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Comparative Studies of Packed Cell Volume, Haemoglobin, Total Protein, Haemagglutination Inhibition Antibodies and Rectal Temperature of Pigeons (*Columbia livia*) Administered Newcastle Disease Virus Through Different Routes

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Abstract: Comparative studies of packed cell volume (PCV), haemoglobin (HB), total protein (TP), haemagglutination inhibition (HI) antibodies and rectal temperature (RT) were carried out on a total of 50 pigeons (*Columbia livia*) that were administered Newcastle disease virus (NDV) Kudu 113 strain through different routes. Fifteen pigeons were administered 0.2 mL each of NDV Kudu 113 strain per os (po), 15 pigeons were inoculated with 0.2 mL of the virus intramuscularly (im), another 15 pigeons were sprayed with about 0.2 mL each of the virus through oculonasal (oc) route, while 15 pigeons served as control. Clinical signs and lesions of Newcastle disease (ND) were observed in some of the infected pigeons. After infection, there was an increase in HI antibodies, reaching the maximum mean values of \log_2 5.2 \pm 0.13, \log_2 5.3 \pm 0.31 and \log_2 5.0 \pm 0.12 in pigeons that were administered NDV Kudu 113 strain through oc, po and im routes, respectively. Similarly, as soon as patent infection was established, the RT continued to rise and attained peak values of 42.31 \pm 0.03, 41.94 \pm 0.06 and 42.18 \pm 0.06°C in pigeons that were administered the virus through po, im and oc routes, respectively. The values of PCV, HB, TP, HI and RT for the control pigeons were relatively constant, while the corresponding values in the infected pigeons fluctuated widely, depending on the route of administration of the virus. It was concluded that vital blood and body parameters, such as PCV, HB, TP, HI and RT were altered during infection of pigeons with NDV Kudu 113 strain.

Key words: Pigeon, newcastle disease virus, vital parameters

Introduction

Most pigeons are strong and swift fliers. These features together with their palatable flesh make them favourite game birds in many countries of the world. However, pigeons are susceptible to many poultry diseases which hinder their maximum output in terms of meat and eggs, when they are reared for food.

Newcastle disease (ND) is a viral infection of birds caused by avian paramyxovirus type 1. Since, its recognition in 1926, ND is regarded as endemic in many countries throughout the world. Newcastle disease is still endemic in Nigeria like other African countries (Sa'idu *et al.*, 2006; Oladele *et al.*, 2008a).

The interaction between organisms and their environment is an old, but very important problem for scientists. Organisms respond to their immediate environment in different ways. For example, NDV is a pantropic virus (Asdel and Hanson, 1960; Kouwenhoven, 1993), as such, during infection of the virus, homeostatic mechanisms of different cells of the infected birds are altered, depending on the type of virus, virulence of the virus and the immune status of the host. It is believed that complex chemical compositions, such as the blood of animals are altered in disease conditions. The blood is a complex medium, characterized not only by its circulating cells with many

functions, but also by organic and inorganic chemicals common to the body as a whole. As a result, its contents are altered in certain disease conditions (Campbell and Coles, 1986; Oladele *et al.*, 2008a).

The total percentage of blood volume composed of packed cellular elements known as the haematocrit or packed cell volume. The erythrocyte contains a very high concentration of haemoglobin, which is also a complex iron-containing conjugated protein that are composed of pigment and single protein. The functions of haemoglobin include carrier of oxygen, assisting in the maintenance of acid-base homeostasis within the body fluids. These functions may be impaired in some disease conditions, depending on causative agents and the effects of these agents on the blood (Reece and Swenson, 2005).

Plasma total protein represents a complex mixture of proteins of different structural and functional properties. The major plasma proteins include albumins, globulins, fibrinogen, nucleoproteins and conjugated proteins, such as lipoproteins and seromucoid proteins (Gentry, 2005). In birds, the bulk of plasma proteins consist of albumin which constitute about 40-60% of the total protein (Campbell and Coles, 1986). Alteration of plasma protein contents in disease can ultimately affect its nutritive, physicochemical or transportive functions (Gentry, 2005).

Temperature is a measure of the heat content of a physical body, and it reflects molecular agitation in the body. In some disease conditions, fever ensues as a result of the action of pyrogens produced by viral or bacterial agents on the hypothalamus (Robertshaw, 2005).

Since, important chemical compositions and functions in the body of birds are being altered in most disease conditions, it is pertinent to determine how some body parameters, such as PCV, HB, TP, HI and RT are also affected in pigeons following infection with NDV.

To our knowledge, there is no existing information on the values of PCV, HB, TP, HI and RT of pigeons infected with NDV Kudu 113 strain. This paper, therefore, reports for the first time comparative values of PCV, HB, TP, HI and RT of pigeons administered NDV Kudu 113 strain through different routes.

Materials and Methods

Birds for experiment: Sixty pigeons, aged between 3-5 weeks, served as the subjects of the experiment. They were purchased from Zaria metropolis in February, 2008. Before the experiment commenced, the pigeons were quarantined for two weeks, during which they were treated with antimicrobial and anthelmintic agents, against any pathogenic agents in their body systems.

At the end of the quarantine, the pigeons were randomly divided into four groups of 15 pigeons each per group. Pigeons in each group were kept in different cages and were supplied with water and feed *ad libitum*.

The challenge virus: Newcastle disease virus Kudu 113 strain was the challenge virus used for the experiment. This virus strain was first isolated from roaming duck in Kuru, Plateau State, Nigeria. The virus has been characterized by Echeonwu *et al.* (1993).

Challenge of pigeons with newcastle disease virus: After quarantine, the pigeons were challenged with NDV Kudu 113 strain as follows:

Group 1: Each pigeon in this group received 0.2 mL of the virus intramuscularly.

Group 2: Each pigeon in this group received 0.2 mL of the virus per os.

Group 3: All the pigeons in this group were first enclosed in a container and sprayed with approximately 0.2 mL of the virus per bird. Pigeons in this group, therefore, received the virus through ocular or nasal (oculanasal) route.

Group 4: This group contained the control pigeons. No virus was administered to pigeons in this group. After the challenge, the pigeons were observed daily for any clinical signs and gross lesions of ND.

Blood sampling: Blood sampling was through wing venepuncture, using 23 gauge sterile hypodermic needles and syringes. About two milliliters of blood were collected on each day of the experiment from each pigeon in the four groups.

Blood for haematological parameters were collected into labeled Bijou bottles, containing ethylene diamine tetra acetic acid (EDTA) at 2 mg mL⁻¹ as anticoagulant. Sera for HI analysis were taken without anticoagulant. The sera were separated by centrifugation at 1,000 g for 10 min and stored frozen in plastic vials until laboratory determinations were made for HI antibody quantification by the procedures of Beard (1989).

Packed cell volume, haemoglobin and total protein determinations:

The PCV values were determined by the microhaematocrit method of Benjamin (1985). Blood samples containing EDTA were aspirated into a set of plain capillary tubes. The tubes were sealed and spun on Hettich centrifuge (Hawksley and Sons Limited, England) at 5,000 g for 5 min. After spinning, the PCV was read as a percentage directly from Graphic Reader (Hawksley and Sons Limited, England). The HB values were also determined. The total protein concentrations were determined by the refractometric methods (Benjamin, 1985).

Measurement of rectal temperature: The rectal temperature was recorded, as an indicator of body temperature, from each pigeon in the morning of each day of the experiment, with the aid of digital clinical thermometer (Hartma, Digital Thermometer, Germany), inserted about 2 cm into the rectum of each pigeon via the cloaca. The thermometer was kept in the rectum of each pigeon until the readings were stabilized.

Statistical analysis: Data obtained were analyzed using Analysis of variance. Values of ($p < 0.05$) were considered significant. The analyzed data were used to plot graphs.

Results and Discussion

Some of the pigeons in the three infected groups developed ND by day five post infection (pi). These birds were dull, anorexic and had droopy and paralyzed wings (Fig. 1).

Muscles of the breast, thighs and legs of the pigeons that died as a result of ND in this study were congested. The carcasses were emaciated, dehydrated and had lesions of ND in various organs (Fig. 2).

The clinical signs and gross lesions observed in the infected pigeons in this study, are in agreement with previous findings in turkeys (Abdul-Aziz and Arp, 1983; Adair *et al.*, 1989), guinea fowls (Haruna *et al.*, 1993; Mohammed *et al.*, 1996), quails (Oladele *et al.*, 2008a) and chickens (Alexander, 1997; Sa'idu *et al.*, 2006; Oladele *et al.*, 2008b).



Fig. 1: Photograph of pigeon infected with NDV Kudu 113 strain. Note the sign of paralysis of the wings

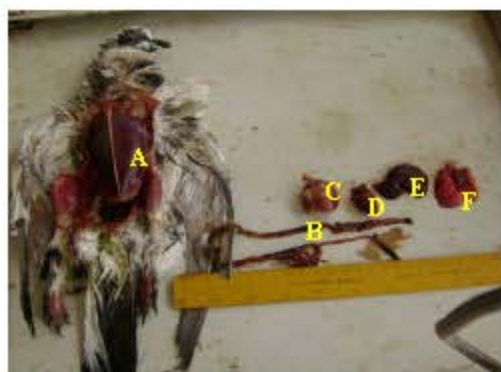


Fig. 2 Gross pathological changes in pigeon which died of ND caused by NDV Kudu 113 strain infection. Note the emaciation and congestion of the muscles of the breast, thighs and legs on the carcass (A); haemorrhagic enteritis (B); and congestion of gizzard (C), heart (D), liver (E) and lung (F)

After infection, there was an increase in HI antibodies, reaching the maximum mean HI antibody titres of $\log_2 5.2 \pm 0.13$, $\log_2 5.3 \pm 0.31$ and $\log_2 5.2 \pm 0.12$ in pigeons that were administered NDV Kudu 113 strain through oc, po and im routes, respectively and correspondingly on days 7, 15 and 21 pi. Gradual decrease in mean HI antibodies was observed from day 28 pi in all the infected groups, except the group of pigeons that were administered the virus po, which still had mean HI antibody titre of $\log_2 5.0 \pm 0.35$ by day 35 pi. The control pigeons that had no HI antibodies to ND at the beginning of the experiment had insignificant increase in HI antibodies from days 21-35 pi (Fig. 3).

The result of HI antibodies to ND in this study is in line with the finding of Mishra *et al.* (2000) in chickens who detected HI antibodies to ND by day 7 pi, with the highest mean HI antibodies by day 21 pi.

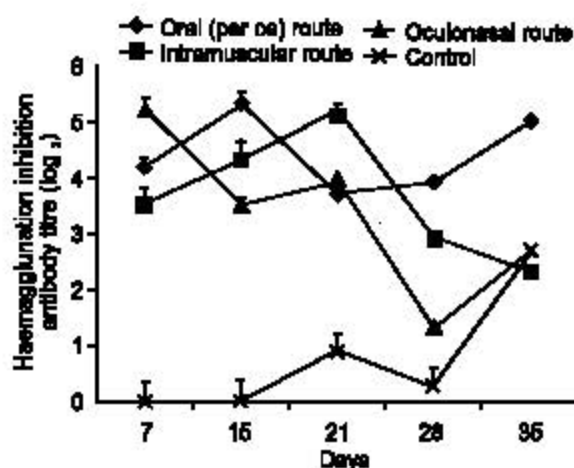


Fig. 3: Changes (mean \pm SE) in haemagglutination inhibition antibody titre of pigeons administered NDV Kudu 113 strain through different routes.

The relatively high HI antibody titre of $\log_2 5.0 \pm 0.35$ recorded on day 35 pi in pigeons that were administered the virus po showed that pigeons in this group could still be shedding the virus in the faeces long after infection. This is because it has been observed that long lasting shedding of NDV might occur in columbiformes (pigeons and doves) and passeriformes in cases where there is chronic infection of some organs by NDV. In this way, pigeons may harbour highly virulent NDV without overt signs and clinical disease (Wakamatsu *et al.*, 2006).

The resurgence of HI antibodies to ND from day 21 pi in the control pigeons that were earlier negative, could be as a result of exposure of the pigeons to the virus probably through the aerosol. This finding is in line with previous findings that NDV can be transmitted by aerosol (Alexander, 1997; Sa'idu *et al.*, 2006) and that different species of birds can be exposed to the virus in endemic areas, irrespective of the possible precautions taken.

As soon as patent infection was established, the RT continued to rise and attained peak values of 42.31 ± 0.03 , 41.94 ± 0.06 and $42.18 \pm 0.06^\circ\text{C}$ in pigeons that were administered the virus through po, im and oc, respectively on days 21 and 28 pi. The RTs of the control group remained relatively constant throughout the period of the experiment (Fig. 4).

In this study, the period of elevated RT values in the infected pigeons coincided with the time of high HI antibodies to ND. This finding is in agreement with the results of previous authors (Kouwenhoven, 1993; Robertshaw, 2005) that following infection with NDV, there is usually viral replication in the tissues of infected birds, which consequently, led to viraemia and corresponding increase in the levels of viral pyrogens and elevated RT thereafter.

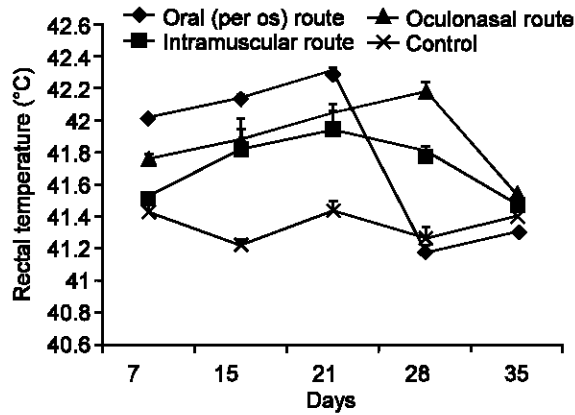


Fig. 4: Changes (mean \pm SE) in rectal temperature of pigeons administered NDV Kudu 113 strain through different routes

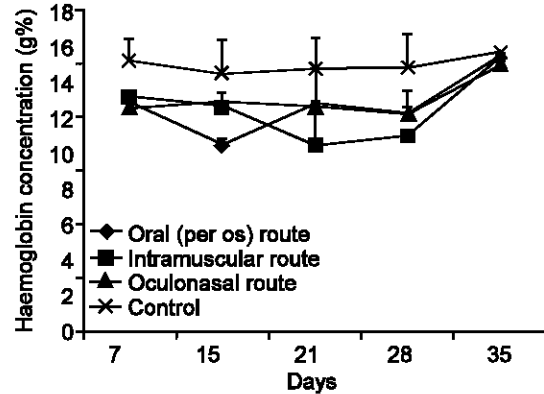


Fig. 6: Changes (mean \pm SE) in haemoglobin concentration of pigeons administered NDV Kudu 113 strain through different routes

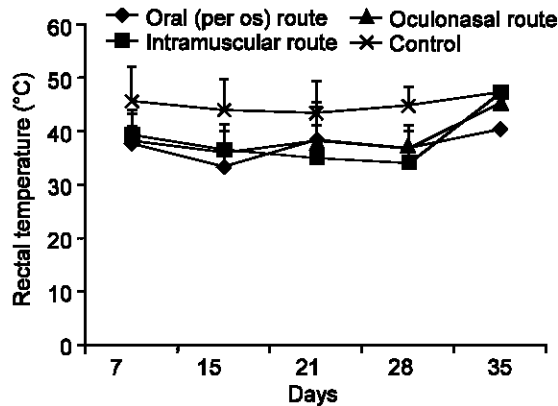


Fig. 5: Changes (mean \pm SE) in packed cell volume of pigeons administered NDV Kudu 113 strain through different routes

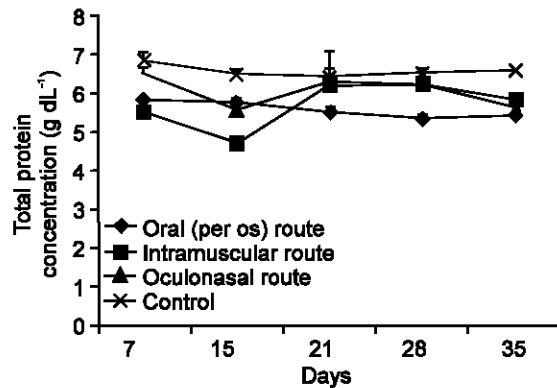


Fig. 7: Changes (mean \pm SE) in total protein concentration of pigeons administered NDV Kudu 113 strain through different routes

The lowest mean PCV value of $33.50 \pm 4.1\%$ recorded among the infected pigeons was obtained on day 15 pi in the pigeons that were administered the virus po, while the highest mean PCV value of $47.40 \pm 8.0\%$ was recorded on day 35pi in the pigeons that were administered the virus im. In general, the PCV values of the control pigeons were higher and relatively constant than the PCV values obtained from the infected pigeons (Fig. 5).

The highest mean HB values among the infected pigeons was recorded on day 35 pi in the group of pigeons that were administered the virus po, while the lowest mean HB value of 10.43 ± 0.52 g% was recorded on day 21 pi in the group of pigeons that were administered the virus im. The HB values of the control pigeons were relatively constant throughout the experimental period (Fig. 6).

The reduction in the values of PCV and HB of the infected pigeons when compared with the control, especially at the time of high mean HI antibodies to ND

in this study, is in line with the results of Cheville and Beard (1972) and Cheville *et al.* (1972) that NDV causes lysis of erythrocytes and consequently, reduction in the values of PCV and HB which serve as important indicators of animals' haemogram.

There were variations in values of TP between the different infected groups of pigeons. For example, the peak value of 6.20 ± 0.03 g dL⁻¹ obtained from the group of pigeons that were administered the virus im was recorded on day 21 pi, while the peak value of 5.81 ± 0.01 g dL⁻¹ recorded from the pigeons that were administered the virus po was obtained on day 7 pi. The group that was administered the virus oc also had its peak value of 6.52 ± 0.13 g dL⁻¹ on day 7 pi. The TP concentrations of the control pigeons remained relatively constant during the experiment (Fig. 7).

In general, the TP values obtained in the infected pigeons (although the values were within the normal range of 3-6 g dL⁻¹) were slightly lower than the TP values obtained in the control pigeons. The slight

reduction in TP values in the infected pigeons could be as a result of tissue pathology induced by NDV Kudu 113 strain in the infected pigeons.

Conclusion: It was observed that vital blood and body parameters, such as PCV, HB, TP, HI and RT were altered during infection of pigeons with NDV Kudu 113 strain.

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