Evaluation of the immunogenicity and protective efficacy of different Newcastle disease vaccination schedules against a Nigerian epizootic strain

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Abstract

The comparative immunogenicity and protective efficacy of four different vaccination schedules employed by poultry farmers for the control of Newcastle disease (ND) in Nigeria were studied. Each vaccination schedule (or group) consisted of 30 day-old-chicks which were sequentially vaccinated at days 7, 28 and 49 of age with live ND vaccines including the lentogenic Hitchner-BI (H) and La Sota (L) and the mesogenic Komarov (K) and R_2B (R) strains. Vaccination schedules involving the use of Komarov strain (H-L-K and L-L-K) elicited the highest mean haemagglutination inhibition antibody titers (log_2) of 6.9 and 8.0, respectively but were not significantly higher (6.4) than the schedule involving R_2B (L-L-R) vaccine. Continuous sequential vaccination with La Sota (L-L-L) appeared to be counterproductive to the stimulation of high levels of antibody (4.9) at day 59 of age. All vaccinated birds were fully protected from morbidity and mortality upon experimental challenge with a recently identified highly virulent genotype XVII ND virus. Ten birds selected from the unvaccinated control group (n=30) all died following experimental challenge after displaying typical clinical signs of ND. However, some vaccinated birds despite being protected from disease, shed ND virus from their oro-pharynx. Vaccination schedules that involved primary vaccination with La Sota and boosted with mesogenic strains like Komarov (L-L-K) or R_2B (L-L-R) are recommended for the control of ND in Nigeria. This is particularly important in birds with high levels of maternally derived antibody. Stringent biosecurity measures should be adopted on commercial farms to prevent the introduction of virulent NDV and other pathogens. This is particularly important during the first 2 weeks of life when antibody titers were observed to be lower and the birds assumed to be immunologically immature.

Keywords: Newcastle Disease, Vaccination Schedules, Protective efficacy, Nigeria.
Running title: Newcastle disease vaccination schedules in Nigeria

Évaluation de l'immunogénicité et de l'efficacité protectrice de différents protocoles de vaccination contre la maladie de Newcastle face à une souche épizootique nigériane

Résume

L'immunogénicité et l'efficacité protectrice de quatre protocoles de vaccination couramment utilisés par les aviculteurs nigérians pour contrôler la maladie de Newcastle (ND) ont été comparées. Chaque protocole (ou groupe) comprenait 30 poussins d'un jour, vaccinés de manière séquentielle aux jours 7, 28 et 49 avec des vaccins vivants atténués, incluant les souches lentogènes Hitchner-B1 (H) et La Sota (L), ainsi que les souches mésogènes Komarov (K) et R_2B (R). Les protocoles incluant la souche Komarov (H-L-K et L-L-K) ont induit les titres moyens d'anticorps inhibant l'hémagglutination les plus élevés (log2), respectivement 6,9 et 8,0, mais sans différence significative par rapport au protocole utilisant le vaccin R_2B (L-L-R, 6,4). En revanche, une vaccination séquentielle continue avec La Sota (L-L-L) s'est avérée contre-productive, ne stimulant qu'un faible taux d'anticorps (4,9) à l'âge de 59 jours. Tous les oiseaux vaccinés ont été totalement protégés contre la morbidité et la mortalité après une épreuve expérimentale avec une souche hautement virulente de NDV de génotype XVII, récemment identifiée. En revanche, les dix oiseaux sélectionnés parmi le groupe témoin non vacciné (n=30) sont tous morts après l'infection,

présentant des signes cliniques typiques de la maladie. Cependant, certains oiseaux vaccinés, bien que protégés contre la maladie, ont excrété le virus de Newcastle par voie oropharyngée. Les protocoles incluant une primo-vaccination avec La Sota suivie de rappels avec des souches mésogènes comme Komarov (L-L-K) ou R_2B (L-L-R) sont recommandés pour le contrôle de la ND au Nigeria, particulièrement chez les oiseaux présentant des niveaux élevés d'anticorps maternels. Des mesures de biosécurité strictes doivent être appliquées dans les élevages commerciaux pour prévenir l'introduction de souches virulentes de NDV et d'autres agents pathogènes. Ceci est particulièrement crucial durant les deux premières semaines de vie, période où les titres d'anticorps sont plus faibles et où les oiseaux sont considérés comme immunologiquement immatures.

Mots-clés : Maladie de Newcastle, Protocoles de vaccination, Efficacité protectrice, Nigéria.

Introduction

Newcastle disease (ND) is a viral disease of poultry caused by Avian *Paramyxovirus* serotype-1 (APMV-1) belonging to the genus *Avulavirus* and family *Paramyxoviridae*. It is prevalent worldwide and affects over 250 species of birds (Alexander, 1997). It is economically important because it results in restriction in trade, losses due to a spectrum of clinical signs and mass mortality in fully susceptible flocks (Roy, 2012). The first report of ND in Nigeria was in 1952 (Hill *et al*., 1953), thereafter several cases have been reported in commercial, rural scavenging, captive and free-living wild birds, making it enzootic across the entire country (Shittu *et al*., 2016a). Previous studies have shown that ND was ranked first among other diseases affecting the poultry industry in Nigeria (Geidam *et al*., 2013).Since there is no effective treatment for ND, the poultry industry relies primarily on stringent biosecurity regimens and vaccination procedures for the control of ND. Various types of live vaccines are used in Nigeria for the control of ND. These include *Hitchner B1* (H-B1) and *LaSota* strains which are commonly used in commercial poultry. The National Veterinary Research Institute (NVRI), Vom recommends the vaccination of birds at 6-8 weeks of age with *Komarov* strain, albeit following primary vaccination with *H-B1* and *La Sota* at 0-1week and 3-4 weeks of age respectively. Despite this recommendation, farmers reported outbreaks of ND on their farms following administration of live *Komarov* vaccine and thus were reluctant to administer *Komarov* (Anosa and Okoroafor, 2024). These outbreaks could be due to inadequate primary immunization and poor biosecurity measures with the presence of concomitant disease agents.

According to the Office International des Epizootics (OIE) master seed of live vaccines used in the control of ND should have an Intracerebral Pathogenicity Index (ICPI) value of less than 0.7. *La Sota* (ICPI=0.4) and *HB1* (ICPI=0.2) are thus recommended for the control of ND. However, mesogenic vaccine strains like *Komarov* (ICPI= 1.4) and R_2B (ICPI= 1.4) are commonly used in countries where velogenic ND is endemic (e.g. Nigeria), but fall within the OIE definition of viruses that cause virulent ND (vND), and are thus not recommended by the OIE for the control of ND. Due to the disease pressures faced by poultry farmers, some farmers resort to frequent use of *La Sota* vaccine (as frequent as every 2-3weeks) while others use imported mesogenic strains like R_2B (Anosa and Okoroafor, 2024) to control ND. In addition, inactivated oil-emulsion vaccines are often used by some farmers, which come as a single package or in combination with other poultry agents (including Infectious bronchitis and Egg-drop syndrome 76 viruses). Some of the vaccines are produced locally by the NVRI, Vom while others are imported from Israel, India and Europe. Despite stringent vaccination of commercial poultry against ND, outbreaks of disease

frequently occur (Saidu *et al*., 2006; Ezema *et al*., 2009; Sadiq *et al*., 2011; Okoroafor *et al.*, 2017). This could be due to several reasons including genotypic and antigenic variation between vaccine and outbreak strains, although all APMV-1 members belong to the same strain. NDV has continued to evolve causing, even more, a threat not only to unvaccinated but the vaccinated flocks inclusive (Shittu *et al*., 2016b). Three genotypes and 2 sub-genotypes have been described (Catolli *et al*., 2010; Snoeck *et al*., 2013) and are responsible for the current ND outbreaks being experienced in the sub region. These genotypes have not been detected outside the sub-region, making them indigenous (Shittu *et al*., 2016). The current vaccination programme in Nigeria and sub region at large needs to be reviewed to accommodate the current circulating strains. This will be the first study of the evaluation of different vaccination schedules which includes *Komarov* against the recently identified circulating velogenic NDV genotype XVII in Nigeria.

The aim of the present study was to evaluate the immunogenicity and protective efficacy of different vaccination schedules involving locally produced live lentogenic and mesogenic ND vaccine strains against a recently identified velogenic NDV strain with peculiarity to West and Central African countries. The efficacy of these vaccination schedules was compared to a schedule involving a frequently administered foreign mesogenic vaccine strain. The ability of the different vaccination schedules to prevent virus shedding was also evaluated.

Materials and methods

Experimental animals

One hundred and seventy 1-day-old commercial layer chicks purchased from a reputable commercial hatchery were used for the study. They were kept on deep litter and housed in a facility which ensured isolation. They were fed with commercial poultry ration and provided water *ad-libitum.* The birds were routinely vaccinated against Marek's disease and infectious bursal disease. They also received prophylactic antibiotics, specifically against *Mycoplasma.*

Experimental design/ vaccination

One hundred and fifty 1-day- old chicks were divided into 5 groups (I-V) of 30 birds each and administered different strains of live ND vaccines at doses prescribed by the manufacturers at days 7, 28, and 49 of age (Table 1). Groups' I-III birds were administered local vaccines (*HB-1, La Sota* and *Komarov* strains) produced by NVRI, Vom while group IV birds were administered imported ND vaccines (*La Sota* and *R_2B*) produced by INDIVAX, India. Group V birds were not vaccinated and served as control. *H-B1* and *La sota* strains were administered by intra-ocular instillation while *Komarov* and *R_2B* were administered by intramuscular injection.

Table 1: Grouping and vaccination schedule

Groups	I	II	III	IV	V
Day 7	H-BI	Lasota	Lasota	Lasota*	-
Day 28	Lasota	Lasota	Lasota	Lasota*	-
Day49	Komarov	Lasota	Komarov	R_2B*	-

*Vaccines produced by INDIVAX, India.

Serology

On day 1, blood was collected terminally by cardiac puncture of 20 randomly selected chickens representing the whole flock, to determine the level of maternal antibodies. Subsequently blood was collected by jugular vein-puncture from 20 randomly selected chickens from each of the 5 groups of chickens at days 17, 38 and 59 of age. Serum was obtained

from clotted blood after about 3hr at room temperature and stored at -20°C.

Serum samples were assayed for ND haemagglutination inhibition (HI) antibodies using the microtitre method (OIE, 2012). HI test was performed using 4HA units of ND vaccine *La Sota* strain as antigen.

Detection of virus shedding

Oropharyngeal swabs were collected from all challenged birds on days 0, 2,4,6,9 and 14 post-challenge (PC). The swabs were tested for NDV using the method of the World Organization for Animal Health (OIE, 2012).

Experimental challenge

At day 65 of age, 10 randomly selected birds from each of the 5 groups were subjected to experimental challenge with a lethal dose (0.1mL) of a velogenic NDV isolate duck/Nigeria/903/kudu-113/1992 by intraocular instillation. It is a genotype XVII NDV (Shittu *et al*., 2016b). The inoculum was provided by NVRI, Vom, and had a median embryo infective dose of 10^6/mL. Following challenge, birds were monitored daily for 20 days for clinical signs of disease and mortality. Any dead chicken was necropsied immediately after death for gross pathology.

Ethical approval

Permission to conduct the study and ethical clearance was obtained from the Medical Research Ethics Committee of the University of Nigeria, Nsukka (FVM-UNN-IACUC-2023-45). All the birds in this study were handled humanely.

Statistical analysis

The data on antibody (HI) titre were summarized as mean geometric titres (MGT) and comparison between vaccination groups (i.e. schedules) at each day of sampling, made by one-way ANOVA. Means were separated using Duncan's multiple range test at a 5% level of probability. Frequencies of virus isolation were analyzed for significance by Fisher's exact test at 5% level of significance.

Results

Immune response

The ND-HI antibody titres of serum collected at day 1 of age (pre-vaccination) ranged from 3 – 7log_2 with a relatively high MGT of 5.2. At day 17 of age (following primary vaccination at day 7 of age) the *La Sota* vaccinated groups (II, III and IV) birds had significantly higher antibody titres than the H-B1 (or group I) vaccinated birds (Table 2). At day 38 of age (following first booster vaccination at day 28) the antibody titres increased with MGT's ranging from 6.1–6.7 in all the vaccinated groups (1–IV) of birds (Table 2). The antibody responses were more variable at day 59 of age (following the second booster vaccination) with the MGT being significantly higher (8.0) in the group IV (L-L-K) birds than the group II (L-L-L) birds (4.9) (Table 2).

There was a steady decline in the antibody titres of the non-vaccinated group V birds with time. In contrast there was an increase in antibody titres in the vaccinated groups of birds with time, except in the group II (L-L-L) birds at day 59 of age, when there was a drop (4.9) in titre (Table 2).

Table 2: Chronologic ND-HI antibody titers (Log_2) elicited by different ND vaccination schedules

Day of Age	Group I	Group II	Group III	Group IV	Group V
17	4.0 ± 0.3^{a}	5.2 ± 0.3^{b}	5.2 ± 0.3^{b}	4.9 ± 0.4^{b}	2.8 ± 0.1^{c}
38	6.7 ± 0.3^{a}	6.2 ± 0.2^{a}	6.1 ± 0.3^{a}	6.6 ± 0.2^{a}	2.0 ± 0.2^{b}
59	6.9 ± 0.2^{ab}	4.9 ± 0.3^{c}	8.0 ± 0.7^{b}	6.4 ± 0.6^{a}	0.3 ± 0.2^{d}
Vaccine Schedule	H-L-K	L-L-L	L-L-K	L^F-L^F-R	Nil

^{abcd}: Different superscripts in a row indicate significant difference between the groups ($P<0.05$)

H = Hitchner B1, L = Lasota, L^F = Foreign Lasota, K = Komarov, R = R_2B.

Protection against challenge

No overt clinical signs of ND were observed among the vaccinated groups of chickens.

Following experimental challenge, there were 10 dead chickens: all from the unvaccinated and challenged group V chickens. The mortalities occurred between 4-7 days post-challenge after displaying typical clinical signs of ND including depression, prostration, greenish watery diarrhoea, ataxia, paralysis of the legs and wings, involuntary shaking of head and torticollis. All vaccinated chickens survived the experiment without displaying any clinical signs of ND (Table 3). Gross postmortem lesions observed in dead birds included haemorrhagic tracheitis, enlargement of the spleen, proventricular haemorrhages and haemorrhages of the ceacal tonsils.

Table 3: Post-challenge mortalities following challenge with genotype XVII NDV

	Group I	**Group II**	**Group III**	**Group IV**	**Group V**
Vaccine Schedule	H-L-K	L-L-L	L-L-K	L^F-L^F-R	Nil
Total	10	10	10	10	10
Dead	0	0	0	0	10
% Mortality	0	0	0	0	100

H = Hitchner B1, L = Lasota, L^F = Foreign Lasota, K = Komarov, R = R_2B.

Virus shedding in vaccinated chickens after fatal challenge

Results of NDV detected from oropharyngeal swabs taken on days 0,2,4, 6, 9 and 14 PC are presented in Table 4. NDV was detected from groups I, II, IV and V birds from day 2 PC. Groups II (L-L-L) and V (unvaccinated-challenged) birds showed the highest positive detection rates of 40 and 70% respectively at day 2 PC. By day 4 PC, the surviving unvaccinated-challenged birds were all positive for NDV while the group III (L-L-K) birds had the lowest detection rate of 10%. No NDV was detected from swabs taken at days 9 and 14 PC in any of the vaccinated groups of birds.

Table 4: Number of birds shedding virus following challenge of vaccinated birds with different ND vaccination schedules

Groups (Vaccine schedule)	**Day 0**	**Day 2**	**Day4**	**Day 6**	**Day 9**	**Day14**
I (H-L-K)	$0/10^a$	$1/10^a$	$4/10^a$	$2/10^a$	$0/10^a$	$0/10^a$
II(L-L-L)	$0/10^a$	$4/10^b$	$6/10^{ab}$	$3/10^a$	$0/10^a$	$0/10^a$
III(L-L-K)	$0/10^a$	$0/10^a$	$2/10^a$	$1/10^a$	$0/10^a$	$0/10^a$
IV(L^F-L^F-R^F)	$0/10^a$	$1/10^a$	$3/10^a$	$2/10^a$	$0/10^a$	$0/10^a$
V(Nil)	$0/10^a$	$7/10^c$	$9/9^c$	$1/1^b$	NS	NS

^{abc}: Different superscripts within a column indicate significant difference between the groups ($P<0.05$)

H = Hitchner B1, L = *La Sota*, L^F = Foreign *La Sota*

K = Komarov, R = R_2B, NS = No survivors

Day x = Day x post challenge

Discussion

The absence of post vaccination clinical reactions following vaccination with *H-B1* and *La Sota* is similar with the results of previous studies (Anosa and Adene, 2007). This could be attributed to the relatively high levels of maternally derived antibody (MDA) among the chicks at day old. Delay of primary vaccination to day 7 of age when the birds would be more immunologically mature, could also have been responsible for the absence of post vaccination tissue reactions in our study.

The ND–HI antibody titres of serum samples collected at day old, showed high prevalence of

maternal antibody to ND in the stock of chicks employed for this study. The endemic nature of vND in Nigeria necessitates the stringent and continuous use of ND vaccines in breeder flocks. This stimulates the production of ND specific antibodies in parent birds which are passed on to the offspring as MDA. Previous studies (Anosa and Adene, 2007; Oni and Adedipe, 2012) shows that day old chicks from some breeder flocks in Nigeria have a high level/prevalence of MDA to ND. When chickens have high levels of MDA, the primary vaccination should be delayed; otherwise, the vaccination may be a failure (Roy, 2012). The half-life of maternal antibody is 4.5 days (Allan *et al*., 1978; Roy, 2012). The significantly higher ND antibody titres observed in the *La Sota* vaccinated groups II, III and IV birds when compared to the H-B1 vaccinated group I birds at day 17 of age, is consistent with results from previous studies (Westbury *et al.*, 1984; Kapczynski and King, 2005; Alexander and Senne, 2008). *La Sota* (ICPI = 0.4) is more pathogenic and subsequently more immunogenic than H-B1 (ICPI = 0.2) (OIE, 2008). *La Sota* vaccines were administered by intraocular instillation in our study to ensure a high and homogenous immune response to vaccination. When vaccination is done through the oral route, infectivity of viruses reduces to 1000 – fold as they pass through the acid pH (around 2.6) of the gizzard (Roy, 2012). Unfortunately, field conditions are often sub-optimal due to stocking densities and varying uptake rates with vaccine success rates reduced to 55% in flocks through aerosol dissemination and 60% when delivered through drinking water drinkers (Degefa *et al*., 2004; Mayers *et al*., 2017). The first booster vaccination of birds with *La Sota* at day 28 of age resulted in improvements in antibody titres in the groups 1 – 1V birds. The foreign and local *La Sota* vaccinated groups showed similar responses and were thus equally effective. The improvements in the antibody titres of the *Komarov* and *R_2B* vaccinated groups I, III and IV birds respectively are consistent with the reputation of these mesogenic vaccines as being of higher pathogenicity and immunogenicity than their lentogenic counterparts (Alexander and Senne, 2008). The immune response increases as the pathogenicity of the live vaccine increases (Alexander and Senne, 2008). Therefore, to obtain the desired level of protection without serious reaction, vaccination programmes are needed that involve sequential use of progressively more virulent viruses or live vaccines, followed by inactivated vaccines (Alexander and Senne, 2008). This may explain the lower immune response elicited by the vaccination schedule (group II) that involved sequential use of only *La Sota* vaccine at day 59 of age (Table 1). As expected, the antibody titres of the unvaccinated control were negligible on day 59 of age. The half-life of MDA in chicks is 4.5 days (Allan *et al.*, 1978; Roy, 2012). The continued outbreaks of velogenic ND in domestic poultry worldwide emphasize the importance for continued research on vaccine efficacy against newly isolated strains (Kapczynski and King, 2005). Following experimental challenge, the post challenge mortalities and any observed clinical sign of ND were used to measure the degree of protection afforded by the different vaccination schedules. The vaccination schedules employed in groups I – IV fully protected the birds from morbidity and mortality upon challenge with the highly virulent genotype XVII NDV. This could be attributed to the moderately high antibody titres among the vaccinated groups of birds. Previous workers have demonstrated a positive correlation between antibody titres (either HI or ELISA) and protection from disease (Kapczynski and King, 2005). The protective role of HI and ELISA antibody against NDV has been described (Mazija *et al*., 2010; Omony *et al*., 2017; Han *et al*., 2017). Allan *et al*., (1978) also described the relationship between the HI test and challenge. Birds with HI titre of 2^2 or less suffered 100% mortality on challenge, birds with

HI titres of $2^2 – 2^5$ had 10% mortality, $2^6 – 2^8$ had serious egg production losses, but those with titres $2^9 – 2^{11}$ remained normal on challenge. Thus, at varying stages of the study the birds might not have been fully protected by the humoral immunity elicited by the various vaccination schedules, particularly during the first two weeks of life. Fortunately, the innate immune response of the bird is capable of exclusion or rapid response to microbes (Kapczynski *et al.*, 2013). Cell mediated immunity; a specific adaptive immunity mediated by T-lymphocytes, has been suggested to be an important factor to the development of protection in chickens vaccinated against NDV and contributes to virus clearance (Kapczynski *et al.*, 2013). NDV was detected among the 4 vaccinated groups of challenged birds from day 2 to day 6 PC. NDV vaccines do not prevent vaccinated birds from becoming infected with virulent NDV and subsequently shedding the virus (Miller *et al.*, 2013). This suggests that vaccinated chickens may still act as reservoirs, causing spread of ND between flocks and farms. The different vaccination schedules significantly reduced the number of chickens shedding NDV when compared to the unvaccinated-challenged group V birds. Live ND vaccines will decrease both the number of chickens shedding virus and the titres shed (Kapczynski and King, 2005).

Conclusion

The four different vaccination schedules studied, fully protected the vaccinated birds from morbidity and mortality following challenge with a virulent genotype XVII NDV. This protection was achieved without resulting in any overt post-vaccination reactions. Vaccination schedules involving the use of *Komarov* vaccines elicited the highest antibody responses but were not significantly different from the schedule involving *R_2B* (foreign vaccine). The vaccination schedule involving the continuous and sequential administration of *La Sota* stimulated lower antibody responses, when compared with those involving mesogenic (*Komarov* and *R_2B*) vaccines. *Komarov* and *R_2B* vaccines are thus recommended for the control of ND in Nigeria. Due to the endemic nature of virulent ND in Nigeria, *La Sota* which is more immunogenic than H-B1 strain should be considered for primary vaccination against ND in Nigeria. This is particularly important in the presence of high levels of MDA. Stringent biosecurity measures should be adopted on commercial farms to prevent the introduction, transmission and spread of virulent NDV and other pathogens.

Conflict of interest

There are no conflicts of interest related to our study

Authors' contributions

The named author's contributions satisfied the ICJME authors' criteria. This included conceptualization of the work, data collection, analysis and interpretation, as well as writing and approval of manuscript.

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