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Vaccination indices and concomitant serological status of Newcastle disease in chickens in Aba and Umuahia of Abia State

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Abstract

The vaccination indices of Newcastle disease (ND) in chickens in Aba and Umuahia towns of Abia state were studied alongside their corresponding antibody status. A total of 296 sera samples were collected from 74 chicken farms. A Haemagglution inhibition (HI) test was conducted to determine the ND virus serum antibody levels. Open and closed-ended questionnaires were administered to staff on the farms selected randomly in the study areas. Information on vaccine types, origin, administering personnel, revaccination interval, and records of ND outbreaks was collected. Whereas 68.9% of the farm carried out vaccination on their own, 27.0% and 4.1% of the vaccination were done by veterinarians and animal health scientists Farms in Aba and Umuahia had average geometric mean titres (GMT) of 166.32 and 100.33, respectively. Approximately 87% of the farms had protective immunity (GMT >8 or $\log 2^3$) against ND. Chickens aged 1-3, 4-8, 9-16, 17 weeks and above had average GMTs of 64.00, 76.99, 283.7 and 197, respectively. Post-vaccination antibody titres were 128.92, 110.63, 52.07 and 43.65, after 1 week, 2-3 weeks, 4 weeks, and above 4 weeks, respectively. Indigenous ND vaccines had an average GMT of 182.55, while foreign ND vaccines had a GMT of 120.82. The Result showed that 77% of farmers used foreign vaccines whereas 23% used indigenous vaccines. On vaccination interval, 40.5% revaccinated for ND every three weeks, 21.6% monthly, 8.1% bi-monthly and 29.7% revaccinated when necessary. About 54% of farmers reported previous ND occurrence. This study identified high seroprevalence of ND antibodies in the flock studied and indicated a high level of awareness and adherence to NDV vaccination among the farmers in the study area. The local vaccines elicited better immunogenic responses than their foreign counterparts. We, therefore, recommend that usage of the local vaccines be adopted, and revaccination is done before a month interval.

Keywords: Newcastle disease, Vaccination, Immune response, Haemagglution inhibition, Geometric mean titre

Introduction

Newcastle disease (ND) is a highly infectious viral disease caused by avian paramyxovirus serotype-I (APMV-1) in the genus Orthoavulavirus, family, *Paramyxoviridae*, with a non-segmented and negative sense single stranded RNA genome (Seal *et al.*, 2000a; Dimitrov *et al.*, 2019). It affects domestic poultry and many other species of birds including some wild ones worldwide causing high morbidity and mortality in unvaccinated flocks especially with the involvement of the velogenic strain of the virus (Alexander, 1997; Kaleta & Kummerfeld, 2012). The disease is one of the most fulminating poultry epidemics in Nigeria and a serious constraint to poultry production throughout the world (Alexander *et al.*, 2012), particularly in developing countries (Samuel *et al.*, 2013). It is enzootic in Africa where it is a major challenge to the traditional poultry industry (Abera *et al.*, 2017). Control of ND is largely dependent on the use of safe and appropriate vaccine. Naturally occurring avirulent strain of ND virus (NDV) has been successfully used as vaccine for more than 70 years (Nayak *et al.*, 2009). Many inactivated and live ND vaccine are currently in use around the world.

In Nigeria, National Veterinary Research Institute (NVRI) Vom, is the only organization producing the three different types of live vaccines, Hitcher B-1 (HB-1), LaSota, and Komarov strains. However, the inadequacy of their production capacity in meeting up with local demand has necessitated the widespread use of imported ones. The immunogenicity of these imported vaccines has been ascertained (Olugasa *et al.*, 2012), but has not been compared with their indigenous counterparts. Reports of frequent subclinical infections and disease outbreaks in vaccinated flocks have led to professionals and farmers resorting to various vaccination schedules with varying results. This study investigated the seroprevalence of NDV and vaccination status with respect to vaccine types in farms in Aba and Umuahia, Abia State.

Materials and Methods

The study was carried out in Aba and Umuahia towns in Abia State from June to September 2018. Close ended questionnaires were administered in 40 broiler and 34-layer farms chosen through a purposive sampling from two Local Government Areas (LGA) each from Aba and Umuahia towns (all the LGAs were involved). Farms with ongoing NDV outbreaks were excluded. Data on vaccination and some epidemiological factors were obtained. These included bird type, vaccine type, route of vaccine administration time at 1st vaccination, revaccination intervals, incidence of ND recorded in the past and periods of vaccine administration.

Haemagglutination inhibition (HI) antibody assay was carried out on birds in the 74 farms. Five chickens were randomly selected from each flock and 1 ml of blood aseptically collected from each by brachial venipuncture. The blood was allowed to clot and the sera harvested and stored at -20℃ for HI test. The antigen used was ND LaSota vaccine, procured from NVRI Vom, Plateau state. 4HAU of the vaccine was used for the subsequent HI test.

Ethical approval

The ethical approval for this work (MOUAU/REC/201809) was obtained from the College of Veterinary Medicine Research and Ethics Committee.

Preparation of chicken red blood cells (RBC)

Chicken blood (5ml) was pooled from three antibody negative healthy unvaccinated birds from the experimental unit of the Department of Veterinary Medicine, Michael Okpara University of Agriculture Umudike through the brachial vein in a sample bottle containing EDTA. The blood was centrifuged at 1500rpm for 15 minutes and the buffy coat was removed with pipette. The pooled blood was washed three times with 0.01M isotonic solution of phosphate buffered saline (PBS). The washed red blood cells were used as a 1% (packed cell v/v) suspension as an indicator for the HI test.

Haemagglutination inhibition (HI) test

Two hundred and ninety-six serum samples were tested for the presence of the antibodies against NDV according to the procedure of O.I.E (2012) with a slight modification. The sera were inactivated by incubating them at 56℃ for 30 minutes to inactivate nonspecific agglutinators. Two-fold serial dilutions of 25ul of serum were made with PBS in U- bottomed microtitre plates. Twenty-five microlitres of 4HA units of the NDV antigen were added to each well and left for 30 minutes at room temperature. Subsequently, 25µl of the 1% RBC were added to each well, mixed gently and allowed to settle for 30 minutes at room temperature (RT). The HI titer was the highest dilution of serum causing complete inhibition of 4 HA unit (HAU) of the antigen. It was expressed as reciprocal of the serum dilution.

Statistical analysis

Data was collected using MS Excel and analyzed with SPSS version 21. Chi-square was used to determine the association of seroprevalence witho vaccine type

and timing of administration with other variables. The statistically significant level was 5%.

Results

The results from the study show that 68.9% of the farm carried out vaccination on their own, while 27.0% and 4.1% of the vaccination were done by veterinarians and animal health scientists, respectively. Different farms had varied intervals of vaccination ranging from the 3rd to the 4th till the 8th week. Most farms, 40.5% were revaccinated at three weeks intervals while 21.6%, 8.1%, and 29.7 % did so at four weeks intervals, eight weeks intervals and at unspecified intervals. Almost 54% of the farms had recorded previous ND outbreaks (based on clinical signs and postmortem lesions), (Table 1). Revaccination of layers at 16 weeks of age in 92% of cases was done using inactivated Komarov strain and thereafter by oral administration of LaSota at specified intervals.

With respect to location, farms in Aba had better antibody response with a titer range of $\log2^2$ –$\log 2^{10}$ and GMT of 166.3, while those in Umuahia had a range of $\log2^1$ –$\log 2^9$ and GMT of 100.3.

Out of the 296 samples under study, 87.8% had ND-HI titres of $\log2^3$ (i.e. 1:8) or above. Low usage of local vaccines of 23% was recorded against 77% usage of the foreign ones. However, ND antibody titre range of $\log2^1$ –$\log2^9$ and $\log2^3$ – $\log2^{10}$ corresponding to GMTs of 120.8 and 182.6 were recorded for foreign and indigenous vaccines, respectively (Table 3).

The HI ND antibody values for the different age groups were equally studied. Chickens between the ages of 9 and 16 weeks had the highest values (GMT of 283.7), whereas those between 1 – 3 weeks had the least (GMT of 64. 0)(Table 4).

Antibody waned very fast in the farms as shown in Table 5. Antibody titer values taken after a month of revaccination in the farm were observed to be about

Table 1: Distribution of vaccination pattern of against ND with respect to vaccination interval and previous outbreak

Frequency of vaccination	Number of farms	Percentages of farms involved	Number of farms with previous pre-vaccination outbreak
Every 3 weeks	30	40.5%	22
Every 4 weeks	16	21.6%	5
Every 8 weeks	6	8.1%	2
Unspecified	22	29.7%	11
Total	74	100%	40

Table 2: Distribution of HI antibody titer in farm of Aba and Umuahia

Location	No. of samples	No. of seropositive	% Sero Positive	Antibody titer by HI Test										
				2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	GMT
Aba	96	88	91.1	-	9	16	13	16	12	15	8	5	2	166.3
Umuahia	200	172	86.0	8	21	17	22	30	55	24	15	7	1	100.3

Table 3: Distribution of ND antibody titre according to vaccine type

Vaccine type	Number of birds	Antibody titre by HI Test										
		2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	GMT
Foreign	228	8	30	25	24	33	53	29	18	8	-	120.8
Local	68	-	-	8	11	13	14	10	5	4	3	182.6

Table 4: Distribution of ND antibody titer with respect to age of birds

Age range (Weeks)	Number of birds	Antibody titer by HI Test										
		2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	GMT
1 - 3	70	6	17	8	6	14	12	7	-	-	-	64.0
4 – 8	86	2	10	16	17	15	13	9	3	1	-	77.0
9 – 16	71	-	-	4	6	7	19	12	13	7	3	283.7
>17	69	-	3	5	6	10	23	11	7	4	-	197.9

Table 5: Distribution of ND antibody liter with respect to duration post-vaccination

The time between Vaccine and HIT	Number of birds	Antibody titer by HI Test										
		2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	GMT
1 day – 1 week before HI	66	-	-	2	4	7	17	15	11	8	2	128.9
2-3weeks before HI	81	-	2	7	10	15	24	10	17	5	1	110.6
1 month before HI	81	1	8	16	14	15	16	8	3	1	-	52.1
>1 month	68	7	20	8	7	9	10	5	2	-	-	43.7

a third of values taken less than a week after vaccination, as shown (Table 5).

Discussion

ND has continued to negatively impact poultry production worldwide (Goldhaft, 1980; Alexander *et al.*, 2012). The disease is panzootic in proportion and has a wide geographic spread (Dimitrov *et al.*, 2016). Biosecurity and vaccinations have been used in the past to control ND and have been successful in reducing the mortality and morbidity associated with the disease. However, these measures do not completely prevent poultry from becoming re-infected or shedding virulent NDV in their feces. The goal of vaccination is always protective immunity; however, this has not been achieved with NDV vaccines (Seal *et al.*, 2000b; Kapczynski, 2013). In this study, the vaccination patterns of ND within Aba and Umuahia were considered alongside the ND antibody in the corresponding flocks.

Results from five vaccination indices considered in this work showed that there is a strong awareness of the disease vaccination dynamics among poultry farmers within the region. This may be due to the recurring problem and the endemicity of ND within the region as established in so many other poultry producing regions of the world (Alders *et al.*, 2001; Adene *et al.*, 2004; Orsi *et al.*, 2010).

The HI test results showed that poultry farms in Aba had better antibody titres than those in Umuahia. Aba is a more advanced town in terms of business enterprise and appears to be more advanced in the poultry enterprise. Out of the 96 samples examined in Aba, only eight had titer lower than GMT of 8 or log 2^3 said to be un-protective for ND specific immunity according to Allan & Gough (1974). On the other hand, 28 out of the 200 samples in Umuahia had un-protective ND antibody. Seroprevalence of 91.7% and 88.0% were recorded for samples from Aba and Umuahia respectively. These results are similar to results obtained in previous studies. Bell & Mouloudi (1988) had recorded antibody levels against NDV in Morocco to range between 5 and 83% (average of 35%) of sampled population while Numan *et al.* (2005) reported 98.07% of sampled population having protective immunity for broilers in Pakistan. However, it should be noted that high antibody titers sometimes may be due to past disease occurrence. In this study, this has been minimized by the avoidance of farms with ongoing ND outbreaks. Thus, the high seroprevalence recorded in the study region can be attributed to vaccinal response due to effective and frequent vaccinations in the farms.

The study revealed that indigenous live ND vaccines produced much better HI ND antibody titer results than their foreign counterparts. Ibu *et al.* (2002) had reported a similar finding that locally produced live ND vaccines in Nigeria, from NVRI, Vom were superior in quality to imported vaccines because the ND strains used for the production of the local vaccines are similar to the prevalent strains in Nigeria. Olugasa *et al.* (2012) and Eniope *et al.* (2020) in their studies on evaluation of immunogenicity of different commercial vaccines against ND in poultry farms in Ibadan, Nigeria recorded significant differences among the imported Lasota vaccines studied. Variations in different methods of production and longer time and distance involved in the sourcing of these foreign vaccines could be contributory to these observed differences. This thus creates a need for the auditing of vaccines imported into the country before their importation. This is therefore an important factor to consider in the choice of vaccines. This finding is against the backdrop of the lower usage of the local vaccines (23%) as against the foreign ones (77%). This low usage of local vaccines has been attributed to frequent unavailability (Ishola, 2012).

The different age groups varied in their ND antibody levels. Chicken aged 9 – 16 weeks showed the highest antibody titres unlike previous studies by Hossain *et al.* (2010) who recorded the highest antibody titres of ND in birds of 1 – 2 weeks in

Bangladesh. This variation may be connected with the vaccination regimen adopted by the farmers in the study region. They administered boaster doses of ND vaccines between the 8th and 16th weeks of age using Komarov (the mesogenic strain of the vaccinal virus). This tended to induce very high ND antibody titres after the initial priming at 1 – 3 days and 21 days of age using the lentogenic strain of the virus. This is in contrast to the low ND titre values recorded within the early stages due to primary antibody response which is usually lower than the subsequent secondary responses. Thereafter, revaccination using the mesogenic strain is discontinued as it tends to affect egg production and hence the reduction in titer levels observed. Less attention is paid to revaccination from the onset of egg production hence the reduction in the titer observed afterwards.

The results of this study show the rapidity with which the antibody levels of NDV wane after vaccination. This should be expected as the general use of the lentogenic LaSota strain in the study region is bound to elicit a weaker and shorter cell-mediated immune response than a more virulent strain (Roy & Koteeswaran, 1999; Rauw *et al.*, 2009). Thus, by the 4th week after vaccination the antibody levels have fallen to less than half of what they were after the 1st week and to about one third after this time. This rapid wane which can arise from the use of low potency vaccine among other factors is the reason for the varied and frequent revaccination. Factors such as genetic variability of the virus or adoption of less optimal conditions like poor vaccine quality and lack of cold chain maintenance of the vaccine leading to vaccination failure, a perennial problem in developing communities like Nigeria (Vui *et al.*, 2002), can be responsible. The presence of immunosuppressive organisms and exposure to mycotoxins will equally adversely affect the outcome of vaccination (Perozo *et al.*, 2012; Santos *et al.*, 2019). Furthermore, Degefa *et al.* (2004) had shown in their studies that only about 60% of the flock were reached during oral application of the vaccines, which is the main route of application of the commonly used LaSota vaccine. Any one or a combination of these factors can be responsible for such incidences.

In conclusion, there is a high prevalence of ND antibodies in the farms within the study area. Furthermore, there is a strong awareness of the disease dynamics and robust vaccination regime adopted by the farmers. The local vaccines proved to be more reliable protective agents than the foreign vaccines. There is, therefore, the need for the government to enact policies that encourage the production of local vaccines, thus enhancing their availability. There may also be a need to audit the foreign vaccines imported into the country to evaluate their potency. The exact cause of post-vaccinal ND outbreaks in the area needs to be further investigated in the face of reoccurring outbreaks.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abera B, Lynch S, Duguma R, Dessie T, Bettridge J, Wigley P & Christley R (2017). Immunogenicity of the Newcastle disease virus vaccine La Sota, in introduced birds under intensive and extensive management conditions. *Livestock Research for Rural Development,* Vol 29 Art 110.

Adene DF, Oladele OA, Akpavie SO & Lawal TW (2004). Field Trial on a Newcastle Disease Vaccine An Example in Quality Assurance and Lawful Marketing. Poultry Health and Production Stirley Horden Pub Nig. Limited. Pp 271 – 275.

Alders RG, Costa R, Dias P Fringe R, Fumo A, Lobo O; Mata BV, Sitra A & Young MP (2001). Investigation into the control of Newcastle disease in village chicken in Mozambique. Review of work done in Mozambique. Report on the ACIAR/INVE. Newcastle Disease Control Project Coordination Meeting, Tofo, Imhambare Province, Appendix 3.

Alexander DJ (1997). Newcastle disease and other Avianparamyxovirus, infections. In: *Diseases of Poultry*, (BW Calnek, HJ Barnes, CW Beard, LR Dougald, YM Saif, editors), tenth edition. Pp 541 – 570.

Alexander DJ, Aldous EW, &Fuller CM (2012).The Longview; A selective review of 40 years of Newcastle disease research. *Avian Pathology,* **41**(2): 329-335.

Allan WH & Gough RE (1974). A standard Haemagglutination inhibition test for Newcastle disease. A comparison of macro and micromethods. *Veterinary Records,* **95**(6): 120 – 123.

Bell JG & Mouloudi S (1988). A reservoir of virulent Newcastle disease virus in village chicken

flocks. *Preventive Veterinary Medicine,* **6**(1): 37 – 42.

Degefa TL, Dadi A, Yami GM & Nassir M (2004). Technical and economical evaluation of different methods of Newcastle disease vaccine administration. *Journal Veterinary Medicine,* doi.10.1111/j.1439-0442.2004.00658.x.

Dimitrov KM, Andrew MR, Xueting Q, Justin B & Claudio LA (2016). Temporal, geographic, and host distribution of avian paramyxovirus 1 (Newcastle disease virus); Infection. *Genetics and Evolution,* **39**(21): 22-34.

Dimitrov KM, Abolnikb C, Afonsoa CL, Albinac E, Bahle J, Bergf M, Briandg FX, Brownh IH, Choii KS, Chvalaj I, Dielk DG, Durrl PA, Helena L., Ferreiraa M, Fusaron A, Gild P, Goujgoulovap GV, Grundq C, Hickse JT, Joannisr TM, Torchettis MK, Kolosovj S, Lambrechtt B, Lewish NS, Liuv H, Liuw H, McCulloughl S, Millerx PJ, Monnen I, Muller CP, Munirz M, Reischakaa D, Sabraab M, Samalac SK, Servan de Almeidad R, Shittur I, Snoecky CJ, Suareza DL, Van Bormt S, Wangw Z, Wongl FYK (2019). Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease. *Infection, Genetics and Evolution*, doi.org/10.1016/j.meegid.2019.103917.

Eniope BO, Oluwole OO & Amos AO (2020). Evaluation of the potency of Newcastle disease vaccines from veterinary outlets in Abeokuta Ogun State, Nigeria. *Science World Journal,* **15**(2): 26-29.

Goldhaft TM (1980). Historical note on the origin of La Sota strain of Newcastle disease virus. *Avian Diseases,* **24**(2): 297-301.

Hossain KMM, Ali MY & Yamato I (2010). Antibody levels against Newcastle disease virus in chickens in Rajshahi and surrounding districts of Bangladesh. *International Journal of Biology,* **2**(3): 102 – 106.

Ibu OJ, Ogunsola D, Aba-Adulugba EP & Echeonwu GON (2002). Quality control and assessment of two Newcastle disease vaccines imported into Nigeria and the locally produced Lasota. *Tropical Veterinarian,* **20**(1): 8-10.

Ishola OO (2012). An appraisal of the use of vaccination for disease prevention in poultry in Ibadan, Nigeria. *Bulletin of Animal Health and Production in Africa,* **60**(1): 19-23.

Kaleta EF & Kummerfeld N (2012). Isolation of herpes virus and Newcastle disease virus from White Storks (*Ciconiaciconia*) maintained at four rehabilitation centers in northern Germany during 1983 to 2001 and failure to detect antibodies against avian influenza A viruses of subtypes H5 and H7 in these birds. *Avian Pathology,* **41**(4): 83-389.

Kapczynski DR, AfonsoCL & Miller PJ (2013). Immune responses of poultry to Newcastle disease virus. *Development and Comparative Immunology,* **41**(3): 447-453.

Nayak B, Rout SN, Kumar S, Khalil MS, Fouda MM & Ahmed LE (2009). Immunization of chickens with Newcastle disease virus expressing H5 hemagglutinin protects against highly pathogenic H5N1 avian influenza viruses. *PLoS One* 4:e6509.

Numan M, Zahoor MA Khan HA & Siddique M (2005). Serological status of Newcastle disease in broilers and layers in Faisalabad and surrounding districts. *Pakistan Veterinary Journal,* **25**(2): 55 – 58.

OIE (2012). Newcastle disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.1.14 http://www,oie. Int/international standingetting/terrestrial-manual/access online, retrieved 02-12-2020.

Olugasa BO, Emikpe BO, Oluwayelu DO, Cadmus SIB, Ayinmode AB & Oluwole OE (2012). Field evaluation of immunogenicity of five commercial vaccines against Newcastle disease in poultry farms in Ibadan, Nigeria. *Nigerian Veterinary Journal,* **33**(2): 475-482.

Orsi MA, DorettoJr L, Camillo SCA, Reischak D, Ribeiro SAM, Ramazzoti A, Mendonça AO, Spilki FR, Buzinaro MG, Ferreira HL & Arms CW (2010). Prevalence of Newcastle disease virus in broiler chickens (*Gallus gallus*) in Brazil. *Brazillian Journal of Microbiology,* **41**(2): 349-357.

Perozo F, Marcano R & Afonso CL (2012). Biological and phylogenetic characterization of a genotype VII Newcastle disease virus from Venezuela: Efficacy of field vaccination. *Journal of Clinical Microbiology*, **50**(4): 1204-1208.

Rauw F, Gardin Y, Palya, van Borm S, Gonze M, Lemaire S, van den Berg T & Lambrecht B (2009). Humoral, cell-mediated and mucosal immunity induced by oculo-nasal vaccination of one-day-old SPF and

conventional layer chicks with two different live Newcastle disease vaccines. *Vaccine*, **27**(27): 3631-3642.

Roy P & Koteeswaran A (1999). Efficacy of the adjuvated mesogenic Newcastle disease vaccine in chicken. *Science Direct,* **17**(20 – 21): 2674 – 2676.

Samuel A, Nayak B, Paldurai A, Sa X, Gilbert. I, Aplogan I, Awoume A, Webby RJ, Ducalez MF, Collins PL & Samal SK (2013). Phylogenetic and pathotypic characterization of Newcastle disease viruses circulating in West Africa and efficacy of a current vaccine. *Journal of Clinical Veterinary Microbiology,* doi. 10.1128/JCM. 02750.12.

Santos PC, Cunha CS & Fernandes JO (2019). Prevalent mycotoxins in animal feed: Occurrence and analytical methods. *Toxins (Basel),* doi.10.3390/toxins11050290.

Seal BS, King DJ & Meinersmann RJ (2000a). Molecular evolution of newcastle disease virus matrix protein gene and phylogenetic relationship among the paramyoxovividae. *Virus Research,* **66:** 1 – 11.

Seal BS, King DJ & Seller HS (2000b). The avian response to Newcastle disease virus. *Development and Comparative Immunology*, doi.10.1016/s0145-305x(99)00077-4.

Vui TO, Lohr JE, Kyule MN, Zessin KH & Baumann MPO (2002). Antibody levels against Newcastle disease virus, infectious bursal disease virus and influencza virus in rural chicks in Vietam. *International Journal of Poultry Science,* **1**(5): 127 – 132.