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THE INFLUENCE OF MATERNAL ANTIBODY ON VACCINATION AGAINST NEWCASTLE DISEASE VIRUS IN CHICKEN

Aniefiok A. Okokon[1], Daniel C. Ezeokoli [2] and Wilfred S. Ezema[3]*

[1]College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; [2]Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, Nigeria and [3]Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

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ABSTRACT

Newcastle disease is one of the most devastating diseases of poultry worldwide. Veterinarians in Nigeria frequently encounter outbreaks of Newcastle disease in vaccinated commercial poultry farms; causing concern about the protective immunity conferred by commercially available vaccines. Such outbreaks in vaccinated flocks could result from, among other things, poor immunologic response arising from immunological interference by maternally derived antibodies (MA). This study sought to evaluate the role of MA in the responses of cockerel chicken to Newcastle disease vaccination, specifically, the rate of decay of MA in chicks from different hatcheries, and the effect of MA on the response of chicks to Newcastle disease vaccination. In the first experiment, 160 day-old-chicks bled at intervals from day 0 to day 21 were used to determine the rate of MA decay, using the haemagglutination inhibition (HI) test. The lowest antibody titre (log 0.4) recorded, was on day 15, however, low levels persisted till day 21. The rate of antibody decay (t½) was 3 days for hatchery A and 4.68 days for hatchery B. In the second study, 200 chicks were used to evaluate the effect of MA on response to Newcastle disease vaccination. Five 5 groups of chicks (A, B, C, D and E) were vaccinated through the intraocular route with Newcastle disease vaccine at different times post-hatching (respectively on days 1, 4, 8 and 14 for groups A, B, C and D); group E remained as unvaccinated control. Serum samples were collected on days 14, 21, and 28 (post vaccination) and tested for antibody levels using the HI test. Birds vaccinated on days 1, 4, and 8 had high antibody titres at vaccination but responded poorly to vaccination. Birds vaccinated on day 14 had low antibody titres at vaccination and responded very well to vaccination. It was concluded that the level of MA at the time of vaccination was critical to the subsequent immunological response. The study also suggested that vaccination protocol for Newcastle disease should take into consideration the decay of MA in the flock, and recommended a practical approach to effective Newcastle disease vaccination.

Keywords: Newcastle disease; Maternal antibody; Vaccination; Haemagglutination Inhibition test.

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***Correspondence: E-mail: wilfred.ezema@unn.edu.ng; Tel: +2348037856356**
ISSN: 2315 – 6856; e-ISSN: 2636 – 5553; *Website: www.jvasonline.com*

INTRODUCTION

Newcastle disease (ND) is a viral disease of poultry affecting domestic and wild avian species and humans [1]. Newcastle disease was first reported in Java, Indonesia in 1926 and subsequently in Newcastle-upon-Tyne (whence it got its name) England in 1927. The first documented outbreak of ND in Nigeria occurred between December 1952 and February 1953 in and around Ibadan [2]. Since then, the disease has become a problem in the country [3]. It is widespread in domestic and exotic chickens [4]. Despite the advances made in the diagnosis of and vaccination against Newcastle disease since it was first described, the disease continues to negatively impact poultry producers by infecting birds worldwide [5,6].

Commercial chicken production in Nigeria uses exclusively exotic chickens, reared intensively or semi-intensively. In most parts of the country, ND is seen and diagnosed throughout the year in commercial flocks and the incidence varies with season [7,8]. Vaccination is regarded as the most effective tool in the control of ND disease [9,10]. However, outbreaks of ND are often reported in many flocks despite rigorous vaccination programs [11]. Important potential causes of such outbreaks include: vaccination in the face of high levels of maternal antibodies, vaccination with vaccines inadvertently inactivated by breaks in the cold chain, which would render such vaccines less effective, emergence of new strains of NDV virus different from the vaccine strains, and poor or inappropriate vaccination programmes [12,13].

Researchers have shown that in the presence of high levels of maternal antibodies during the first two weeks of life, the vaccine virus may be neutralized [14,15], resulting in vaccination failures. Many current ND vaccination protocols may not have taken into account the fact that day-old chicks inherit different levels of maternal antibodies at hatching, therefore the decay and disappearance of the maternal antibodies will vary in birds acquired from different hatcheries, and even different batches from the same hatchery, thereby influencing the immune response of such flocks to currently used ND vaccination programmes.

A better understanding of maternal antibody decay and influence of maternal antibody on response to vaccination will enable clinicians know the best vaccination programmes to follow. This study aims to determine the rate of maternal antibody decay in chicks from different hatcheries and evaluate the effect of different levels of maternal antibodies in the response of chicks to ND vaccination.

MATERIALS AND METHODS

Experimental birds

Three hundred and sixty unvaccinated cockerel chicks were bought at day old from two commercial hatcheries and used for this study. The birds were reared in cages. Water and commercial feed were provided *ad libitum* to all the chickens. Prophylactic medications against bacteria and *Eimeria* species infections were administered from day 8 to day 12 of the experiment using gentamicin (Pfizer pharmaceutical company) and sulphaquinoxaline (Pfizer pharmaceutical company) at 1.0 mg/kg/day and 1g/l drinking water respectively. Both drugs were administered orally in drinking water.

Newcastle disease virus antigen for haemagglutination (HA) antigen.

Newcastle disease virus-antigen was obtained from the National Veterinary Research Institute (NVRI), Vom and used as antigen for the HA and Haemagglutination–inhibition (HI) tests.

Newcastle disease vaccine i/o (Hitchner B1)
The vaccine was sourced from NVRI (Umudike office, Abia State).

Experimental design
The study was conducted in two stages.

Experiment 1: To determine maternal antibody titre and rate of maternal antibody decay.
One hundred and sixty unvaccinated cockerels (80 chicks from each hatchery) were used for this study. Blood was obtained from ten tagged chicks (from each hatchery), on days 1, 3, 6, 9, 12, 15, 18, and 21 post hatchery (PH).

Experiment 2: To study the effect of Newcastle disease virus maternal antibody on immune response of chicks to vaccination with the Newcastle disease vaccine.
Two hundred unvaccinated cockerels from one hatchery, were used for this experiment. The chicks were randomly assigned to 5 groups (A, B, C, D, and E) of 40 chicks each, and were vaccinated as follows: Groups A was vaccinated on day 1, Group B on day 4, Group C on day 8 and Group D on day 14. Group E served as unvaccinated control. Pre-vaccination blood samples were obtained on day 1 from ten tagged chicks in each Group to determine initial NDV maternal antibody titres. Thereafter, the Groups (A, B, C and D) were vaccinated on days 1, 4, 8 and 14 respectively by instilling one drop (0.05 ml) of NDV (Hitchner B1 strain vaccine) reconstituted as specified by the manufacturer (10 ml of saline to 200 dose vial) into the eye of each chick. Subsequently, blood samples were obtained from ten tagged chicks in each group on days 14, 21, and 28 (post vaccination). All the blood samples were kept at an angle of 45^{o} to clot. The serum was decanted into properly labeled sample bottles and stored at -20°C until tested for antibody content using the Haemagglutination -Inhibition (HI) test.

Serological tests
The chicken blood cells used for haemagglutination (HA) test, were collected in the anticoagulant sodium citrate. The red blood cells (RBC) were washed with phosphate buffered saline (PBS) of pH 7.0 by centrifuging the blood-PBS mixture at 3000 rpm for 5 minutes. The washing was repeated three times and the supernatant was discarded at each wash. Thereafter, the hematocrit value was determined using the micro-capillary tube centrifugation method. To prepare 0.6% RBC concentration the formula: X = PV/H was used [16,17] .
Where:
X = Volume of the washed blood to be added,
P = Percentage of RBC concentration needed (0.6%).
V = Volume of RBC solution to be prepared.
H = Hematocrit value of the washed RBC.

The Newcastle disease virus antigen obtained from NVRI was used for haemagglutination (HA) test as described [18]. Also, haemagglutination inhibition (HI) test was performed as described by (18).

Data analysis

Calculation of antibody decay half-life (exponential decay)

The antibody half-life was calculated according to the method described in matrixlab calculations (www.matrixlab-example.com).

The antibody titre obtained for each day of sampling was expressed as geometric mean titre. Log geometric mean titre for each day of bleeding was plotted against age of the birds to show the extent of decay of the maternally derived antibody. The One-way Analysis of Variance (ANOVA) was used to assess the significant differences (at 5%) between the 5 groups on day 21 and day 28.

RESULTS

Maternal antibody decay

In experiment 1 which evaluated the decay of maternal antibodies in chicks from two commercial hatcheries (A and B), the geometric mean titres (GMT) of the antibody levels from day old (Day 0) to the end of the study (Day 21) are presented in Fig I.

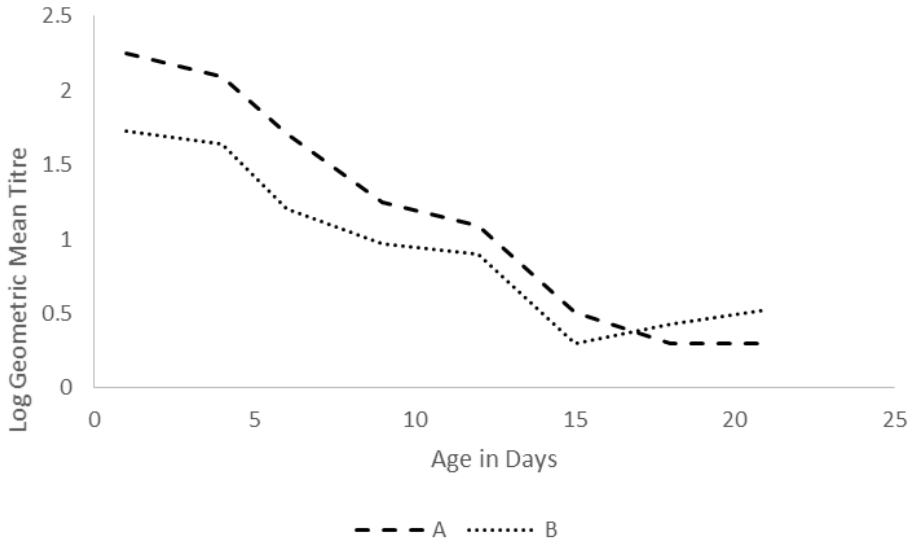

Figure I: Log of the geometric mean titre of antibodies against ND in unvaccinated chicks from two commercial hatcheries A and B.

Day-old chicks from the two hatcheries have different levels of maternal antibodies at the time of procurement from the hatchery, but the rates of decay of maternal antibodies from both hatcheries were similar; HI antibody titres were ≥ log 2.0 in both hatcheries on day 1, subsequently this decreased gradually to ≤ log 0.5 at day 15 of age. The rate of decay (t½) of maternally derived antibody in the chicks from Hatchery A was about half every 3 days (t½=3), in Hatchery B it was about half every 4.68 days (t½=4.68). Antibody titres persisted at low levels in the birds from both hatcheries from day 15 until the end of experiment on day 21.

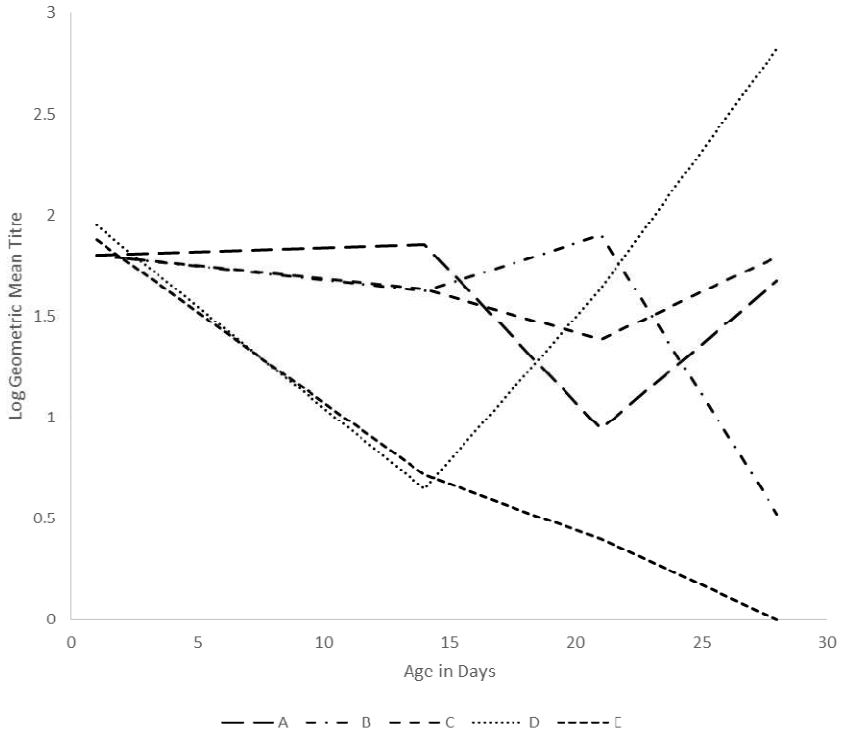

Figure 2: log geometrical mean titres of antibodies obtained from chickens vaccinated with Newcastle disease vaccine on various days post hatchery. A = Birds vaccinated on Day 1; B = Birds vaccinated on Day 4; C = Birds vaccinated on Day 8; D = Birds vaccinated on Day 14; E = Non-vaccinated control.

Effect of maternal antibodies on immune response

The results of experiment 2 which evaluated the effect of maternal antibodies on the response of chicken to ND vaccine are reported in Figure 2. In group A vaccinated on day 1, titres lingered around the pre-vaccination levels of log 1.81 until day 14 of age, then dropped rapidly to < log 1.0 by day 21, before rising again to pre vaccination levels by day 28. In Groups B and C (vaccinated on day 4 and day 8 respectively), titres stayed at pre-vaccination levels (log 1.7) till day 15; then rose slightly in Group B before dropping rapidly to log 0.5. In Group C, titres dropped slowly to < log 1.5 by day 20, then rose to log 1.7 by day 28. The response was dramatically different in Group D vaccinated on day 14. Group D, showed the classic antibody response to vaccination which is rapid and steady rise in titre. Antibody titres rose rapidly from the pre-vaccination level of log 0.64 to >log 2.80 within 2 weeks of vaccination (by day 28 of age).

Table I shows titres at Day 1 and Day of vaccination, to highlight the differences in the titres between day of vaccination and the response at day 21 and day 28. Groups A, B, and C have high levels of antibody on the day of vaccination (log 1.70 – log 1.81); only Group D has low antibody titres (log 0.64) at the time of vaccination, and subsequently showed increasing immune response on days 21 and 28 following vaccination. By day 21, three weeks after vaccination of Group A, the antibody titre had actually gone down, indicating that there had been no or minimal immune response, though by day 28, the titre increased. However, antibody titres went down

drastically in Group B. In Group C, the titres remained at the pre-vaccination levels even at day 28.

Table I. Titres at Day 1 post hatchery (PH) and Day of vaccination, to highlight the differences in the titres between day of vaccination and the antibody response on days 21 and 28 post vaccination (PV).

Groups	Titre on D1 (PH)	Day vaccinated (PH)	Titre on Day of vaccination	Titre on D21 (PV)	Titre on D28 (PV)
A	1.81	D1	1.81	0.98 ± 0.29^{a}	1.73 ± 0.52^{b}
B	1.81	D4	1.70	1.43 ± 0.97^{a}	0.60 ± 0.35^{a}
C	1.81	D8	1.70	1.43 ± 0.38^{a}	1.81 ± 0.25
D	1.95	D14	0.64	1.66 ± 0.30^{a}	2.86 ± 0.52^{c}

DISCUSSION

Based on the results of the study in both sets of chickens obtained from two different hatcheries, the maternal antibody titres were highest immediately after hatching, and showed a time-dependent progressive decay within the first 15 days of the chicks hatching. This is because over time, maternal antibody titres decline and the antibodies get metabolized (normal physiological hypogammaglobulinaemia) and do not protect any longer [19]. The HI antibody titres ($\geq \log 2^5$) recorded in both hatcheries at day old is usually presumed to provide protection to the chicks against ND for up to two weeks of age. Spradbrow [20] and Alexander *et al*., [21] stated that HI titre of $\geq \log 2^3$ (whether maternal or following vaccination) has been considered protective against virulent ND virus. Other studies also stated that high maternal antibody titres protect young chickens against viral diseases [22, 23,24].

Other studies have reported half-life in a range between 4-7 days. Rahman *et al*., [25] had estimated that each two-fold decay in maternally derived Newcastle disease antibody takes about 5 days, whereas Allan *et al*., [26] and Darbyshire and Peters [27] reported 4.5 days as two-fold decay of maternally antibodies in chicken. Gharaibeh and Mohmoud [28], reported 4.7 days as the half-life of maternal antibody (NDV) which is in agreement with our observation of (t½) of 4.68 days in birds from Hatchery B in our study.

We observed the lowest antibody titres at 15 days in both hatcheries, and this is in agreement with Islam *et al*.[29], who reported that maternally derived antibodies can persist up to 15-20 days of age in chicken. Similarly, Siwek and Knoll [30] found that newborn chicks catabolize maternally transmitted immunoglobulin by the 14^{th} day of life after hatching.

The implications of our findings in the first study are that (a) chickens with high antibody titres early in life would be more likely to respond poorly to vaccination than later in life when maternal antibody levels would have gone down, (b) regardless of the initial antibody titre at day old, antibody levels dropped to their lowest levels by 15^{th} day of life and (c) the decay rate from both hatcheries also suggest that by day 15 posthatch, antibody levels would be too low to interfere with vaccination.

Gharaibel *et al*., [31] had concluded that maternal antibodies play crucial role in the health status of modern-day broiler-chicken industry, and play a major role in modulating early life vaccines strategy for commercial poultry flocks. Chu and Rizk [32], showed that maternal antibodies against Newcastle disease virus decrease the severity of adverse live vaccine reactions and at the same time decrease the immunity following vaccination. In the same vein, Naqi *et al*., [33] reported that high levels of maternal antibody against certain infections may neutralize vaccine effect and result in complete failure of immunization, suggesting that the effect of maternal antibody on the development of active immunity in vaccinated chicks varies, depending on the level of maternal antibody titre and on the type of infecting viral pathogen.

In the second study, we evaluated the effect of different levels of maternal antibody on the response of chicken to ND vaccine.
The erratic response of the birds in Groups A, B, and C is particularly interesting, and may be an indicator to the reasons for the apparent 'vaccine failures' observed in commercial poultry farms. The common denominator in Groups A, B, and C is that they received the ND vaccine at a time when maternally derived antibody levels were still high (log1.70 to log 1.81) as compared to Group D which was vaccinated when maternal antibody level was low (log 0.64).

Our study indicates that the presence of maternal antibodies at levels > log 0.60 would result in poor immune response to subsequent vaccination. The poor response to vaccination can be attributed to the effect of maternal antibodies, which interfered with the vaccination to diminish the development of primary immune response arising from days 1, 4 and 8 post vaccination. Our findings in this study are in agreement with the reports of previous studies [34, 35, 36]. The results also suggest that adequate response to vaccination would be elicited if the vaccine is given to birds when maternal antibody levels have fallen lower than log 0.6. The dilemma is that the birds cannot respond to vaccination unless the maternal antibody titres drop to a reasonable level, but we cannot predict when such drop would happen.

Gillepsie *et al*., [37] studied a similar problem involving vaccination of dogs against canine distemper, specifically the relationship between the antibody titre of the mother and the antibody delivered to the offspring and the effect such maternal antibodies have on the vaccination of puppies. They found that a mother with high titre transferred more antibodies than a mother with low titre, and that generally protection was found to last more than 1 week but less than 2 weeks. However, puppies with higher titres showed a longer persistence of antibodies than puppies with low titres. They also found that puppies would not develop immunity until they have lost the colostral protection and become susceptible to distemper. Gillepsie and colleagues then developed a '*Nomogram*' system for distemper vaccination in dogs whereby the earliest age for vaccination can be predicted in advance by the titre at whelping of every dog so as to select the best date for vaccination. This procedure was quite cumbersome. They also showed that puppies that inherit minimal antibodies lose such maternal antibodies early (between 6-9 weeks) and would respond well to vaccination given at those times, whereas in puppies that inherit maximum antibodies, the decay of the maternal antibodies will last till 12 to 14 weeks. Their observations led to the practice of giving puppies vaccination at 6 weeks, 9 weeks and 12 weeks with the expectation that those puppies that received minimal antibodies will seroconvert from the 6-week vaccination, whereas those that receive a little more maternal antibodies will respond to the 9-week vaccination, and finally those that receive maximum dose from their mother will

respond positively to the vaccine given at 12 or 14 weeks of age. In this way it is unnecessary to determine the individual dam's antibody titre at whelping.

The distemper situation can be emulated for poultry vaccination. Studies, including ours, have shown that by 15 days after hatching, the maternal immunity has decayed sufficiently to permit successful vaccination. The problem then is that between day-old and 15 days, flocks that got minimal or no maternal antibodies would be susceptible to infection and disease from virulent ND virus. Most hatcheries would vaccinate at day-old, so that flocks that did not acquire maternal immunity could respond. We suggest a second vaccination between 5 to 8 days to cater for the flocks that get low levels of maternal antibodies. The final vaccination would be given on or after day 15. This last dose of vaccination will provide for those flocks that got high levels of maternal antibodies at birth, and serve as booster vaccine for those flocks that had been successfully immunized with the first two doses at day-old and day 5.

In conclusion this study showed that (i) the level of maternal antibody in chicks may vary from hatchery to hatchery, that is, it is management dependent (ii) maternal antibody decay in chicks post hatching is expected and known as normal physiological hypogammaglobulinaemia. (iii) The decay of maternal antibodies is completed in about 15 days, with half-life ranging between 3- 4.7 days. (iv) The difference in the response to ND vaccination can be attributed to the presence or absence of maternal antibodies. Hence the suggestion for vaccination at day 1 and/or day 5 for flocks with low levels of MA at birth and a booster vaccination on day 15,when MA has significantly waned, for flocks with high levels of maternal antibodies at birth.

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