

EVALUATION OF THE POTENCY OF NEWCASTLE DISEASE VACCINES FROM VETERINARY OUTLETS IN ABEOKUTA, OGUN STATE, NIGERIA

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ABSTRACT

Vaccination against Newcastle disease (ND) is the most reliable tool in its prevention and control, it is therefore important that vaccines used are of good quality. This study was conducted to assess the quality of live ND vaccines sold in Abeokuta over a six months' period. Live Newcastle disease vaccines were purchased from ten different veterinary sales outlets and their antigenic titres were determined using Hemagglutination assay (HA). The results revealed a difference in antigenic titres amongst the various ND vaccines sold in Abeokuta with only 20.33 % having high titre, 55.9 % having an average titre, and 23.7 % having low titre. The vaccine titres varied between different brands with storage and handling as contributing factors to the variations. Antigenic titre contained in vaccines properly stored showed significantly ($p < 0.05$) higher antigenic titre and vaccines that were close to their expiration dates had lower titres. Newcastle disease vaccines in Abeokuta are of average titres, it is therefore necessary that adequate evaluation of ND vaccines be recommended before use in flocks and owners of vaccine sales outlets be educated on the need for proper handling and storage of these biologicals.

Keywords: Evaluation, Newcastle disease vaccine, potency, Abeokuta

INTRODUCTION

Newcastle disease (ND) is one of the most important poultry disease in the world; both for the number of animals affected every year and the severe economic impact on the poultry industry (Thompson, 2015). It is a highly devastating disease of domestic poultry and wild birds (Alexander, 2000; Alexander, 2009; Alexander *et al.*, 2012) caused by Newcastle disease virus (NDV), also known as Avian paramyxovirus serotype-1 (APMV-1). The major clinical sign associated with ND are loss of appetite, depression, weakness, greenish diarrhea, gasping, coughing, paralysis of wings and legs, torticollis and cyanosis of comb and wattle (Pazhanivel *et al.*, 2002).

In Nigeria, ND is presently one of the most important endemic diseases of poultry causing high morbidity, mortality, decrease in egg production and it constitutes a major constraint to the development of rural poultry production (Shittu *et al.*, 2016). Poultry egg production enterprise is profitable in Ogun state as indicated by the gross margin of N 2,118,280.10 and a net income of N 2,011,857.16 per annum (Afolabi *et al.*, 2013). The profitability ratios further reveals that for every N 1 investment made in the enterprise there is a potential return of N 0.43 net farm income (Afolabi *et al.*, 2013). Vaccination against ND is the most reliable tool in its prevention and control; and animal health

professionals and farmers alike have encountered several challenges with the use of this means at preventing this disease. The effect of quality vaccines cannot be overemphasized, in that they help confer immunity against diseases by eliciting enough antibody responses and thus help reduce disease outbreaks in flocks. Phatak (2000) reported that quality of vaccines, conditions of their transportation, storage, distribution, time interval of vaccinations, presence of maternal antibody, age of chicks at vaccination, level of stress at the time of vaccination, immunosuppression factors and routes of vaccination determine failure or success of the vaccination.

Among the factors that affect potency of vaccines, Abbas *et al.* (2006), documented that Newcastle disease virus vaccines of different manufacturers illicit varying levels of antibody titre in broiler chicken in Pakistan while Dairo & Osizimete (2016) noted that improper handling has been identified as one of the major reasons for the decline in vaccine potency at the time of administration. Loss of potency becomes evident when vaccinated flocks contract the diseases the vaccines were meant to prevent.

In Nigeria, poultry chickens are routinely vaccinated against ND using various routes of vaccination such as drinking water, intraocular, intranasal and aerosol spray. Although antibody response to vaccinal antigen depends on factors such as the immune status of the chicks at vaccination and the level of maternally derived antibody, the quality of the vaccine, in terms of its antigenic titre, severely affect protection conferred by vaccines. There have been concern in the poultry industry that ND vaccines used in Nigeria may not be provoking desired level of antibody response and as such the required protection. The aim of this study was to assess the antigenic titre of some of the ND vaccines sold in Abeokuta, Ogun state, Nigeria

MATERIALS AND METHODS

Vaccines

A total of fifty-nine Newcastle disease vaccines (LaSota strain) were purchased from ten different Veterinary shops in Abeokuta once in a month for a six months' period, from July 2018 to January 2019. The vaccines were stored in an ice packed cooler from the point of purchase to ensure that cold chain was maintained and the titre assessment tests were carried out on the day of purchase. Storage conditions, brand names, manufacturing, batch numbers and expiry date were noted at the point of purchase.

Evaluation of Newcastle Disease Antigen Titres of some ND vaccines

The hemagglutination (HA) test to determine the vaccines antigenic titre was done as described by OIE, Terrestrial Manual (2012). Briefly, a 0.5 % washed red blood cells (RBCs) was prepared and each vial of the vaccines was diluted initially with 1ml of normal saline. Normal saline (25 µl) was dispensed into the well of U-bottom microtitre plates with three rows representing a vaccine sample (test was done in triplicates) using a multichannel micropipette. A serial dilution of 25 µl of the diluted vaccines was made across the wells in each row and 25 µl of washed RBCs was added. Two wells were used as negative and positive controls for each row.

Statistical analysis

Data were analyzed using SPSS 16 software by employing one-way analysis of variance (ANOVA) to analyze the difference in the titres among different outlets and significance difference was taken at p <0.05 level while other results were presented in bar graphs using the same software.

RESULTS

Newcastle disease vaccine titres from different locations per different time points in Abeokuta

The antigenic titres of the vaccines purchased from ten veterinary outlets in Abeokuta at different time points (Table 1) revealed that the titres varied at different times in a particular location. The lowest vaccine titre was 1 log₂ while the highest was 9 log₂ with 23.7 % (14/59) having low titres (1 log₂ – 3 log₂), 55.9 % (33/59) having an average titres (4 log₂ – 6 log₂) and 20.33 % (12/59) have high titres (above 7 log₂). Locations E and J had the lowest average ND vaccine titres though some vaccines with high titre were purchased from some locations at some time points.

Table 1: Titres of the different vaccine purchased from ten locations in Abeokuta

Locations	27/7/18	4/9/18	19/10/18	23/10/18	5/11/18	15/1/19	Average Titre
A	6 log ₂	6 log ₂	5 log ₂	5 log ₂	4 log ₂	5 log ₂	5.2 log ₂
B	-	7 log ₂	5 log ₂	7 log ₂	5 log ₂	7 log ₂	6.2 log ₂
C	6 log ₂	7 log ₂	7 log ₂	6 log ₂	6 log ₂	6 log ₂	6.3 log ₂
D	6 log ₂	4 log ₂	2 log ₂	7 log ₂	5 log ₂	5 log ₂	4.8 log ₂
E	7 log ₂	2 log ₂	3 log ₂	3 log ₂	2 log ₂	1 log ₂	3 log ₂
F	7 log ₂	6 log ₂	5 log ₂	7 log ₂	6 log ₂	6 log ₂	6.1 log ₂
G	5 log ₂	5 log ₂	9 log ₂	3 log ₂	5 log ₂	4 log ₂	5.1 log ₂
H	7 log ₂	5 log ₂	3 log ₂	6 log ₂	5 log ₂	5 log ₂	5.1 log ₂
I	7 log ₂	4 log ₂	2 log ₂	3 log ₂	1 log ₂	1 log ₂	3 log ₂
J	5 log ₂	5 log ₂	3 log ₂	5 log ₂	3 log ₂	6 log ₂	4.5 log ₂
Total	9	10	10	10	10	10	4.9 log ₂

The mean titre of the different brands of ND vaccines in Abeokuta

A total of five brands of ND vaccines (V1-V5) were sold in Abeokuta during the period of this study (Figure 1) and were all imported (foreign) vaccines. Majority (43 %) of vaccines were of the V3 brand with an average titre of 5.3 log₂. The brand with the lowest number purchased (2 %) was V4 with a titre of 9 log₂ while V1 with the lowest average titre (2.8 log₂) represented 21.4 %. Three of the vaccines purchased did not have labels on them but had an average titre of 4.1 log₂.

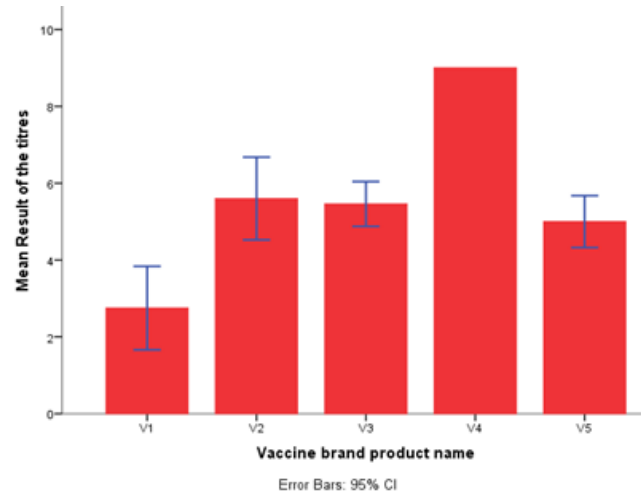


Figure 1: Average titres of the different vaccine brands purchased in Abeokuta

Observations at veterinary outlets during the purchase of ND vaccine in Abeokuta

Based on the features of the vaccines purchased (Table 2), it was observed that vaccines stored in refrigerators before been sold had the highest titre of 5.5 log₂ (Figure 2). The vaccines with the lowest average titres were those sold without ice packs (4.1 log₂) and those stored in freezers before sale (4.2 log₂) respectively.

Table 2: Features of the vaccines purchased

Groups	Description
1	vaccines sold in cold water without ice packs
2	vaccines without labels
3	vaccines stored in freezers
4	vaccines sold with ice packs
5	vaccines stored in refrigerator
6	vaccines with intact labels
7	vaccines with unknown storage method

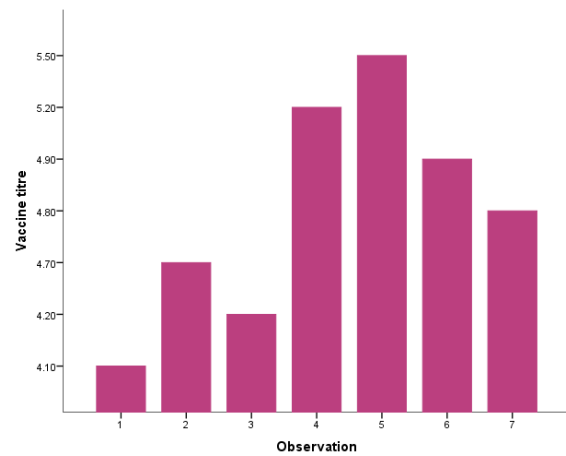


Figure 2: Average titre of the vaccines based on their features

Months to expiry dates of the ND vaccines sold versus their average titres

Only fifty-six out of the fifty-nine vaccines purchased had their labels intact. Sixty-six percent of the ND vaccines sold in the veterinary outlets during the period of this study were 13-18 months away from their expiry dates with some of their titres being as low as $1 \log_2$ (Figure 3). Seven percent of vaccines were 19-24 months to their expiry dates and they had the highest average titre ($6.8 \log_2$) while 27 % were 7-12 months from expiration date. Three of the vaccines did not have labels and so their expiry dates were not known.

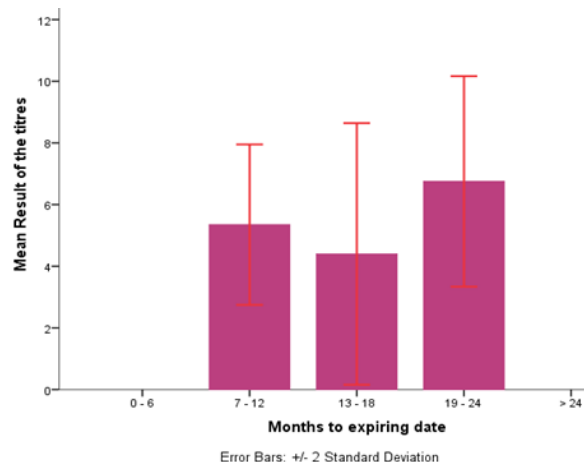


Figure 3: Average titre of vaccines with respect to their expiry dates

DISCUSSION

Newcastle disease has both epizootic and enzootic patterns in different flocks but mostly epizootic in intensive poultry. It is prevalent in Nigeria with as high as 100 % prevalence in Ogun state (Oyekunle *et al.*, 2006) and so it is very important to prevent or control it by determining factors that could contribute to variations in the antigenic titres present in the ND vaccines.

It was observed from this study that there are significant ($p < 0.05$) variations in the titre of live ND vaccines sold in Abeokuta among the outlets. Two outlets sold consistently low titre ($0-3 \log_2$) ND vaccine and others between average to high titre vaccines (above $5 \log_2$) over the period of six months but mostly, fluctuation in the titres of vaccines sold. This may be due to the availability of electricity in the areas with high titre live ND vaccine versus the epileptic supply in areas where outlets with low titre ND vaccines are located. The implication of this is that outbreaks of ND will continue to occur in Abeokuta and farmer will not trust outlets selling these live ND vaccines for good quality products. Only two out of the ten veterinary outlets consistently sold vaccines with high titre. Having only two dependable veterinary outlets in Abeokuta is poor as Abeokuta not only serve many other small towns in terms of being the centre of purchase of vaccine, but also have a lot of small and large scale poultry farmers that need these vaccines.

Live ND vaccines with high titre induce a high antibody production which protects birds from mortality and morbidity associated with ND at single use (Muhammad *et al.*, 2018). In Abeokuta, majority (56 %) of the live ND vaccine purchased during this study had the average titre ($4-6 \log_2$), 24 % were in the range of low titre, while

20 % ($\geq 7 \log_2$) had a high titre. This means that about 80 % of the live ND vaccines sold in Abeokuta will not adequately protect birds from ND. This accounts for the repeated use of live ND vaccines on many farms as vaccine protection is directly related to its antigenic titre (Spradbrow *et al.*, 1988; Senne *et al.*, 2004). The repeated use of live ND vaccines in turn leads to the abuse of vaccine use recorded on farms and increase cost of poultry production. This is not the first report of a very low live ND vaccine titre in Abeokuta, Oni *et al.* (2017) reported a low vaccine titre in Abeokuta. This indicates that situation of the ND vaccine titre in Abeokuta has not improved.

Locally produced live ND vaccines in Nigeria, from NVRI, Vom, have been reported to be superior in quality to imported vaccines (Ibu *et al.*, 2002) because the ND strains used for the production of these local vaccines are similar to the prevalent strains in Nigeria. In this study, all vaccines gotten, which represented what was sold in the outlets at that time, were all foreign vaccines.

In assessing the different vaccine brands sold in Abeokuta, it was observed that the titres amidst the brands differs. V1 brand consistently had a low titre in all the outlets, which suggests that V1 vaccines may have been poorly produced or that the particular batch was bad. Also, some vaccine brands (V3 and V5), with the same batch numbers but purchased at different outlets had two extreme titres (very low in one outlet and high in another) which may be as a result of improper handling and storage of vaccines at different outlets. The age of the vaccines, on shelf, was observed to have no effect on the titre of live ND vaccine although few older vaccines (13-18 months on the shelf) had a high titre and some younger vaccines (< 6 months old on the shelf) had low titres.

Storage facility and pattern are essential in maintaining the cold chain and ultimately the potency of vaccines. Live vaccines can either be stored in refrigerators or freezers depending on the manufacturer's instruction but a lot of people do not store them properly. As seen in this study, some outlets in Abeokuta store their vaccines in freezers at -20°C . Most live vaccines are not stored in the freezer because low temperature (-20°C) will cause shock and destroy the live vaccine viruses reducing the vaccine antigenicity (Bosha & Nongo, 2012). Storing vaccines in freezers may be responsible for their lower titre when compared to those stored in refrigerator in this study.

Break in cold chain, including freezing and thawing, which occurs as vaccines are exposed to high temperature, is majorly responsible for poor vaccine potency. Therefore, it is important to keep intact the cold chain of the vaccine from the point of purchase to usage (Abbas *et al.*, 2006). Almost all of the outlets requested for a thermoflask before the sale of vaccines, but while some added ice packs to aid the maintenance of cold chain of vaccines on transit, others did not. Cold water will cause a vaccine to thaw quicker in transit than ice packs, especially when the location where vaccine is to be purchased is far from where it will be administered. Another salient observation was that certain vaccines were purchased without labels. Vaccines without labels means vaccines without the appropriate information needed for successful vaccination (Bermudez & Stewart-Brown, 2003).

Conclusion

This study revealed that the quality of most ND vaccines sold in Abeokuta are of average quality and different vaccine brands have inconsistent titres. Live ND vaccines from different outlets in Abeokuta have significantly different titres and their titres are

affected by storage and handling. Vaccines sold in icepacks have higher titres, live ND vaccines that are closer to expiration dates have low titers and Abeokuta veterinary outlets sell mostly foreign ND vaccine. The evaluation of ND vaccines is thus recommended before vaccinating flocks; Veterinarians should enlighten and encourage farmers on the need to evaluate ND vaccines before administering them to their birds. Also veterinary outlets where ND vaccines and other vaccines are sold should employ the best practices while storing and handling the vaccines.

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