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Effects of a Velogenic Newcastle Disease Virus on Packed Cell Volume, Total Protein and Hemagglutination Inhibition Antibody Titres of Vaccinated Shikabrown Cocks

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Abstract: Fifty 20 week old Shikabrown cocks consisting of 22 red Shikabrown and 28 white Shikabrown cocks were purchased from the National Animal Production Research Institute, Shika and used for this study. Twenty-five of the cocks made up of 12 red and 13 white cocks selected on basis of weight were infected with 2 ml of $10^{6.0}$ EID₅₀ of a Velogenic Kudu 113 strain of Newcastle disease virus intranasally and orally. The remaining twenty-five cocks made up of 14 red and 11 white served as control. Blood samples were taken from the wing veins of both infected and control cocks and centrifuged in a Hermle Z364 centrifuge at 251.6 g for packed cell volume, total protein and Newcastle disease antibody titres. There was no significant difference in the packed cell volume of the control and infected red Shikabrown cocks. Similarly there was no significant difference in the packed cell volume of the control and infected white Shikabrown cocks, although the infected cocks had slightly lower values. Total protein did not show any significant difference between the control and infected red cocks and between the control and infected white cocks. The antibody titres of the control red and white cocks were significantly ($p < 0.05$) lower than those of the infected red and white cocks. This finding showed that the challenged red and white cocks had high antibody titres and a slight drop in packed cell volume. The mean antibody titres of 1.9 ± 0.7 to 4.6 ± 0.4 log₂ provided protection to the Shikabrown cocks against the velogenic Newcastle disease virus since none of the challenged cocks died. This study suggests that in an endemic environment like Zaria, poultry farmers keeping Shikabrown chickens should vaccinate them against Newcastle disease. Challenging the red and white Shikabrown cocks with the velogenic Newcastle disease virus increased their protection against the Newcastle disease.

Key words: Shikabrown cocks, Newcastle disease virus, Immunization of chickens

INTRODUCTION

Immunization of chickens with vaccines can improve egg production; prevent losses from clinical disease and reduction in mortality. The design of vaccination programmes to accomplish protection for the vaccinated animals must be carefully done to minimize interference from maternal antibody (Ivanyi, 1970; Beard and Brugh, 1975; Westbury *et al.*, 1984). The titration of serum for Newcastle Disease Virus (NDV) Hemagglutination Inhibition (HI) antibody is a convenient way to evaluate the response to the vaccination (Beard and Brugh, 1975).

The degree of protection from morbidity or mortality is related to the virulence and dose of the challenge NDV, the route of infection, the age of the chicken at the time of infection and the previous immunization history (Beard and Brugh, 1975). Most chickens with negative antibody titres (1:10) die if infected with virulent virus, while those with titres of 1:20 to 1:40 have a high probability of survival.

The ability of NDV and other paramyxoviruses to agglutinate red blood cells is due to the binding of the hemagglutinin-neuraminidase protein to receptors on the

surface of the red blood cell (Burnet, 1942). Chicken red blood cells are usually used in Hemagglutination (HA) tests, but Newcastle disease virus causes agglutination of all amphibians, reptilian and avian cells (Lancaster and Alexander, 1975). Winslow *et al.* (1950), showed that human, mouse and guinea pig red blood cells were agglutinated by all NDV strains tested but the ability to agglutinate cattle, goat, sheep, swine and horse cells varied with the strain of NDV. NDV and other paramyxoviruses may bring about hemolysis of red blood cells or fusion of other cells by essentially the same mechanism (Ackerman, 1964). Hyperproteinaemia can be caused by dehydration which is common in acute and chronic inflammation, liver disease, neoplasia, viral and rickettsial disease, fungal and protozoal disease (Bush, 1991). Hypoproteinaemia results from inadequate protein consumption, small intestine malabsorption, liver disorder, congestive heart failure and increased protein loss through hemorrhage and burns (Bush, 1991).

Vaccination with live vaccine strains leads to increase in antibodies (Timm and Alexander, 1977). It has been shown that vaccinated chickens challenged with NDV

can become infected due to stress, nutritional deficiencies, toxins, genetic immuno-deficiency, poor storage and administration of vaccines and rough handling of birds (Abdu *et al.*, 2006). Information on the effect of NDV challenge on vaccinated Shikabrown cocks is not available. This study was therefore designed to determine the effect of a velogenic Newcastle disease virus on the packed cell volume, total protein and hemagglutination inhibition antibody titres of vaccinated Shikabrown cocks.

MATERIALS AND METHODS

Location: The research was conducted at the Faculty of Veterinary Medicine, Ahmadu Bello University, Samaru, Zaria. Samaru is situated in the Northern Guinea savannah zone between latitudes 11° and 12°N and between longitudes 7° and 8°E, at an elevation of 650 meters above sea level. Samaru has two seasons; dry (October to April) and rainy (May-October) with an annual rainfall of 1107 mm (Rekwot, 2000).

Experimental cocks: Fifty 20 week-old Shikabrown cocks consisting of 28 white and 22 red strains were purchased from the National Animal Production Research Institute Shika, Zaria and used for this study. The cocks had been routinely vaccinated against ND, using the Vom produced vaccine (National Veterinary Research Institute, Vom) before purchase.

Management of cocks: The cocks were kept in pairs in cages and fed a layer mash containing 18% crude protein, 95.6% dry matter, 17% crude fibre and 3% nitrogen *ad libitum*. Water was provided *ad libitum*. All the necessary veterinary screening and treatment for ecto- endo- and hemoparasites were carried out according to standard procedure. For a period of six weeks the cocks had their cloacal temperatures taken, using a digital thermometer and were weighed weekly. The cocks were infected with 2 ml of $10^{5.0}$ EID₅₀ of a velogenic 113 strain Kudu of Newcastle disease virus (National Veterinary Research Institute, Vom) intranasally and orally, after screening.

Blood sampling: Blood samples were collected from the fifty cocks, consisting of 22 red Shikabrown and 28 white Shikabrown for 6 weeks pre- and post-infection. Twenty-five of the cocks consisting of 8 red Shikabrown and 17 white Shikabrown were infected with the virus. 2 ml of blood samples were taken from the wing vein for Packed Cell Volume (PCV) and total protein determination. The blood was centrifuged at 251.6 g for 15 min to harvest sera for the determination of antibodies against Newcastle disease virus using hemagglutination inhibition test.

Determination of Newcastle disease antibody titres: Hemagglutination test was carried out by micro test

method using two-fold serial dilution wells of 50 µl of reconstituted vaccinal virus antigen and 50 µl of 1% chicken red blood cells (Beard, 1980). An equivalent volume of chicken red blood cells suspension was also added to wells containing PBS with pH 7.2 alone to serve as control. The plate was gently tapped to mix the contents and after 45 min of incubation at room temperature, the end point of the Hemagglutination (HA) was read. The titre was taken as the reciprocal of the highest dilution giving a 100% agglutination of the 1% chicken red blood cells. The last well that showed a complete hemagglutination is said to contain one Hemagglutinating (HA) unit. If the seventh well (1:64) is the last to show complete hemagglutination, then the original material contained 64 (2^6) HA units. One HA unit is defined as the highest dilution of antigen, which will completely agglutinate a test dose of red blood cells under standard conditions of temperature and time of incubation.

Determination of packed cell volume and total protein: Hematocrit centrifuge technique was used to determine the packed cell volume (Schalm *et al.*, 1975). Blood was collected once a week from the wing vein of the cocks using heparinized capillary tubes on each day of collection. The capillary tubes were placed on a centrifuge pad covered and centrifuged at 251.6 g for 15 min and PCV was determined using the hematocrit reader.

After determination of the PCV, the portion of the capillary tube containing the plasma was separated from the packed red cells. The plasma was then poured into the Goldberg refractometer or Total Solids (TS) meter and the reading taken.

Statistical analysis: Data on packed cell volume, total protein and hemagglutination inhibition test were subjected to analysis of variance using the Statistical Analysis System (SAS) and differences between treatment means were determined by Duncan's multiple range tests (Helwig and Council, 1979).

RESULTS

The packed cell volume of the control red cocks (41.9±0.9%) were slightly higher than that of the control white cocks (41.2±1.0%) and infected red (41.1±1.2%) and infected white cocks (39.5±0.8%) at week 1 post infection. The infected red (41.1±1.2) and control white cocks had almost the same percentage packed cell volume value at week 1 (41.2±1.0%). At week 2 the PCV of the control and infected red cocks were almost the same (Table 1). The control white cocks had slightly lower packed cell volume (34.5±0.8%) than the infected white cocks (38.5±0.7). Both the control and infected red cocks had higher PCV than the control and infected white cocks at week 2 (Table 1).

Table 1: Packed cell volume (PCV), hemagglutination inhibition antibody titres (HIT) and total protein of control and infected Shikabrown cocks

P	B	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
		C	I	C	I	C	I	C	I	C	I	C	I
HIT	RSB	4.5±0.5 ^a	7.0±0.7 ^b	4.0±0.5 ^a	9.4±0.6 ^b	3.1±0.4 ^a	8.5±0.5 ^b	2.9±0.4 ^a	8.1±0.5 ^b	2.9±0.4 ^a	7.0±0.5 ^b	1.5±0.3 ^a	5.4±0.4 ^b
(Log ₂)	WSB	3.4±0.6 ^a	8.0±0.5 ^b	4.0±0.5 ^a	9.4±0.4 ^b	2.4±0.4 ^a	7.8±0.3 ^b	2.2±0.4 ^a	8.4±0.4 ^b	2.6±0.4 ^a	7.4±0.4 ^b	1.1±0.3 ^a	5.5±0.3 ^b
PCV	RSB	41.9±0.9	41.1±1.2	39.6±0.7 [*]	39.0±0.1	41.7±1.3	41.5±1.7	41.5±0.1	41.1±1.3	43.9±1.1	40.6±1.5	43.5±0.9	43.1±1.2
(%)	WSB	41.2±1.0	39.5±0.8	34.5±0.8 [*]	38.5±0.7 ^b	46.1±1.5 [*]	40.0±1.2	46.3±1.1 [*]	41.0±0.9	46.0±1.2 [*]	42.0±1.0 ^b	39.2±1.0	40.3±0.8
TP	RSB	4.6±1.6	4.5±2.0	4.5±0.1	4.5±0.2	4.8±0.1	4.9±0.2	4.5±0.1	4.8±0.2	4.6±0.2	4.5±0.4	4.7±0.1	4.7±0.2
(g/dl)	WSB	4.7±1.8	4.1±1.4	4.6±0.1	4.4±0.1	4.9±0.1	4.6±0.1	4.6±0.2	4.7±0.1	4.7±0.2	5.0±0.1	4.8±0.2	4.9±0.1

abMeans with different letter superscripts within treatments and asterisks(*) between treatments are significantly (P<0.05) different within rows.

Where P = Parameter; B = Breed; C = Control; I = Infected

The packed cell volume of the control white cocks at week 3 were higher than that of the control red cocks and infected red and white cocks. The PCV of the control and infected red cocks was about the same at week 3. At week 4 the PCV of the control white cocks was higher than that of the control red cocks and infected red and white cocks. The PCVs of the control red cocks and infected red and white cocks were almost of the same value.

The PCV of the control white cocks was again higher than that of the control red cocks and infected red white cocks at week 5 (Table 1). The infected red cocks had the least PCV followed by the infected white cocks. The PCVs of the control and infected red cocks were almost the same at week 6. The PCV of the control white cocks was the lowest. There was no significant (p>0.05) difference in the PCV of the control and infected red cocks; the infected cocks had slightly lower PCV (Table 1).

The total protein values of the control and infected red cocks were about the same at week 1, 2, 3, 4, 5 and 6 (Table 1). The total protein of the infected white cocks was slightly lower than that of the control cocks at week 1, 2, 3 and slightly higher at week 4, 5 and 6. The control red cocks had significantly (p<0.05) lower antibody titres than the infected red cocks at week 1, 2, 3, 4, 5 and 6. Similarly, the control white cocks had significantly (p<0.05) lower antibody titres than the infected white cocks throughout the six-week period of the research work.

DISCUSSION

In this study there was no significant decrease in the mean packed cell volume post infection throughout the six-week period of observation in both the red and white cocks. This disagreed with the findings of Oladele (2004) who found a decrease in the packed cell volume 2 days post infection and which continued up to day 11. The cocks used in this study were vaccinated hence had immunity against Newcastle disease virus which prevented destruction of their red blood cells by the velogenic Newcastle disease virus.

There was no significant difference between the mean total protein values of the control and infected red and white cocks used in this study. Oladele (2004), however found a decrease in the total protein values from day 3 and it reached its lowest value of 2.6±0.15 g/dl by day

seven post infection. This was followed by a gradual increase until day 21 when there was a slight drop in value and stabilized by day 42 post infection. His result disagreed with the present finding probably due to the differences in breed of chickens used and the vaccination history of the cocks used by Oladele (2004) and in the present work. In this study, vaccinated Shikabrown cocks at 20 weeks old were used while Oladele used day old Shaver brown unvaccinated cockerels.

The high mean antibody titres of the infected red and white cocks showed that challenging the cocks with the velogenic virus increased the antibody titres of the cocks giving them more protection from infection by the Newcastle disease virus. The highest mean antibody titre in this work was 9.4±0.6 log₂ for infected cocks, at 2 weeks post infection and thereafter decreased to the lowest level of 5.4±0.4 log₂ at week 6-post infection. This agrees with the findings of Oladele (2004) who infected Brown Shaver birds with Kudu strain 113 NDV and reported that antibody titres increased by day 2-post infection up to a maximum value of 9.7±1.2 log₂ by day four-post infection and thereafter decreased.

The mean antibody titre of 1.9±0.7 to 4.6±0.4 log₂ provided protection to the red and white cocks against the velogenic Newcastle disease virus. In an endemic environment like Zaria farmers keeping red and white Shikabrown chickens should vaccinate them against Newcastle disease. The red Shikabrown cocks with slightly higher mean antibody titres withstood the challenge by the velogenic Newcastle disease virus better than white Shikabrown cocks. The packed cell volume of the red Shikabrown cocks was slightly higher than that of the white Shikabrown cocks because they had higher mean antibody titres and withstood the challenge of the Newcastle disease virus better than the white Shikabrown cocks.

The red Shikabrown cocks reacted better to vaccination than the white Shikabrown cocks. The slightly higher antibody titre in the red Shikabrown cocks is an indication that they responded better to previous vaccination against Newcastle disease; based on this finding it was expected that the red Shikabrown cocks would better resist infection by the Newcastle disease virus than the white Shikabrown cocks. This speculation has been strengthened by the fact that the changes in the mean packed cell volume of the red Shikabrown

cocks was not affected as much as that of the white Shikabrown cocks after challenge with the velogenic Newcastle disease virus.

From this study hemagglutination inhibition Newcastle disease antibody titres of as low as $2 \log_2$ were able to protect birds challenged with the velogenic Newcastle disease virus used. This value was below the recommended value of $5 \log_2$ (Allan *et al.*, 1978). It is therefore possible that birds with antibody titres of less than $5 \log_2$ will be protected when exposed to a velogenic Newcastle disease virus.

The study also confirmed that vaccinated birds could be infected with velogenic Newcastle disease virus when exposed. The challenge of the red Shikabrown and white cocks with the velogenic Kudu 113 strain of Newcastle disease virus in this study led to an increase in antibody titres in the cocks hence more protection from Newcastle disease infection.

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