

# Comparison of the Serum Proteins and Immune Responses of Velogenic Newcastle Disease Virus Infected Chickens and Ducks

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## Abstract

The effect of velogenic Newcastle disease virus (vNDV) on the immune responses and serum proteins was investigated in six-week-old ducks and chickens. Results showed that weight loss was markedly significant ( $p < 0.05$ ) from days 3 - 21 (PI) in chickens and mild ( $p < 0.05$ ) on days 3 and 15 PI in ducks. The antibody response obtained showed significant ( $p < 0.05$ ) increase in infected chickens (IC) than those of the infected ducks (ID). While the total serum protein and serum globulin increased significantly ( $p < 0.05$ ) in IC on days 7 and 14 PI, they decreased significantly ( $p < 0.05$ ) in ID only on day 21 PI. The immune responses and serum protein values in this experiment X-ray showed less susceptibility of ducks when compared with the chickens. This may be related to marked anorexia and severe dehydration observed in the latter consequent upon serum concentration. Ducks could be maintaining the endemicity of Newcastle disease (ND) as reservoir host.

## Keywords

Velogenic Newcastle Disease, Experimental Infection, Immune Response, Serum Proteins, Ducks, Chickens

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## 1. Introduction

Proteins are the most abundant compound in the serum comprising amino-acid building blocks and are in turn,

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building blocks for cells and tissues [1]. They are vital components of enzymes, hormones, antibodies and clotting agents [1]-[3]. Some proteins function as transporters, osmotic pressure and acid-alkaline balancers, and reserved energy sources. Due to these multiple functions, any condition that alters the serum proteins extends its tentacles on these roles played by proteins. Newcastle disease (ND) is a highly contagious and infectious disease that affects almost all avian species including poultry, cage and wild life bird species [4]-[6]. It is widespread affecting many continents of the world namely: Asia, Africa, Europe and America [7]. ND causes considerable economic losses, not only due to high flock mortality but also through the economic impact arising from trade restrictions and embargoes [8] [9]. It has been reported to affect other birds such as guinea fowls, quails, turkeys, pheasants and peacock [4]. Mortality due to ND ranges from negligible to as high as 100% depending on the form or pathogenicity of the virus [10]. Kudu 113, (Kuru duck 113) a strain of NDV isolated from apparently healthy ducks and characterized by Echeonwu *et al.* [11] in Nigeria, has been studied in chickens and guinea fowls [12]. Anseriformes are suspected to maintain the endemicity of ND around the world [7]. In view of these facts, a comparative study of the serum proteins and immune response of vNDV infected ducks and chickens was done.

## 2. Materials and Methods

### 2.1. Flock History

One hundred and seventy day old birds hatched the same day were obtained comprising 70 ducklings from poultry section of National Veterinary Research Institute NVRI Vom and 100 cockerel chicks from Zartech Hatchery. Brooding was done separately for the chicks and ducklings on deep litters under the same environmental conditions. The cockerels received IBD vaccine by intraocular route at days 10 and 24 post hatch (PH). Chicks' mash was given ad libitum to the birds from day old to 8 weeks PH. Growers' mash was given ad libitum also from 9 weeks PH until the end of the experiment. Water was allowed free choice.

### 2.2. NDV Inoculum

The VNDV strain, (Kudu-113) was acquired and used in the challenge experiment.

### 2.3. Experimental Design

At six weeks of age the chicks and ducklings were each randomly assigned into two groups of infected chicks (IC), uninfected chicks (UC) and infected ducks (ID), uninfected ducks (UD). The inoculum was reconstituted to give embryo lethal dose (ELD<sub>50</sub>) titre of  $10^{6.36}$  per ml. The chicks and ducks in groups IC and ID were inoculated intramuscularly (IM) with 0.2 ml of the inoculum (infected groups). The chicks and ducks in groups UC and UD received 0.2 ml of phosphate buffered solution IM (control groups). The infected and control groups were housed at different locations and maintained on deep litter system.

### 2.4. Live Body Weight

At day 3 PI, 10 birds from each group were randomly selected, marked, weighed and the weight recorded as live body weight (LBW) of each group. The marked birds were re-weighed at days 6, 9, 12, 15 and 21 PI.

### 2.5. Serology

Blood samples (3ml/bird) were collected from 10 birds in each group on days 0, 7, 14, and 21 PI through the wing vein, using sterile syringe. The sample bottles were stoppered, laid on near horizontal position, and allowed to clot. The serum samples were harvested into 2 ml vials. The duck sera were inactivated at 56°C in water bath for 30 minutes and with the chicken sera, use in the serological analysis.

#### 2.5.1. Washing of Erythrocytes

Two ml of ND sero-negative chicken blood was collected from adult bird in a test tube containing EDTA and washed according to standard procedure. Using the formula  $CV = RxV/O$ , where CV = calculated volume (ml) of washed RBC, R = required % of RBC (0.5 %), V = volume (ml) of PBS intended to be used in the dilution and O = original PCV (%) of the RBC after washing, 0.5% of the washed chicken RBC was prepared [13].

### 2.5.2. Haemagglutination (HA) Test

The standard HA technique as described by Beard, [13] was used and two hundred doses of La Sota ND vaccine was diluted in 10 ml of PBS (pH 7.0). The titre was then adjusted to 4 hemagglutinin units (HAU) for the HI test.

### 2.5.3. Haemagglutination Inhibition (HI) Test

The standard HI technique as described by Beard, [13] was used. The arithmetic method of geometric mean titre (GMT) was calculated mathematically for 2 fold dilution with the following formula:

**GMT = antilog[(A – 1) × logB + logC]** where A= average end point well number, B = the factor (2.0) and C = the reciprocal of first dilution (2.0) [14].

## 2.6. Serum Proteins

At days 0, 7, 14 and 21 PI, 10 birds from each group were randomly selected and at least 3 ml blood samples collected through the wing vein, into plain test tubes and serum samples harvested from the clotted blood into 2 ml vials and stored at –40°C for serum protein studies.

### 2.6.1. Determination of Total Serum Protein (TSP)

The TSP was determined using direct Biuret method. Biuret reagent containing NaOH, potassium iodide, copper (II) sulphate and sodium—potassium tartarate and Standard containing aqueous solution of protein, equivalent to 5 g/dL (50 g/L) were used [15].

The total proteins were calculated as follows: **Total protein (g/dL) = absorbance of sample × 5/absorbance of standard.**

### 2.6.2. Determination of Serum Albumin Level (SAL)

The SAL was determined using bromocresol green method. Bromocresol green reagent containing bromocresol green, succinate buffer (pH 4.2), surfactants, preservatives and stabilizers, and standard containing aqueous solution equivalent to 5 g/dL (50 g/L) of albumin were used [15].

The serum albumin was calculated as follows:

**Serum albumin (g/dL) = absorbance of sample × 5/absorbance of standard.**

### 2.6.3. Calculation of Serum Globulin Fractions (SGF)

The globulin fraction was calculated as the difference between total serum proteins and serum albumin level; **SGF (g/dL) = TSP – SAL.**

## 2.7. Data Analysis

The LBW, HI and serum protein values between and within groups were subjected to statistical analysis using independent sample t-test and the level of significance was determined and accepted at  $p \leq 0.05$  for all the results using statistical product for service and solution (SPSS) version 16.0 computer software. The mean ± standard error of mean (SEM) of the results obtained in the experiment were calculated and presented in tables, graphs and charts.

## 3. Results

### 3.1. Live Body Weight (LBW)

The result of live body weights of both species are shown on **Table 1**. The mean weights of IC were significantly lower ( $p < 0.05$ ) than those of the control on days 3, 12, 15 and 21 PI, while the mean weights of ID were significantly lower ( $p < 0.05$ ) than those of the control on days 3 and 15 PI.

### 3.2. Hemagglutination Inhibition (HI)

The PI HI values were determined and the results are shown in **Table 2**. On day 0 PI all the groups recorded zero HI titre. The HI titers of IC sera were significantly higher ( $p < 0.05$ ) when compared with ID at days 7—

**Table 1.** Mean live body weight (g)  $\pm$  SEM of chickens and ducks.

| Days PI | Infected chickens   | Control chickens    | Infected ducks      | Control ducks      |
|---------|---------------------|---------------------|---------------------|--------------------|
| 0       | 680.00 $\pm$ 23.81  | 680 $\pm$ 23.805    | 314.00 $\pm$ 9.91   | 314.00 $\pm$ 09.91 |
| 3       | 632.00 $\pm$ 18.61* | 746.00 $\pm$ 17.59  | 283.00 $\pm$ 12.65* | 349.50 $\pm$ 19.30 |
| 6       | 679.00 $\pm$ 28.61  | 745.00 $\pm$ 20.12  | 386.00 $\pm$ 24.13  | 356.00 $\pm$ 16.61 |
| 9       | 763.00 $\pm$ 51.45  | 835.00 $\pm$ 23.63  | 360.00 $\pm$ 20.98  | 384.50 $\pm$ 16.34 |
| 12      | 763.00 $\pm$ 46.60* | 996.00 $\pm$ 31.70  | 363.00 $\pm$ 19.22  | 386.00 $\pm$ 19.56 |
| 15      | 795.00 $\pm$ 47.40* | 1030.00 $\pm$ 41.95 | 358.00 $\pm$ 27.36* | 414.50 $\pm$ 20.42 |
| 21      | 867.00 $\pm$ 38.73* | 1044.00 $\pm$ 34.13 | 450.00 $\pm$ 19.72  | 448.50 $\pm$ 20.17 |

\*Means values significantly different at  $p < 0.05$  along the same row.

**Table 2.** Hemagglutination inhibition in chickens and ducks.

| Days   | Geometric mean titre $\pm$ SEM |                    |                   |                    |
|--------|--------------------------------|--------------------|-------------------|--------------------|
|        | IC                             | UC                 | ID                | UD                 |
| D0 PI  | 000.00 $\pm$ 00.00             | 000.00 $\pm$ 00.00 | 00.00 $\pm$ 0.00  | 000.00 $\pm$ 00.00 |
| D7 PI  | 105.60 $\pm$ 20.27             | 000.00 $\pm$ 00.00 | 22.40 $\pm$ 4.89* | 000.00 $\pm$ 00.00 |
| D14 PI | 537.60 $\pm$ 59.73             | 000.00 $\pm$ 00.00 | 32.00 $\pm$ 0.00* | 000.00 $\pm$ 00.00 |
| D21 PI | 119.20 $\pm$ 17.64             | 000.00 $\pm$ 00.00 | 36.80 $\pm$ 6.33* | 000.00 $\pm$ 00.00 |

\*Means values significantly different at  $p < 0.05$ .

(105.60  $\pm$  20.27 and 22.40  $\pm$  4.89), 14—(537.60  $\pm$  59.73 and 32.00  $\pm$  0.00), and 21—(119.20  $\pm$  17.64 and 36.80  $\pm$  6.33), PI respectively. Control chickens and UD had zero titre throughout the period of the experiment.

### 3.3. Serum Proteins

The results of the serum proteins are shown in **Table 3**. The total serum proteins in infected chickens were significantly higher ( $p < 0.05$ ) than the control on day 7 and 14 PI. By the day 21 PI, the infected chickens presented significantly lower TSP ( $p < 0.05$ ) than the control group. In the infected ducks, the TSP were significantly lower ( $p > 0.05$ ) than the control on days 7 and 21 PI.

The results of the SGF are shown in **Table 4**. The serum globulin protein fraction in infected chickens were significantly higher ( $p < 0.05$ ) than the control on days 7 and 14 PI, but was significantly lower ( $p > 0.05$ ) than the control on day 21 PI. In the infected ducks, the SGF was significantly lower ( $p > 0.05$ ) than the control only on day 21 PI.

The results of the SAL are shown in **Table 5**. The serum albumin level in infected chickens was not significantly different ( $p > 0.05$ ) when compared with the control throughout the experimental period. In the infected ducks, the SAL was significantly lower ( $p > 0.05$ ) than the control only on day 7 PI.

## 4. Discussion

Since maternal antibody could be detected in chickens up to 3 weeks of age [4], the choice of 6 weeks as the age of inoculation in this experiment was to ensure that the antibodies did not interfere with the susceptibility of the birds in these two species [9]. The results of the HI tests showed that the virus elicited a progressive serological response and sero-conversion detected earlier from day 7 PI which is different from day 10 PI first response recorded by Piacenti *et al.* [16] and Igwe [9]. Significantly higher antibody titers were observed in the chickens when compared with the ducks, indicating that the immune system in chickens may be more responsive, but were more stable in ducks than that in the chickens since there was rapid antibody decay in the latter. However, both species presented protective levels of antibodies ( $>2^3$ ) at day 21 PI as reported by Villagas and Purchase [14]. The results underscore variations between both species and may be due to dehydrations suffered by chickens. The response was in tandem with the results of the study of vNDV in chickens by Mishra *et al.* [17], Okoye *et al.* [12] and Piacenti *et al.* [16] who recorded the highest antibody response on days 15 and 21 PI. Contrary to this result, Oladele *et al.* [18] detected the highest HI titers by day 4 PI using Kudu-113 strain. The variations may be due to differences in the immune status of the chickens [19] [20] and the laboratory procedures used in

**Table 3.** Total serum proteins  $\pm$  SEM (g/dL  $\times$  10).

| Days | IC               | UC              | ID               | UD              |
|------|------------------|-----------------|------------------|-----------------|
| 0    | 4.09 $\pm$ 0.17  | 4.09 $\pm$ 0.17 | 5.25 $\pm$ 1.66  | 5.25 $\pm$ 1.66 |
| 7    | 6.00 $\pm$ 0.14* | 5.34 $\pm$ 0.11 | 5.44 $\pm$ 0.12  | 5.76 $\pm$ 0.09 |
| 14   | 3.13 $\pm$ 0.12* | 2.55 $\pm$ 0.40 | 3.81 $\pm$ 0.25  | 3.37 $\pm$ 0.13 |
| 21   | 2.55 $\pm$ 0.09* | 3.61 $\pm$ 0.14 | 3.13 $\pm$ 0.05* | 4.35 $\pm$ 0.13 |

\*Means values significantly different at  $p < 0.05$ .**Table 4.** Serum globulin proteins  $\pm$  SEM (g/dL  $\times$  10).

| Days | IC               | UC              | ID               | UD              |
|------|------------------|-----------------|------------------|-----------------|
| 0    | 2.63 $\pm$ 0.19  | 2.63 $\pm$ 0.19 | 3.44 $\pm$ 0.11  | 3.44 $\pm$ 0.11 |
| 7    | 3.96 $\pm$ 0.27* | 3.29 $\pm$ 0.21 | 4.10 $\pm$ 0.99  | 3.93 $\pm$ 0.17 |
| 14   | 1.63 $\pm$ 0.20* | 1.13 $\pm$ 0.05 | 2.41 $\pm$ 0.24  | 1.80 $\pm$ 0.22 |
| 21   | 1.03 $\pm$ 0.12* | 1.75 $\pm$ 0.21 | 1.42 $\pm$ 0.05* | 2.61 $\pm$ 0.19 |

\*Means values significantly different at  $p < 0.05$ .**Table 5.** Serum albumin proteins  $\pm$  SEM (g/dL  $\times$  10).

| Days | IC              | UC              | ID               | UD              |
|------|-----------------|-----------------|------------------|-----------------|
| 0    | 1.46 $\pm$ 0.10 | 1.46 $\pm$ 0.10 | 1.81 $\pm$ 0.10  | 1.81 $\pm$ 0.10 |
| 7    | 2.04 $\pm$ 0.16 | 2.03 $\pm$ 0.17 | 1.30 $\pm$ 0.07* | 1.81 $\pm$ 0.10 |
| 14   | 1.50 $\pm$ 0.11 | 1.42 $\pm$ 0.56 | 1.40 $\pm$ 0.06  | 1.59 $\pm$ 0.08 |
| 21   | 1.49 $\pm$ 0.06 | 1.87 $\pm$ 0.19 | 1.68 $\pm$ 0.29  | 1.74 $\pm$ 0.12 |

\*Means values significantly different at  $p < 0.05$ .

the HI test. One of the mechanisms by which antibodies fight against pathogens is neutralization, particularly viruses [21]. Neutralized viruses are unable to attach to surface receptors of target cells and are thus prevented from replication [22] [23].

The serum proteins elevation was more in chickens that presented higher antibody response and this may be associated with hyper-globulinemia due to sero-conversion to immunoglobulin [1] [3] [24] or hemo-concentration associated with the dehydration occasioned by the infection and reduced feed and water intake [25]. Serum proteins have been known to play important roles in infection following invasion of the body by pathogens such as virus, bacteria etc. [1] [2] [18] and as vital substrate for antibody formation [26]. Low level of albumin in serum in livestock may be due to heavy loss in urine or loss due to enteropathy (ulcers and diarrhea), or decreased production by the liver probably because of insufficient intake of protein in diet [1] [25] [27]. Serum albumin proteins which are the most abundant proteins in the clear fluid portion of the blood, typically decrease during inflammation [28] [29] and in a condition of anorexia, albumin is said to depress leading to osmotic disequilibrium and dehydration [29] [30]. There is a strong indication that ducks are far less susceptible to ND than chickens and may likely be maintaining the disease in poultry.

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