group	
Mean value during experiment	= 6.40±0.24 g dL^{-1}	

a,b = In each group above, data along the same column with different superscript alphabets are statistically significant ($p<0.05$), while data with the same superscript alphabets are not statistically significant ($p>0.05$)

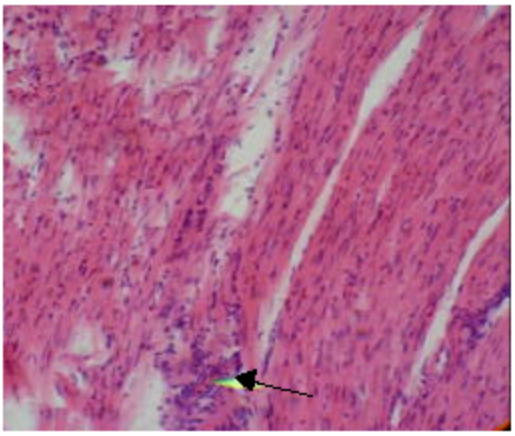

Fig. 3: A section from the heart of quail infected with NDV Kudu 113 strain. Note the focal area of myocardial degeneration and some inflammatory cells infiltration (arrow head). H and E stain×400

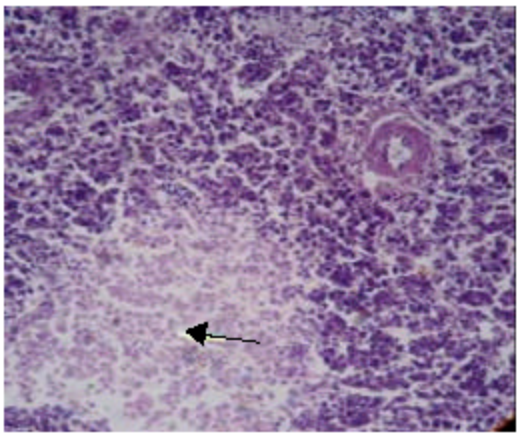

Fig. 4: A section from the spleen of quail infected with NDV Kudu 113 strain. Note the area of depleted lymphocytes (arrow head). H and E stain×400

After infection, there were variations in values of total protein concentration. The lowest value of 4.73±0.34 g dL^{-1} was obtained on day seven pi in quails administered 0.1 mL im of NDV Kudu 113 strain, while the highest value of 6.38±0.35 g dL^{-1} was obtained on day 28 pi in quails administered 0.1 mL of the virus po (Table 4).

In general, the histopathological lesions observed were focal areas of necrosis, mononuclear cells infiltration and depletion of lymphoid tissues. However, in some organs, specific lesions were found. For example, in the brain, there were neuronal degeneration and vacuolation of the cerebrum (Fig. 1).

In the liver, there were focal areas of necrosis and fatty degeneration (Fig. 2).

There were focal areas of myocardial degeneration and mononuclear cells infiltration in the heart (Fig. 3).

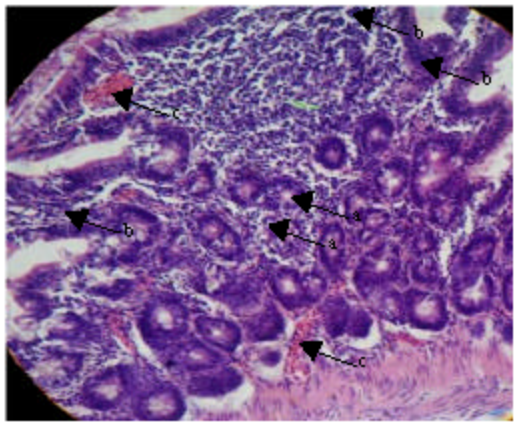

Fig. 5: A section from the intestine (duodenum) of quail infected with NDV Kudu 113 strain. Note the necrosis of duodenal glands (arrow heads a) and villi (arrow heads b) and congested blood vessels (arrow heads c) H and E stain×400

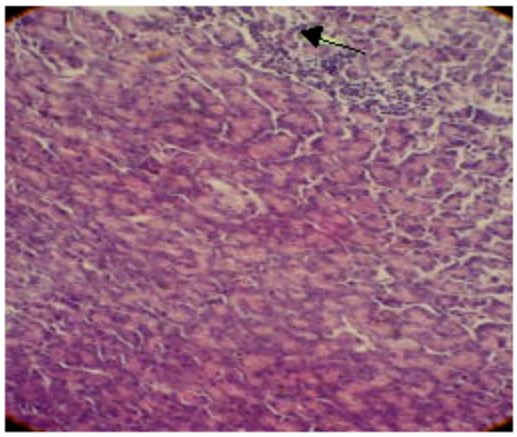

Fig. 6: A section from the pancrease of quail infected with NDV Kudu 113 strain. Note the focal area of necrosis and mononuclear cells infiltration (arrow head). H and E stain×400

Areas of depleted lymphocytes were observed in the spleen (Fig. 4).

In the intestine, there were focal areas of necrosis, involving the duodenal glands, villi and tunica muscularis (Fig. 5).

Focal areas of necrosis and monocuclear cells infiltration were observed in the pancrease (Fig. 6).

DISCUSSION

The lower values of haematocrit and haemoglobin concentration obtained in the infected quails following infection with NDV Kudu 113 strain could be as a result of the effect of NDV on the erythrocytes of the infected

quails. This finding supports the previous results where the anaemia that was observed in chickens infected with NDV was attributed, 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). Furthermore, the anaemia observed in ND was also attributed to the removal of erythrocyte surface sialic acid by neuraminidase of NDV, which led to erythrophagocytosis of desialylated erythrocytes by macrophages and consequently, resulting in anaemia (Oladele *et al*., 2002; Oladele, 2005).

The sharp rise in haemagglutination inhibition antibody titre to NDV in Japanese quails, following infection with NDV Kudu 113 strain in this study shows that the virus replicated in the tissues and organs of the quails and was responsible for the clinical signs and lesions of ND observed and changes in the vital body parameters observed in the infected quails in this study. Furthermore, the maximum mean HI antibody titre of $\log_2$ 10.56±0.29 and $\log_2$ 9.89±0.48 obtained from quails administered NDV Kudu 113 strain through im and po, respectively were similar to maximum mean HI antibody titre obtained from chickens inoculated with the same virus strain (Oladele *et al*., 2005).

The slight reduction in total protein concentration, following infection with NDV Kudu 113 strain in this study could be as a result of a decrease in albumin content, resulting from tissue pathology, such as hepatic necrosis and fatty degeneration observed in the infected quails. This is in line with the result of Kaneko (1989) who found that total protein concentration is likely to decrease in birds with hepatic damage, thereby inhibiting the synthesis of albumin, which constitutes 40-60% of the total protein concentration in birds (Campbell and Coles, 1986).

It is interesting to note that the histopathological lesions observed in the infected quails in this study were similar to those reported previously in chicken infected with NDV (Alexander, 1991; Okoye *et al*., 2000; Oladele *et al*., 2005). Also, histopathological lesions, such as neuronal degeneration, vacuolation and necrosis observed in the brain of infected quails further support the fact that the nervous system of the quails was affected by NDV Kudu 113 strain as evidenced by neurological signs observed in the infected quails in this study.

CONCLUSION

In conclusion, this study has demonstrated that some vital body parameters were also altered in quails that were infected with NDV Kudu 113 strain. This result further confirms that NDV also replicates in the body systems of quails, thereby inducing some injuries on the tissues and organs of infected quails during NDV infections.

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