Journal of Animal and Veterinary Advances 5(6) : 503-506, 2006

Newcastle Disease Antibodies in Parent Stock, Yolk and Chicks

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Abstract: Parent stock, Yolk and chicks from five commercial hatcheries at Zaria and Kaduna were evaluated for antibodies to Newcastle Disease (ND). The ND vaccination history and the age (s) of the breeders were found out from the hatcheries. Haemmagglutination Inhibition (HI) test was used for the detection and quantification of antibodies (AB) to ND in the parent stock, yolk and in the day old chicks. The HI titre of each bird, yolk and chick was determined and expressed in $\log_2$ and the mean titres for the parent stock, yolk and chicks were calculated. The ages of the parent stock ranged from 30 weeks for hatchery A to 95 weeks for hatchery B, all the hatcheries used to administer ND vaccine Hitchner B1 (intra ocular) at day old, La Sota at 3-5 weeks, Komorov at 6-8 weeks and another dose of komorov at 16-18 weeks. There after the birds were given booster ND vaccination using ND La Sota vaccine or killed oil emulsion Komorov vaccine. The HI.AB titre for the parent stock ranged from 5.8±1.6 to 7.6±1.6 $\log_2$ and the titre for the egg also ranged from 5.1±0.9 to 6.2±1.7 $\log_2$. The HI titre for the chick ranged from 3.4±1.4 to 5.3±1.1 $\log_2$ at 1day (d) old and it declines to a lowest value of 1.1±1.2 $\log_2$ at 28d. The study shows that all the parent stock had protective AB titre (5.8±1.6 to 7.6±1.6) and there is a correlation between the AB titre in the serum of the parent stock and that of the yolk. The AB titre in the chicks declines to non-protective level (1.5±1.1 $\log_2$) at 14 d. It was concluded that although the titres of the parent stock is within protective level, it is still low and that the yolk of fertile eggs can be used to determine the AB titre of the parent stock.

Key words: Newcastle disease, parent stock, yolk and chicks

INTRODUCTION

Newcastle Disease (ND) is a viral disease that affect a wide range of poultry species irrespective of age. The disease is a major set back in poultry production throughout the world. In Africa and Asia it is a major constraint to the development of both industrial and village poultry production[1,2].

Newcastle disease was reported to be endemic in Nigeria and there is seasonality in its prevalence (10 ; 7). In order to control the disease various vaccination programmes against the disease are employed in different parts of Nigeria. In Zaria the vaccination programme is as follows, Hitcher B1 (I/O) at 1-7d, La Sota at 3 week (WK) and Komorov at 6 and 16WK[3]. After the 16th WK Breeders and layers are revaccinated. Usually with NDV La Sota vaccine at different intervals ranging from 4WK to 12WK.

Breeders vaccinated against ND or infected with field ND virus are able to transfer antibodies to the embryo through the yolk[4-6]. Chicks from immunized parents possess high level of Maternal Antibodies (MA) which protect the chicks against virulent and vaccine virus[4,5,7]. It was also reported that MA are protective[7] and the MA are also reported to neutralize vaccine virus if the chicks are vaccinated in the presence of high level of MA[8,6].

Since the MA are transferred to the embryo through the yolk, it was suggested by earlier workers that the level of HI antibodies in the yolk may be similar to the HI antibody titers in the serum of the parent stock. The level of HI antibodies in the yolk may serve as an indicator of the HI AB in the parent stock and that it may not be necessary to bled the parent stock for seromonitoring instead the fertile eggs can be used[9]. This study was designed to measure the level of HIAB titres in parent stock, eggs and chicks and to Find out whether there are correlations between the titres in the serum of parent stock and yolk, so that the yolk can be used to determine the titre of the flock instead of bleeding the hens. as well as to determine the age at which the MA level in chicks is not protective.

MATERIALS AND METHODS

Parent stock: Twenty-five (25) female parent stock were randomly bled from 4 out of the 5 commercial hatcheries used in this study. Three of the commercial hatcheries are

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at Kaduna and 2 are at Zaria. A total of 100 females parent stock were bled for seromonitoring for AB to ND using the haemmagglutination Inhibition (HI) test. One of the hatcheries did not allow the parent stock, to be bled the ages of the parent stock and ND vaccination history were obtained from the hatcheries.

Chicks: 50-day-old cockerels were bought from each of the five hatcheries. Making a total of 250 day old cockerels. The chicks were not vaccinated at the hatchery before purchase.

Unfortunately the 50 cockerel obtained from hatchery A were vaccinated with Hitchner B1 vaccine, therefore they were not used for the study.

Eggs: A total of 25 fertile hatchery rejected eggs were purchased from each of the hatcheries studied. A total of 125 fertile eggs were used for the test.

Serum samples: Twenty-five females parent stock were randomly bled through the wing vein and 2 mL of blood was collected in to a test tube, serum was extracted from the blood by centrifugation at 2,000 rpm for 5 min. The sera were stored at -4°C in the freezer until used. Twenty-five chicks out of the 50 obtained from each of the 4 hatcheries were bled at 1 d, 7 d, 14 d, 21 d and 28 d. The chicks were bled intracardially on 1 d, 7 d,, 14 d and there after through the wing vein. A minimum of 0.5 mL of blood and a maximum of 1 mL of blood was collected, serum was extracted from the blood by centrifugation at 2,000 rpm. for 5 min; the sera were stored at - 4°C in the freezer until used.

Egg yolk: The egg is aseptically broken into a clean Petri dish. 1 mL of yolk was collected using a clean syringe and transferred into a clean test tube. 2 mL of PBS (PH 7.4) was added to the 1ml of the yolk. Giving a mixture of 1 part yolk and 2 parts PBS the test tube is shaken gently so that the mixture will mix well. The mixture was centrifuged at 2,500 rpm for 15 min. Thereafter the mixture was allowed to stand. The clear fluid between the lipid droplets on the top and yolk solids at the bottom of the test tube was used for the haemagglutination inhibition (HI) test as described by Allan and Gough[10].

Chickens Red Blood Cells (RBC): Five millilitre of chicken RBC was collected in to 5 mL of Alsever's anticoagulant solution and gently mixed. The RBCs were washed 3 times with Phosphate Buffered Saline (PBS) pH 7.4 by centrifugation at 2,000 rpm for 5 min. A 0.25% solution of RBC was prepared by transferring 0.05 mL of RBC into 20 mL of PBS for use in Haemmagglutination (HA) and HI tests as described by Allan and Gough[10].

Antigen: ND La Sota virus obtained from the National Veterinary Research Institute, Vom was used as the antigen for the HI test. The HA titre of the ND La Sota virus was determined as described by Allan and Gough[10,7] and diluted to contain 4 HA units. This concentration of the antigen is also used for the HI test.

Serological Test (HI test): The HI test was used for the detection and quantification of antibodies against ND virus as described by Allan and Gough[10]. Sera from all the female parents and chicks were tested by this procedure. So also the yolk. The HI titre of each parent, chick and yolk was expressed in $\log_2$ and the mean titre for each was calculated. The HI test was performed using beta-technique (constant virus and varying serum) against 4 HA units of ND virus computed from the results of the HA titration. Double dilutions (0.05 mL) of the different sera or yolk extract were reacted with 0.05 mL of the 4 HA unit of the antigen per well. The mixture was allowed to stand for 30 min at room temperature for antigen-antibody reaction to take place, 0.05 mL of 0.25% dilution of washed chicken RBC was added to all the wells and shaken gently to mix and incubated at room temperature for 45 min. Negative control wells were also included. The titres were taken as the reciprocal of the serum or egg yolk dilutions at which there was complete HI of the chicken RBC.

RESULTS

Age of parents stock and vaccination history: The ages of the parent stock ranges from 30WK for hatchery A to 95 WK for hatchery B the hatcheries used to administer Newcastle disease vaccine intraocular, at day old, La sota at 3-5 WK, Komorov at 6-8 WK and another dose of Komorov at 16-18 WK there after the birds were given booster ND vaccination using ND La Sota or killed oil emulsion Komorov vaccine.

Mean HI titre in parent stock: Table 1 shows the shows the mean HI AB titre for parent stocks, the mean titre for the hatcheries were: 5.8±1.6 log2, 6.8±1.3 log2, 7.6±1.62 log2 and 5.8±0.8 log2, for parent stock from hatcheries A, B, C and D, respectively. The parent stock from hatchery E were not tested.

Mean HI titre of fertile eggs: Table 2 shows the mean ND HI AB titre of fertile eggs. The mean titres for the eggs

Table 1: Mean newcastle disease HI antibody titre in parent stock from Zaria and Kaduna

Hatchery	No. tested	No. positive (%)	Mean antibody titre $\log_2$
A	25	25(100)	5. 8±1.6
B	25	25(100)	6.8± 1.3
C	25	25(100)	7.6±1.6
D	25	25(100)	5.8±0.8
E	NT	NT	NT

NT = Not tested

Table 2: Mean newcastle disease HI antibody titre in fertile eggs from Zaria and Kaduna

Hatchery	No. tested	No. positive (%)	Mean ntibody titre ($\log_2$)
A	25	25(100)	6.2±1.7
B	25	25(100)	5.4±1.6
C	25	25(100)	5.1±0.9
D	25	25(100)	5.6±0.8
E	25	25(100)	6.0±0.2

Table 3: Mean newcastle disease HI antibody titre in chicks from Zaria and Kaduna

	Age in days Mean HI Anti body titer in (log 2)				
Hatchery	1	7	14	21	28
A	NT	NT	NT	NT	NT
B	5.3±1.1	3.6±0.9	1.8±1.7	1.8±1.5	1.1±1.2
C	4.8±1.7	3.3±0.9	2.9±0.8	2.3±1.2	1.8±1.3
D	3.8±1.0	2.0±1.1	2.0±1.2	1.3±0.9	1.2±1.1
E	3.4±1.4	3.0±1.0	1.5±1.1	1.2±1.0	1.1±1.2
Overall mean titre	4.3± 1.0	2.9± 1.5	2.0± 1.3	1.9± 1.3	1.3±1.6

NT: not tested

from the different hatcheries are as follows: 6.2±1.7 log2, 5.4±1.6 log2, 5.0±0.9 log2, 5.6±0.8 log2 and 6.0±0.2 log2 for hatcheries A, B, C, D and E, respectively.

Mean HI titre of chicks: The chicks from hatchery A were not tested because they were vaccinated against ND with Hitchner B1 before collection. The HI AB titre for chick from hatchery B. was 5.2±1.1 log2, 3.6±0.9,Log2 1.8±1.7 log2 and 1.1±1.2 log2 at 1d, 7d,14d and 28d, respectively. The mean HI AB titers for hatchery C chick were: 4.8±1.7 log 2, 2.0±0.8 log 2 and 1.8±1.3 log 2 at 1d, 7d and 28d, respectively. The mean HI titer for hatchery D chick were: 3.8±1.0log 2, 2.0±1.2 log 2 and 1.2±1.0 log 2 at 1d, 14d and 28d, respectively. While the mean HI titer for hatchery E chicks were: 3.4±1.4 log 2, 1.5±1.1 log 2 and 1.1±1.2 log 2 at1d, 14d and 28d, respectively. (Table 3)

DISCUSSION

The study shows that all the parent stock in all the hatcheries tested had protective level of HI antibodies. It was[11] reported that a titre of 3.0 $\log_2$ may be considered as protective, it is important to note that the titres of the birds from all the hatcheries range from 5.8±0.8 to 7.6±1.6 $\log_2$. These titre levels may protect against mortality but may not protect against drop in egg production[11].

The tires in the yolk are similar to those of the parent stock. This finding is similar to that of[9] and further support the projection that the HI AB titres in the yolk may be used to evaluate the Anti body titres of the parent stock. It is worthy to note that the egg yolk titres recorded in this work are slightly lower than the HI AB titres in the parent stock. Pearson correlation co-efficient show that there is a correlation between the HI AB titres of the parent stock and those of the yolk.

It is important to note that at 1d all the chicken from the 5 hatcheries have titres from 3.4 to 5.3 $\log_2$. It is only chicks from hatchery B that have ND AB higher than the protective level as reported by[11]. This is slightly different from what was reported by[12].

The HI, AB titres in the chick from all the hatcheries gradually decay from 1 d to 28 d. This finding is similar to that of[12]. It was reported by [5]that the MA AB from chicks from vaccinated parent decline below protective level within 15-20 d but in this work the MA declines to below the protective level within 14 d. Earlier workers like[13] suggested that chicks in an area where ND outbreak is not a threat should not be vaccinated before the age of 5 wk when the MA has completely waned. In an area like Zaria and Kaduna where ND is endemic and there is the possibility of early exposure the suggestion of early vaccination followed by revaccination four or five weeks later[13] is the most laudable suggestion. Based on this, the present practice of vaccinating the day old chick with Hitchner B1 vaccine intraocular or through aerosol is adequate but farmers must boost their flocks at 3-5 weeks as is currently practiced. It was concluded that although the HI AB titres in all the hatcheries are at the protective level, nevertheless the titres are at the borderline for protection and there is a correlation between the HI AB titer in the parent stock and yolk therefore the yolk may be used to assess the AB titre of parent stock. There by avoiding the stress of seromonitoring parent stock So also the MA in all the chicks from the commercial hatcheries tested decayed to below p rotective level by 14d.

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