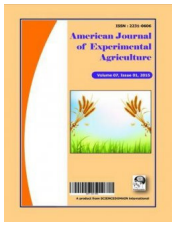

American Journal of Experimental Agriculture
13(3): 1-6, 2016, Article no.AJEA.26944
ISSN: 2231-0606

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Potency and Efficacy of Commercial Brands of Newcastle Disease Vaccines in Nigeria

I. O. Olatoye[1,2*], D. O. Oluwayelu[3], J. Y. Adeseko[1], I. A. Adebiyi[3] and S. O. Adeyemi[4]

[1]Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria.
[2]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, USA,
[3]Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria.
[4]Soptimal Ventures Nigeria Ltd, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors IOO, DOO and JYA designed the study. Authors IOO and DOO wrote the protocols and authors IOO and JYA wrote the first draft of the manuscript. Authors IOO and DOO reviewed the experimental design and all drafts of the manuscript. Authors IOO, JYA and IAA managed the analyses of the study. Author SOA sourced the vaccines used in the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2016/26944
Editor(s):
(1) Monica Rosa Loizzo, Department of Pharmacy, Health Sciences and Nutrition of University of Calabria, Italy.
Reviewers:
(1) Ogbonna Ijeoma Joy, Micheal Okpara University of Agriculture, Umudike, Abia State, Nigeria.
(2) Musa Usman, Usmanu Danfodiyo University, Sokoto, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/15500

Original Research Article

Received 11th May 2016
Accepted 6th July 2016
Published 24th July 2016

ABSTRACT

Aims: To evaluate the potency and efficacy of four commercially available brands of Newcastle disease (ND) vaccines in southwest Nigeria.
Study Design: Cross sectional survey of commercial ND (Hitchner and LaSota) for *in-vitro* and *in-vivo* analysis.
Place and Duration of Study: Vaccines were obtained from manufacturers' representatives in southwest Nigeria while chicks were obtained from commercial hatchery, reared at the Teaching and Research Farm and analysis was carried out at Department of Veterinary Microbiology in University of Ibadan.
Methodology: Isa Brown cockerel chicks divided into five groups (A-E) comprising 60 birds each were used. Chicks in groups A-D were vaccinated primarily with four different brands (ND-Sevac®;

**Corresponding author: E-mail: olatoye@vetmed.wsu.edu;*

ND-Jovac®, ND-Biovac® and ND-Fort Dodge®) of Hitchner B1 (HB1) vaccine at one-day-old (Day 1) and later with four corresponding brands of LaSota (booster) vaccine at day 21. Chicks in the fifth group which served as control received no vaccine and were used to assess the rate of maternal antibody decay. Sera were collected on days 1, 9, 16, 32, and 39 of age and the chickens were challenged with Kudu strain of ND virus (NDV) on day 33. Haemagglutination (HA) and haemagglutination inhibition (HI) tests were used to determine NDV titre and antibody levels in the vaccines and sera respectively.

Results: NDV maternal antibody level was highest (64±18.2) on day 1 and persisted to a minimum protective level (8±5.7) on day 16 before decaying to non-protective levels by day 32. All the four vaccine brands were potent with Biovac® (Vaccine C) yielding the highest NDV titres [HB1 (1536±724.1), LaSota (512±0.0)]. Also, following the administration of primary and booster (secondary) vaccinations, all the vaccines elicited protective immunity with the booster doses producing higher immune response in all the groups. However, chicks that received ND-Jovac® and ND-Biovac® were best able to overcome the effects of experimental challenge as they gave the lowest mortality rate of 33.3% with mean NDV antibody titres of 9 $\log_2$ (512±313.5) recorded seven days post-challenge. Chicks that received Sevac® and Fort Dodge® (Vaccines A and D) gave mortality rates of 55.0% and 50.0%, respectively with mean antibody titres of 7 $\log_2$ (154±57.3) post-challenge.

Conclusion: All the tested vaccines were potent and elicited protective antibody. However, ND outbreaks in vaccinated flocks in southwest Nigeria may not necessary be due to lack of potency of the vaccines but other factors such as virus strain (s) used in the production of the vaccines, vaccine storage and handling and biosecurity among others may play a role. Therefore, there is a need for routine isolation and characterization of the enzootic strain(s) of NDV in Nigeria and production of ND vaccines with such circulating strain(s) to offer optimal protection against the disease.

Keywords: Newcastle disease; vaccine; potency; chickens.

1. INTRODUCTION

The importance of poultry production in solving the challenge of protein insufficiency in Nigeria cannot be overemphasized. Poultry eggs and meat which provide acceptable form of animal protein to most people throughout the world are able to bridge this gap due to their short generation time. Many developing countries including Nigeria have adopted intensive poultry production in order to meet the demand for this form of animal protein [1]. However, Newcastle disease (ND) remains one of the major obstacles to poultry production in Nigeria despite routine vaccination by poultry farmers [2]. It is a deadly viral disease of poultry that has been reported worldwide since its first isolation in England in 1926. It is also considered as one of the major economic threats to poultry population because of its high morbidity and mortality which varies from 90-100% depending on the strain of NDV involved [3].

Effective control of ND relies on biosecurity as well as the use of safe and effective vaccines. Live vaccines prepared with lentogenic and mesogenic strains of NDV are now more commonly used in both broiler and layer birds because these vaccines can be produced on a large scale at relatively low cost [4]. Additionally, they are easy to administer on a large scale, and rapidly stimulate humoral, cell-mediated and mucosal surface immunity in vaccinated birds [5].

The southwest Nigeria is hub of poultry industry in Nigeria with Ibadan being a major city from where poultry (day-old chicks, broilers, and point-of-lay pullets) and poultry inputs (vaccines, drugs and feed ingredients) are distributed to other parts of the country. Many backyard, small-scale and commercial poultry farms as well as hatcheries which utilize different brands of poultry vaccines exist in the city [6]. Also, locally produced and imported brands of NDV Hitchner B1 (HB1) and La Sota vaccines are routinely used in the poultry industry. Despite all efforts aimed at prevention and control of this disease through the administration of vaccines at recommended doses and intervals, outbreaks of the disease still occur in vaccinated birds [7]. However, efficacy of these vaccines, especially the imported brands, in relation to their distribution, transportation and prevailing climatic conditions are not properly monitored and documented, raising doubts on their protectiveness in vaccinated chickens. Moreover,

despite enabling laws, vaccines, vaccination procedures and sales and distribution of these vaccines are unregulated while the vaccines are administered indiscriminately. This study was therefore designed to investigate the potency and efficacy of four imported NDV vaccines commercially available in Ibadan, southwest Nigeria.

2. MATERIALS AND METHODS

2.1 Newcastle Disease Virus Vaccines

Eight imported lyophilized NDV vaccines were used for this study. They included four brands of Hitchner B1 (Cevac®(A), Jovac®(B), Biovac®(C) and Fort Dodge®(D)vaccines and their corresponding brands of La Sota vaccines obtained directly from manufacturers' representatives in Nigeria. Cevac® NEW B1 L & NEW L (Ceva Sante Animale, France) contained La Sota lentogenic strain of NDV in life freeze-dried form, 1000 dose per vial Jovac® vaccines (Jordan Bioindustries Center, Jordan) contained $10^{6.0}$ EID_{50} of freeze dried live attenuated NDV prepared from a refrence strains of B1 and La Sota (1000 dose per vial); Biovac vaccines (Biovac, Israel) contained $10^{6.5}$ EID_{50} of freeze dried live attenuated NDV prepared from a reference strains of B1 (*VIR 106*) and La Sota (*VIR 116*) 1000 dose per vial; Fort Dodge vaccine (Fort Dodge Animal Health, Fort Dodge, USA) contained at least $10^{6.3}$ EID_{50} of B1 and La Sota strains NDV.

2.2 Experimental Chickens

A total of 300 day-old cockerel chicks with a history of ND vaccination of the parent stock were obtained from a reputable hatchery in Ibadan, Oyo state. The chicks were housed in an experimental animal pen that was thoroughly cleaned and disinfected at the University of Ibadan Teaching and Research Farm. They were divided into five groups (A-E) of 60 birds each and managed on deep litter with strict enforcement of biosecurity measures. The chicks were brooded and reared for a period of 14 weeks with optimal temperature, humidity, space, light and ventilation provided. They were given chick starter feed adequately with sufficient amount of water daily. Other routine vaccination such as that against infectious bursal disease was carried out while vitamins and other necessary medications were also administered.

2.3 Vaccination Schedule

Primary and booster ND vaccination guide of the National Veterinary Research institute (NVRI), Nigeria was followed as our schedule. Chicks in each of groups i - iv were vaccinated at day-old against Newcastle disease with four different brands (Sevac®, Jovac®, Biovac® and Fort Dodge® A, B, C, D) respectively of Hitchner (B1) strain using the intra-ocular route while secondary (booster) vaccination with the corresponding brands of LaSota strain was done at day 21 post-HB1 vaccination using the oral route. Chickens in group v which served as controls received no vaccine.

2.4 Experimental Infection

Chicks in groups i-v were challenged intraocularly at day 33 with 0.2 ml inoculum containing $10^{8.46}$ ELD_{50} per milliliter of NDV (Kudu 113 strain) obtained from the NVRI. The virus was originally isolated from an apparently healthy duck in Plateau State, Nigeria [8].

2.5 Blood Collection

About 2 ml of blood was aseptically collected through the jugular vein of five randomly selected chicks from each group at day-old (before vaccination), on day 9 and 16 post- Hitchner (B1) vaccination and on day 7 and 14 post-ND La Sota vaccination (i.e. day 32 and 39 of age). The collected blood samples were transferred into labeled sample bottles without anticoagulant, allowed to clot at room temperature and separated sera were stored at -20°C until tested.

2.6 Haemagglutination (HA) and Haemagglutination Inhibition (HI) Tests

The potency of the four vaccines was determined by subjecting them to haemagglutination (HA) test using 0.5% washed chicken red blood cells (RBC) while collected sera were screened for NDV antibodies by haemagglutination inhibition (HI) test using 4 haemagglutinating units (4HU) of the antigen. Both the HA and HI tests were performed according to standard protocols [5].

2.7 Statistical Analysis

Data obtained were subjected to One-way ANOVA using the GraphPad Prism version 4.0 (San Diego, USA) and Turkey's Multiple

Comparison post-test for multiple comparisons in order to assess the significance of differences between all possible pairs of groups with significance levels set at $P \leq .05$.

3. RESULTS AND DISCUSSION

A Newcastle disease HA titer of 3.0 $\log_2$ (i.e. 1:8) and above is generally accepted as indicative of specific immunogenicity [5,9,10]. Using this criterion in the present study, all the four vaccine brands showed evidence of antigenic potency as HA titre range of 5.6 $\log_2$ (48±22.6, GMT)-10.6 $\log_2$ (1536±724.1, GMT) and 6.6 $\log_2$ (96±43.3, GMT) - 9.0 log2 (512±0.0, GMT) were obtained for HB1 and LaSota vaccines, respectively (Table 1). Statistically, vaccine C was significantly higher in potency ($P = .02$) compared to vaccines A, B and D for both vaccine strains.

Maternally derived antibody (MDA) measured over a period of 39 days at day 1, 9, 16, 32 and 39 as shown in Fig. 1 persisted at protective level (≥3.0log2) from day 1 to day 16 and then waned to non-protective levels (≤3.0log2) from 21 days (Fig. 2), during which the chickens could be highly susceptible to ND. This finding is consistent with earlier reports by several researchers [11,12,13] that detected high levels of ND MDA in chickens were protective up to day 15 to 27 of age. The variations could be dependent on the ND vaccination and seroconversion status of the parent flocks.

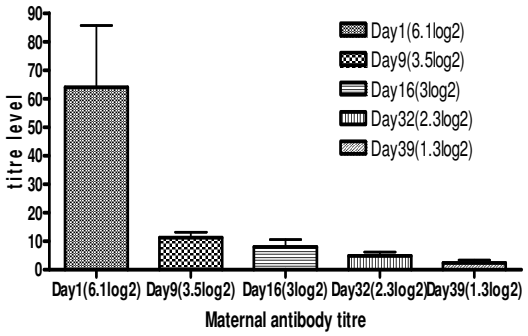

Fig. 1. Maternally derived antibody levels in the unvaccinated chicks

Also, following the administration of primary and booster (secondary) vaccinations, all the vaccines elicited protective immunity with the booster doses producing higher immune response in all the groups. However, chicks that received Jovac® and Biovac® (Vaccines B and C) were best able to overcome the effects of experimental challenge as they gave the lowest mortality rate of 33.3% with mean NDV antibody titres of 9 $\log_2$ (512±313.5) recorded seven days post-challenge. Chicks that received Sevac® and Fort Dodge® (Vaccines A and D) gave mortality rates of 55.0% and 50.0%, respectively with mean antibody titres of 7 $\log_2$ (154±57.3) post-challenge (Fig. 2). Statistically, vaccine C was significantly higher in potency ($p<0.05$) compared to vaccines A, B and D for both vaccine strains. After secondary vaccination it was also observed that the antibody titres further increased in all groups except control group v where the antibody level declined. Kafi et al. [14] found the highest HI titres after secondary vaccination with BCRDV. Also, Shuaib et al. [15] reported that secondary vaccination with La Sota produced significantly higher HI titres than chickens vaccinate with single dose. The results also showed that the LaSota strain of different brands of ND vaccines produced higher immune response than the B_1 strain. This finding is strongly supported to the findings of Almassy et al. [16] who reported that LaSota strain provided superior antibody production after vaccination compared to B1. Westbury et al. [17] also observed that LaSota is much more immunogenic than the Hitchner B1.

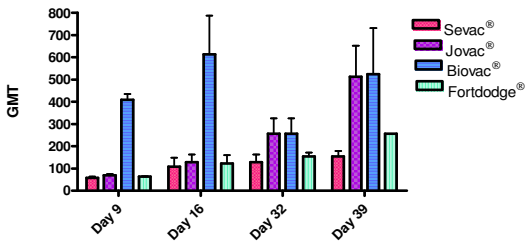

Fig. 2. Antibody titre (GMT) in chicken vaccinated ND vaccines

Table 1. Antigen titres (GMT±SD) of brands of NDV vaccines (*in vitro* HA test)

Brand of ND vaccines	Hitchner B1 titre (GMT±SD)	ND LaSota titre (GMT±SD)
Sevac® (A)	5.6 log2 (48±22.6)	6.6 log2 (96±43.3)
Jovac® (B)	8.0 log2 (256±0)	8.6 log2 (384±181)
Biovac® (C)	10.6 log2 (1536±724.1)	9.0 log2 (512±0)
Fort Dodge® (D)	7.6 log2 (192±90.5)	7.0 log2 (128±0)

The findings of this study revealed that vaccinated chicks in all the groups did not respond similarly to vaccination and individual variations in the humoral immune response were observed within the same group of birds at the same test interval following both primary and secondary vaccination. This is corroborated by earlier findings of Saifuddin et al. [18] and Banu et al. [10]. After primary vaccination with Hitchner B1 strain, an increased immune response was observed for all tested vaccine brands at day 9 and a further increase at day 16. This is in agreement with the OIE manual [5] that single vaccinations with live lentogenic virus produce a response in susceptible birds of about 4–6 $\log_2$. The actual titres obtained and their relationship to the type of protection and duration of immunity for a given flock and programme are however difficult to predict. Variations in HI titres may occur for non-specific factors. For instance, due to antigenic variation, infection with other avian paramyxoviruses AMPVs (e.g. APMV-3) may result in significantly increased titres to NDV [5]. Comparative analysis of the four brands of ND Hitchner B1 vaccine strain revealed that Biovac® vaccine C was significantly higher in immunogenicity compared to Sevac®, Jovac® and Fort Dodge® vaccines A, B and D (Fig. 1). These variations may be due to the presence of variable MDA in chicks or varying degree of susceptibility of immune mechanism to ND antigen [13]. After secondary vaccination it was also observed that ND antibody titres further increased in birds of all vaccinated groups with the immune response of birds in groups C and B being significantly higher than the other two vaccinated groups (A and D). Similarly, Shuaib et al. and Kafi et al. [14,15] reported that secondary vaccination yielded HI titres that were significantly higher than the HI titres after single vaccination. A comparative analysis of all the tested ND vaccines of different brands in this study revealed that the LaSota strain produced higher immune response than those of the HB1 strain. This suggests that the LaSota strain of ND vaccine is more immunogenic [19] and elicits superior antibody production [15] than the HB1 strain.

4. CONCLUSION

This study has shown that all the tested vaccines elicited relatively high antibody titres. However, overall consideration among the vaccinated groups revealed that HI antibody titres were highest in birds vaccinated with Hitchner B1 strain of Biovac® (vaccine C) and corresponding booster La Sota strain than in all other vaccinated groups. These findings suggest that Newcastle disease vaccine failures, vaccine failures or morbidity and mortality occurrences in vacated flocks could be due to variation in the strain of virus that is enzootic in the environment and that used for vaccine production. These variations in antigenicity of different circulating strains suggest a need to tailor vaccines more carefully to be antigenically related to prevalent field virus [16]. Therefore, based on the findings of this study, we advocate:

i) Identification of enzootic strains of ND virus for production of appropriate vaccines. This would ultimately reduce the incidence of Newcastle disease in southwest Nigeria.
ii) Vaccination of chickens against ND using the most potent brands of Hitchner B1 and La Sota vaccines as obtained in this study for Jovac and Biovac vaccines C and B after routine quality tests.
iii) Effective control of ND in Nigeria through veterinary supervision of handling, distribution and administration of vaccines to ensure preservation of their potency.
iv) Appropriate regulatory control of importation of ND and other poultry vaccines and biologicals into Nigeria in order to avoid introduction of exotic strains of live viruses into the country.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mengasha M. Biophysical and the socio-economics of chicken production. Afri. Jour. Acad. Res. 2013;8(18):1828-1836.
2. Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. Clin Exp Allergy. 2002;32(4):489-98.
3. Aldous EW, Alexander DJ. Detection and differentiation of Newcastle disease virus (avain paramyxvirus type 1). Avian Pathol. 2001;30:117-128.
4. Hauqe MH, Hossain MT, Islam MT, Zinnah MA, Khan MSR Islam MA. Isolation and detection of Newcastle disease virus from field outbreaks in broilers and layer chickens by reverse transcription-polymerase chain reaction. J. Vet Med. 2010;8(2)87-92.

5. OIE World Organisation for Animal Health Terrestrial Animal Health Code - Newcastle Disease. 2012;Chapter 2.3.14. OIE, Paris.
6. Olatoye IO. Antibiotics use and resistance patterns of *Salmonella* spp in poultry in Ibadan Nigeria. Trop. Vet. 2011;29(3):28-35.
7. Abbas AK, Lichtman AH, Pober JS. Cellular and molecular immunology. 4th ed. Philadelphia: Saunders; 2001.
8. Ugochukwu EI. Post-vaccination Newcastle disease outbreak. Nigerian Veterinary Journal. 1982;11(1):24-29.
9. Echeonwu GON, Iroegbu GU, Emeruwa A. Recovery of velogenic Newcastle disease virus from dead and healthy free-roaming birds in Nigeria. Avian Pathol. 1993;22: 383-387.
10. Allan WH, Gough RE. A standard haemagglutination-inhibition test for Newcastle disease: A comparison of macro-and micromethods. Veterinary Record. 1974;95:120-123.
11. Islam MR, Huque QML, Giasuddin MJ, Rahman MM. Assessment of maternally derived antibody of commercial flock against Newcastle disease. Proceedings of 3rd International poultry show and seminar, Bangladesh china friendship conference center, Dhaka, Bangladesh 28th February-2nd March; 2003.
12. Shil NC, Amin MM, Rahman MB. Evaluation of antibody production following single vaccination with BCRDV, Izovac B_1 Hitchner and Cevac New L in broiler chicks. M.S. Thesis submitted to the department of Microbiology and Hygiene, Faculty of Vet. Science, BAU, Mymensingh. 2006;205.
13. Banu NA, Islam MS, Chodhury MMH, Islam MA. Determination of immune response to Newcastle disease virus vaccines in layer chickens. J. Bangladesh Agric. Univ. 2009;7(2):329-334.
14. Kafi MA, Rahman MB, Amin MM, Islam MR, Rahman MM, Rahman MK. Comparative serological responses and protection conferred by vaccination with V4HR and BCRDV in chicken. Bang. J. Vet. Med. 2003;1(1):25-27.
15. Shuaib M, Ashfaque M, Sajjad-ur R, Mansoor MK, Yousaf I. Comparative immune response of broiler chicks to Newcastle disease vaccine (LaSota strain).Pak. Vet. J. 2003;23:91-93.
16. Almassy K, Barhouma N, El-sabbagh A, Ibrahim SN, Boktor N, Khashaba E, Gawad SA. Comparative immunization experiments with lentogenic Newcastle disease vaccine strain. J. Egypt Vet. Med. Asso. 1979;35:95-104.
17. Westbury HA, Parsons G, Allan WH. Comparison of immunogenicity of Newcastle disease virus strains V4, Hitchner B1 and La Sota in chickens. 2 Tests in chickens with marternal antibody to the virus. Aust. Vet. J. 1984;61:10-13.
18. Saifuddin M, Sarker AJ, Amin MM, Rahman MA. Studies on the efficiency of Newcastle disease vaccines and their vaccination schedule. Bang. Vet. J. 1986;20(3):67-74.
19. Miller PJ, King DJ, Afonso CL, Suarez DL. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. Vaccine. 2007;25(41):7238-7246.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15500