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Haemagglutination inhibition antibody responses of pullet and broiler chickens (*Gallus gallus domesticus*) to Newcastle disease virus LaSota vaccination

AO Igwe[1]* & Agbakwuru IO[2]

1. *Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, PMB 7267 Umuahia, Abia State, Nigeria*
2. *Department of Veterinary Anatomy, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria*

*Correspondence: Tel.: +2348034509991; E-mail: docoleji@yahoo.com

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Abstract

Newcastle disease outbreaks still occur sporadically in commercial vaccinated flocks and remains a constant threat to poultry producers despite advances in vaccination against the disease. Another aspect that can be a complementary control strategies or that is well recognized but is often neglected is the differences in immune response due to genetic or breed/type variation. This study investigated the immune responses to LaSota vaccination in light weight type or breeds of chickens (pullets) and heavy weight type or breeds of chickens (broilers) used in commercial poultry production. Fifty seven-week-old White Marshall broilers (Br) and 50 Isa Brown pullets (Pu) of the same age were randomly divided into 4 groups viz: vaccinated broilers chickens (VaBr), unvaccinated broiler chickens (UBr), vaccinated pullet chickens (VaPu) and unvaccinated pullet chickens (UPu). Chickens in groups VaBr and VaPu were vaccinated with LaSota vaccine while groups UBr and UPu were not vaccinated. The chickens were observed for clinical signs and lesions. Serum samples were collected from the chickens in all the groups on days 0, 7, 14, 21, 28 post vaccination (PV), and assayed for haemagglutination inhibition (HI) antibodies. The geometrical mean antibody titres (GMT) of the pullets were 2 to 3 times higher than those of the broilers on days 7 to 28 PV. Vaccination produced neither clinical signs nor lesions. The above observations show that naturally pullets produce higher antibodies than broilers, and suggest breed-based variation on immune responses to Newcastle disease vaccination. The knowledge from the present study may lead to genetic approach to vaccine development and development of more effective vaccination strategies to be used in commercial poultry production.

Keywords: Broilers, Haemagglutination inhibition antibody, LaSota vaccination, Pullets

Introduction

Newcastle disease (ND) is regarded throughout the world as a contagious and often fatal infectious viral disease affecting most species of birds, with chickens being the most susceptible (Miller & Koch, 2013; Igwe *et al*., 2014). This is not only due to the serious disease and high flock mortality that may result from some Newcastle disease virus (NDV) infections, but also because of the economic impact that may ensue due to trading restrictions and embargoes placed on areas and countries where outbreaks have occurred

(Aldous & Alexander, 2008). ND is listed as notifiable by the Office International des Epizooties (OIE) due to the severe nature of the disease and the associated consequences (OIE, 2012). The disease is caused only by infections with virulent strains of NDV or avian paramyxovirus serotype-1 (APMV-1). In the current virus taxonomy NDV or APMV-1 is an enveloped, non-segmented, single-stranded RNA virus classified in the genus *Avulavirus*, sub-family *Paramyxovirinae,* family *Paramyxoviridae*, and order *Mononegavirales* (Lamb *et al*., 2005; Afonso *et al*., 2016). Although contained in one serotype, all NDV strains show marked genomic variability (Diel *et al*., 2012***a***). Phylogenetically, NDV strains are classified into two major groups: classes I and II (Czeglédi *et al*., 2006; Diel *et al*., 2012***a***), with only one genotype of class I, and 18 genotypes of class II. Historically, grouping NDV strains into genotypes based on the similarities of the genomes began as a way to provide epidemiological information (Lomniczi *et al.*, 1998). Protection against NDV is through the use of vaccines generated with low virulent NDV strains. Immunity is derived from neutralizing antibodies formed against the viral haemagglutinin and fusion glycoproteins, which are responsible for attachment and spread of the virus (Miller & Koch, 2013).

Based on the virulence for chickens, five pathotypes have been distinguished: asymptomatic, lentogenic, mesogenic, neurotropic velogenic, and viscerotropic velogenic (OIE, 2012). Infection with virulent Newcastle disease virus (vNDV) has been associated with severe systemic disease and accompanied by high morbidity and with 100% mortality in poultry (Okoye *et al*.,2000; Miller & Koch,2013; Igwe *et al*., 2018a).Therefore, ND is a major threat to food security in many countries, especially in areas of Africa, Asia, the Middle East, and in some countries of North, Central and South America, causing recurrent outbreaks in poultry production facilities resulting in considerable economic impact (Afonso & Miller, 2013; Shittu *et al*., 2016).

Different approaches and measures have been adopted to control the spread of ND in many parts of the world. These include vaccination, movement restrictions and other biosecurity measures that prevent vNDV from contacting poultry, destruction of infected animals and surveillance (Alexander, 2001). For efficient control of ND where it is endemic, vaccination regimen is routinely undertaken in commercial poultry and restriction of the movement of sick animals and their products is crucial (Afonso & Miller, 2013; Shittu *et al*., 2016). However, ND outbreaks still occur sporadically in commercial vaccinated flocks and remains a constant threat to poultry producers across the globe on a daily basis, especially in developing countries where the disease is endemic despite advances in vaccination against the disease (Dimitrov *et al*., 2017). This could be attributed to poor flock immunity due to inadequate vaccination practices that may be responsible for outbreaks and spreading of virulent NDV field strains (Dortmans *et al*., 2012). In developing countries, the free-range chickens have been implicated in harbouring velogenic strains of the virus which have been considered a threat to the commercial poultry and problems associated with effective vaccination with conventional vaccines due to unreliable cold chain to keep the vaccines at 4 ºC during the distribution process (Harrison & Alders, 2010; Shittu *et al*., 2016). Despite decades of research and development towards formulation of an optimal ND vaccine, improvements are still needed (Kapczynski *et al*., 2013).

Provision of immunological protection against ND through use of vaccines is regularly and routinely practiced by all major poultry industries (Kapczynski *et al*., 2013; Igwe *et al*., 2018***b***). Since humoral immunity from vaccination is critical to ND control (Kapczynski *et al*., 2013), another aspect that can be a complementary control strategy or that is well recognized but is often neglected is the differences in immune response due to genetic or breed/type variation. However, very little is known regarding the antibody response to ND LaSota vaccination among breed of chickens. The aim of this study was to investigate the haemagglutination inhibition antibody responses of pullet and broiler chickens (*Gallus gallus domesticus*) to Newcastle disease virus LaSota vaccination.

Materials and Methods

Experimental design

All animal studies were approved by the Institutional Committee on Medical and Scientific Research Ethics given by the University Committee on Medical and Scientific Research Ethics, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Fifty day-old White Marshall broiler chicks and fifty day-old Isa Brown pullet chicks (*Gallus gallus domesticus*) procured from a reputable local commercial hatchery were used for the study. Both breeds (groups) were hatched the same day. The groupings were broiler chickens (Br) and pullet chickens (Pu). Each of the groups was brooded separately under the same environmental conditions at the departmental poultry experimental facilities. Brooding of all the chickens was done on deep litter

and they were not vaccinated against any disease. Feed and water were supplied *ad libitum*. General care of the birds was provided in accordance with the Institutional Animal Care and Use Committee, as outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Vaccination of chickens

The LaSota (Lentogenic strain of NDV) vaccine used was produced by and obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The vaccine had a medium embryo infective dose (EID_{50}) of $10^{6.9}$ per ml. The viability of the stock vaccine was checked using HA test (OIE, 2012).

At 7 weeks of age (day 0 post vaccination), the chickens were found to be free from detectable or negative for NDV HI maternal antibody using the methods of OIE (2012). They were randomly assigned into four groups of 25 chickens each. The groupings and their treatments were vaccinated broiler chickens (VaBr); unvaccinated broiler chickens (UBr); vaccinated pullet chickens (VaPu); unvaccinated pullet chickens (UPu). LaSota was administered by diluting a vial containing 200 doses with 200 mL of sterile water for injection. One mL of the reconstituted vaccine was administered orally by drenching each chicken in groups VaBr and VaPu. Each chicken in groups UBr and UPu was drenched orally with one mL of the diluent used as placebo. Chickens in all the groups were observed twice daily for clinical signs for 10 days post vaccination (PV). On day 3 PV, three chickens in each group were humanely sacrificed by cervical dislocation and examined for gross pathological changes. Samples of the bursa, spleen and thymus were fixed in 10% formalin saline for 48 hours, trimmed and processed for histopathology as described by Bancroft & Stevens (1982).

Serology

One mL of blood was collected from the jugular veins of 10 randomly selected chickens in each group, on days 0, 7, 14, 21 and 28 PV. The serum samples were harvested and assayed for haemagglutination inhibition (HI) antibodies using the method of OIE (2012). The antigen used for the HI test was a PBS suspension of LaSota vaccine which had 4 HA unit of antigen. The antibody titers were reciprocal of highest dilution of sera that gave complete inhibition of the chicken red blood cells.

Statistical analysis

On day 0 PV, the weights of pullets were compared to those of broilers using Student's t –test. On day 4 PV the weights of the vaccinated and unvaccinated pullets and broilers were subjected to one-way analysis of variance (ANOVA) and variant means were separated using the least significant difference (LSD) method. All tests were performed with a 5% level of significance ($P < 0.05$). The geometric mean haemagglutination inhibition antibody titres of the vaccinated pullets and broilers were calculated and compared. Results were presented as means with standard deviations (SD) or geometrical mean titres as appropriate.

Results

Clinical signs and lesions

The chickens in all the groups showed neither clinical signs nor lesions (Plates I & II). There was no significant ($P>0.05$) weight gain in groups VaBr and VaPu when compared with their respective controls,

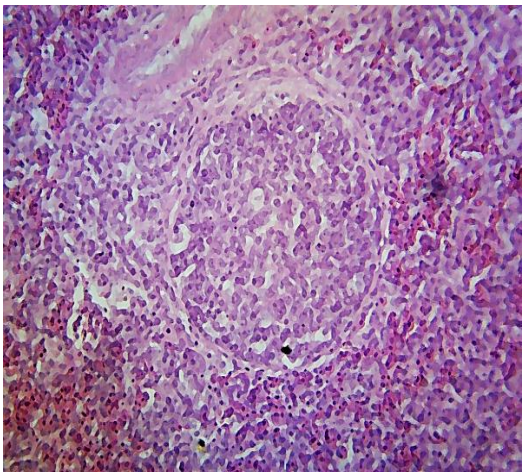

Plate I: Photomicrograph of spleen of vaccinated pullet showing a normal architecture on day 3 post vaccination. H & E, X200

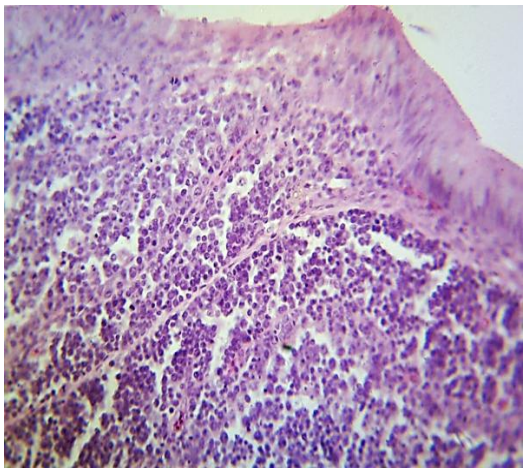

Plate II: Photomicrography of bursa of Fabricius of vaccinated broiler showing a normal architecture on day 3 post vaccination, H&E, X200

Table 1: Mean body weights of birds (g) ± Standard deviation

Days PV	Treatment groups			
	Non vaccinated pullets	Vaccinated pullets	Non vaccinated broilers	Vaccinated broilers
0	433 ± 66.67 [a]	NA	1450 ± 145.30 [b]	NA
4	500.00 ± 57.74 [a]	500.00 ± 57.74 [a]	1766.67± 44.10 [b]	1600.00 ± 50.00 [b]

[a, b] Alphabetical superscripts in a column indicate significant differences between the means of the groups, P< 0.05
[PV] Post vaccination
[NA] Not applicable

UBr and UPu, on days 0 and 4 PV (Table 1).

Serology

The GMT of the HI antibody of the pullets were much higher than those of the broilers on all the days assayed, days 7-28 PV (Figure 1). Throughout the experiment, titres of the control groups, UPu and UBr chickens remained negative.

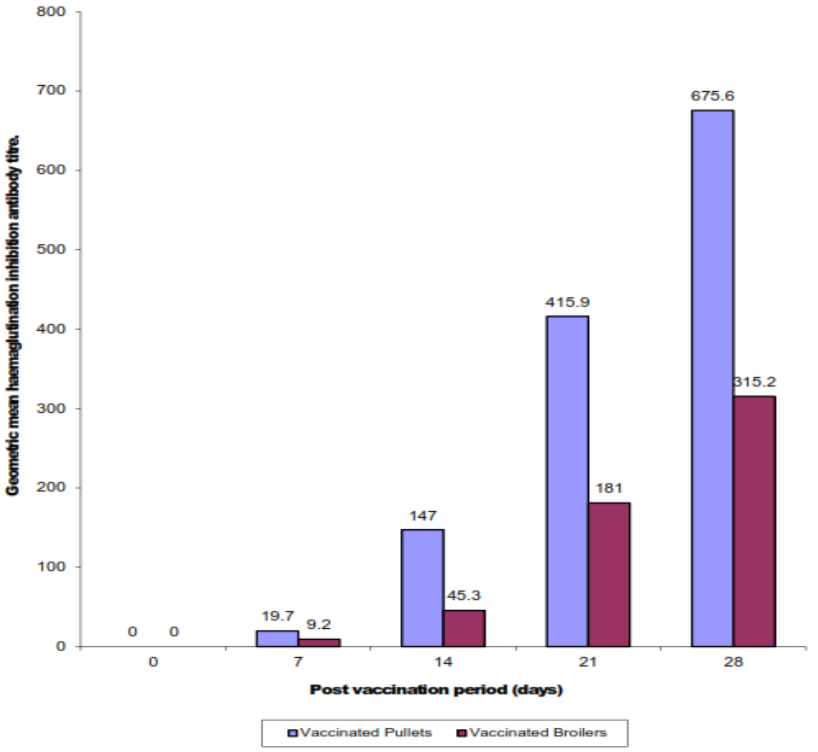

Figure 1: Geometric mean haemagglutination inhibition antibody titers of vaccinated and unvaccinated pullets and broiler chickens on days 0-28 post vaccination

Discussion

The results of the present study showed that LaSota vaccine was able to produce sufficient immunity in antibody-free birds. Doses of the LaSota vaccine of 10^6 EID_{50} or higher produced strong humoral immune responses (Cornax *et al*., 2012). Dimitrov *et al*. (2017) reported that when administered correctly to healthy birds, ND vaccines formulated with low virulence or virus-vectored vaccines that express the NDV fusion protein are able to prevent clinical disease and mortality in chickens upon infection with virulent strain. The sufficient immunity (HI antibody responses) recorded in the present study in pullets and broilers dictates that the vaccine is protective, indicating no increase in titers will be observed after challenge, and suggesting little to no replication of any challenge vNDV. Therefore, cases of vNDV outbreaks in vaccinated flocks sporadically reported in Nigeria or in other developing countries where the disease is endemic (Shittu *et al*., 2016) or developed countries where the disease is exotic (Miller & Koch, 2013) could be due to such other factors like maternal antibody interference with live vaccination thereby neutralizing the vaccine virus in young birds (Giambrone & Closser, 1990). Although, after hatch, the administration of a killed or live ND vaccine to birds that were vaccinated *in ovo* with rHVT-ND vaccine, increases the level of immunity to facilitate more complete protection and helps decrease the amount of virulent NDV shed after challenge (Palya *et al*., 2014). However, worldwide, the most commonly used ND vaccines are live vaccine viruses formulated with strains isolated in the 1940's and 1960's. Viruses circulating in poultry were the source of the LaSota, B1, and VG/GA vaccines. All of those viruses belong to genotype II and are genetically and antigenically highly related among themselves (>98% nucleotide identity). The main differences among those vaccines are the tropism and the capacity to replicate in naive chickens, which is highest in LaSota and results in higher levels of neutralizing antibodies compared to other strains (Meulemans, 1988). Thus, the LaSota strain is nearly always used in countries where virulent NDV is endemic (Diel *et al*., 2012b).

Improper handling, transportation and storage of these vaccines especially under the present incessant power failure in Nigeria could lead to loss of potency. Studies of lesions and mortalities of ND have shown that the strain of ND in Nigeria is comparatively very virulent (Igwe *et al*., 2018***a***). It is therefore possible that the vaccine failures could be as a result of vaccine immunity being overcome by over large dose of very virulent field strain of the virus.

Clinically, the pullet and broiler chickens vaccinated with lentogenic strain of NDV (LaSota) exhibited no overt signs of disease at day 3 post vaccination and throughout the experimental period, when compared with the unvaccinated group, by which time vNDV being an acute disease should have produced significant weight loss and clinical disease (Igwe *et al*., 2014; Igwe *et al*., 2018a, Igwe *et al*., 2018b). This corresponds with the findings of Brown *et al*. (1999), who reported no overt disease in birds inoculated with lentogenic pathotypes. All these are expected from a lentogenic NDV infection which is mildly pathogenic, and correlates with lack of gross and histologic lesions in the visceral organs of vaccinated groups when compared with the controls. Neither necrosis nor depletion of lymphocytes was observed in bursal follicles, thymus, spleen and intestinal lymphoid tissues and on day 3 and 5 PV by which time velogenic NDV should have been produced lymphocidal effects. This shows that the vaccine is not lymphocidally pathogenic. However, lymphoid follicles and lobules were prominent at day 3 PV. This phenomenon is probably indicative of non-specific immuno-stimulation of the lymphoid organs perhaps by some vehicle or preservative in vaccine. It could also be suggestive of the continuous replication of lymphocyte precursors in the lymphoid organs (Day, 2010).

The present study showed that naturally the HI antibody response to LaSota vaccination was 2-3 times higher in pullets than in broilers. The comparatively higher serum antibody could be due to an earlier report that pullets (light breed of chickens) have higher lymphoid organ weight indices than broilers (heavy weight breed of chickens) used in commercial poultry production (Okoye & Aba-Adulugba, 1998; Sá e Silva *et al*., 2016; Igwe, 2018b). This is likely to translate to more disease resistance in some diseases which are not acute and protective antibody response is allowed to build up before mortalities occur. Igwe *et al*. (2018***b***) reported that such resistance may not develop before mortalities occur in some acute diseases like velogenic NDV infection. King (1996) reported that the SPF White Leghorn layers were more susceptible to velogenic neurotropic NDV infection than White Rock broilers. Hassan *et al*. (2004) reported that the Mandarah local Egyptian breed of chicken was more resistant to virulent NDV infection than other local breeds. These demonstrations of genetic susceptibility and resistance to specific disease producing agents aid in focusing attention upon the important role that genetic constitution of a stock plays in its overall performance. Bishop (2010) reported that host genetic variation in disease resistance invariably exists, due in large part to the variability in host immune responses to infection. In the same way it is expected that the observations made in the pullets in this study will apply to other light weight chickens.

In conclusion. the present study showed that naturally pullets produce higher antibodies than broilers, suggesting breed-based variation on immune responses to ND vaccination. Considering that protective immunity against NDV is largely based on the production of antibodies directed at viral proteins involved with attachment and fusion, the variation could be utilized in selective breeding and genetic approach to vaccine development by poultry geneticist and immunologist, identifying such natural gene(s) possessed by these light weight breeds of chickens (pullets) that enable them produce higher antibodies that can be used in effective vaccination strategies in commercial poultry production.

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