

**Original Article****Open Access*****In vitro* assessment of the potency of some Newcastle disease vaccine brands in Ibadan, Nigeria**¹Okanlawon, A. A., ^{*2}Ameen, S. A., ²Kadir, R. A., ²Ambali, H. M., ²Baba, Y. A., ³Azeez, O. M. and ⁴Owoade, A. A.¹Veterinary Diagnostic Laboratory for Poultry and Livestock Diseases, CHI Farms Ltd, Ibadan, Nigeria²Department of Veterinary Medicine, University of Ilorin, Nigeria³Department of Physiology and Biochemistry, University of Ilorin, Nigeria⁴Department of Veterinary Medicine, University of Ibadan, Nigeria*Correspondence to: drsaameen@yahoo.com; ameen.sa@unilorin.edu.ng; +2348125064091;ORCID: <https://orcid.org/0000-0002-7453-1133>**Abstract:****Background:** Newcastle disease (ND) is a very common and economically important disease of poultry. There is no drug for treatment of the disease during an outbreak in poultry flocks, and prevention by vaccination is one of the recommended control measures. However, post vaccination outbreaks have been observed on many occasions in chicken flocks and one of the causes has been attributed to possible failure of vaccine to confer immunity. This study was designed to evaluate the potency of ND vaccines available in Ibadan, Nigeria.**Methodology:** Haemagglutination (HA) technique and elution phenomenon were employed to evaluate the potency of ND vaccines randomly selected in Ibadan. A total of 45 vaccines comprising 9 brands and 5 different strains were selected for potency test. The vaccine brands included 'Vireo 116' (n=10), 'ABIC' (n=5), 'Biovac' (n=9), 'Nobilis' (n=3), 'NVRI' (n=12), 'R2B' (n=2), 'BAL-ND' (n=2), 'Forte dodge' (n=1) and 'Jovac' (n=1), while the vaccine strains in the brands included Lasota, B1, Clone, Komarov, Hitcher, and an unknown strain.**Results:** Thirty-five of the 45 (77.8%) ND vaccines tested had more than 4 HA titer (>64) and were therefore regarded as potent. All the 15 (100%) ND Lasota vaccine strain, 7 out of 10 (70%) ND Komarov strain, 4 out of 5 (80%) ND clone and 5 out of 8 (62.5%) ND B1 strains were potent. None of the ND brand 'R2B' vaccine as well as Hitcher strain from 'Nobilis' brand was potent, but all 5, 2, 1 and 1 vaccines tested from brands 'ABIC', 'BAL-ND', 'Fort dodge' and 'Jovac' respectively were potent. Similarly, 9 of 10, 6 of 9, 2 of 3 and 9 of 12 vaccine strains tested from brands 'Vireo 116', 'Biovac', 'Nobilis' and 'NVRI' were respectively potent.**Conclusion:** The occurrence of ND vaccines that are not potent in this study may be contributing to post vaccination failure. It is advisable to subject vaccines to potency test before use.**Key words:** *in vitro*, assessment, potency, Newcastle disease, vaccine brands, vaccine strains

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Abstrait:

Contexte: La maladie de Newcastle (ND) est une maladie très courante et économiquement importante des volailles. Il n'existe aucun médicament pour le traitement de la maladie lors d'une épidémie dans des troupeaux de volailles, et la prévention par vaccination est l'une des mesures de contrôle recommandées. Cependant, des flambées post-vaccination ont été observées à de nombreuses reprises dans des troupeaux de poulets et l'une des causes a été attribuée à un éventuel échec du vaccin à conférer l'immunité. Cette étude a été conçue pour évaluer la puissance des vaccins contre la MN disponibles à Ibadan, au Nigéria.

Méthodologie: La technique d'hémagglutination (HA) et le phénomène d'élution ont été utilisés pour évaluer la puissance des vaccins contre la MN sélectionnés au hasard à Ibadan. Un total de 45 vaccins comprenant 9 marques et 5 souches différentes ont été sélectionnés pour le test d'activité. Les marques de vaccins comprenaient 'Vireo 116' (n=10), 'ABIC' (n=5), 'Biovac' (n=9), 'Nobilis' (n=3), 'NVRI' (n=12), 'R2B' (n=2), 'BAL-ND' (n=2), 'Forte dodge' (n=1) et 'Jovac' (n=1), tandis que les souches vaccinales des marques comprenaient Lasota, B1, Clone, Komarov, Hitcher et une souche inconnue.

Résultats: Trente-cinq des 45 vaccins contre la MN testés (77,8%) avaient plus de 4 titres en HA (>64) et étaient donc considérés comme puissants. Toutes les 15 (100%) souches de vaccin ND Lasota, 7 souches sur 10 (70%) ND Komarov, 4 sur 5 (80%) clones ND et 5 sur 8 (62,5%) souches ND B1 étaient puissantes. Aucun des vaccins 'R2B' de marque ND ni la souche Hitcher de la marque 'Nobilis' n'étaient puissants, mais tous les vaccins 5, 2, 1 et 1 testés des marques 'ABIC', 'BAL-ND', 'Fort dodge' et 'Jovac' respectivement était puissant. De même, 9 des 10, 6 des 9, 2 des 3 et 9 des 12 souches vaccinales testées des marques 'Vireo 116', 'Biovac', 'Nobilis' et 'NVRI' étaient respectivement puissantes.

Conclusion: La présence de vaccins contre la MN qui ne sont pas puissants dans cette étude peut contribuer à l'échec post-vaccinal. Il est conseillé de soumettre les vaccins à un test de puissance avant utilisation.

Mots-clés: *in vitro*, évaluation, puissance, maladie de Newcastle, marques de vaccin, souches vaccinales

Introduction:

Newcastle disease (ND) is a highly contagious viral disease of domestic and wild birds. The disease is caused by the Newcastle disease virus (NDV) of the avian paramyxovirus 1 (APMV-1), a single-stranded, negative-sense ribonucleic acid (-ssRNA) virus belonging to the family Paramyxoviridae, sub-family Paramyxovirinae and genus Rubulavirus (1,2,3). The virus exhibits hemagglutinin/neuraminidase (HN) and fusion (F) glycoprotein spikes at its surface (2,4). These proteins are important in determining the virulence and infectivity of the virus (2). Also, the hemagglutination property is an important aid in the laboratory for virus detection by hemagglutination test (5).

Newcastle disease was first recognized in Indonesia and England in 1926 (6), though earlier existence of the disease dating back to the mid 19th century had been postulated (7). Since then it has become worldwide in distribution (2). It was first reported in Nigeria in 1951 with laboratory confirmation in 1953 (8). Since its recognition, the disease has been regarded as endemic in Nigeria. The disease have been reported as the most important viral disease of poultry in the world (9), and a

major constraint to the poultry industry in Nigeria and Africa in general (9,10), in which it can cause devastating losses in both commercial and village chickens (11).

The disease is characterized by digestive, respiratory and/or nervous signs. The disease has a number of strains that differ in the severity of their clinical signs, ranging from in apparent infection to a rapidly fatal condition (2,6,12). There are many ND vaccines suitable for use in commercial poultry, many of which are available in Nigeria. However, these vaccines can be broadly grouped into two; the first are live/attenuated ND vaccines which are vaccines made with strains of virus of low to moderate virulence that are able to infect cell, but have been substantially modified to lose pathogenicity while maintaining immunogenicity. They are given by intraocular, intranasal, oral and intramuscular routes, and they mirror natural infection and induce cell mediated, humoral and local immunity. The second are killed/inactivated ND vaccines, which are inactivated organisms usually combined with an oil emulsion or aluminium hydroxide adjuvant. They are usually administered to individual birds by intramuscular or subcutaneous injection, and mainly elicit hum-

oural immunity (5,13).

It has been reported that procedure for the production of ND vaccine may differ from one producer to the other in terms of cultures and degree of passage. While some use cell culture for propagation, others may use chicken embryo. Some producers may have short passage while others may perform long passage for vaccine seed attenuation, and this may have effect on potency of the resulting vaccine. From literature on ND vaccines, it has been observed that titres of infective dose vary from one brand to the other, which again could have effect on the potency of the vaccine. Fluctuations in storage temperature of vaccines may also have deleterious effect on vaccine potency. In view of unstable electricity power supply in Nigeria, it is most appropriate to investigate how potent are vaccines sold at various outlets in Ibadan. ND outbreaks in vaccinated flocks are common occurrence in poultry flocks and the causes of such vaccine failure are many, one of which is lack or loss of potency of vaccine. This study was designed to determine the potency of selected ND vaccines available to poultry farmers at the point of purchase in Ibadan.

Materials and method:

Study setting

The study was carried out at the Biotechnology Laboratory of the Department of Veterinary Medicine, University of Ibadan.

Sources of vaccines and materials for testing

Newcastle disease vaccines (a total of 45 vaccines with 9 brands and 5 known strains) were randomly obtained from National Veterinary Research Institute branch office at Mokola, Ibadan, Nigeria, and from private veterinary shops and clinics where poultry vaccines are sold. The materials used included samples of nine brands of ND vaccines, phosphate buffered solution (PBS) tablets, Chicken red blood cells (Crbc), 50 μ l single and 8-channel pipette, distilled water, and U-bottom microtitre plates.

Determination of haemagglutination (HA) titre

First, 0.5% of washed Chicken RBC (Crbc) was prepared in PBS. Then, each vaccine was diluted with PBS to give 250 doses

per ml of PBS. The wells (1-12) in rows of the microtitre plates were filled with 50 μ l of PBS and 50 μ l of the diluted vaccine was added to the PBS in the first well (well 1) in the row and diluted serially up to well 11. Well 12 served as negative control. 50 μ l of 0.5% Crbc was then added to each well 1 to 12. The reaction was allowed to continue until Crbc in the control well 12 completely settles (buttoning). Haemagglutination titre was taken as the reciprocal of the dilution number of last well to show 100% haemagglutination (complete carpeting of well by red blood cells). Each vaccine was tested in triplicate.

Haemagglutination inhibition test

Vaccines that showed haemagglutination were reacted with standard serum containing ND antibodies obtained from chicken challenged with ND vaccine. Inhibition of the haemagglutination was shown by settling of Crbc

Interpretation of results

Observation of complete haemagglutination indicated presence of potent haemagglutinating substance in the vaccine, and the haemagglutination titer indicated potency of the vaccine. Inhibition of the haemagglutination by standard ND serum confirms ND vaccine virus.

Elution test:

The last well to show haemagglutination for each vaccine brand during the haemagglutination test was observed for the period of time it takes for the Crbc to settle. Any vaccine in which haemagglutination disappears, resulting in Crbc settling, has elution. Any vaccine without elution after 30 minutes was regarded as potent and the shorter the complete elution time, the less potent the vaccine

Results:

A total of 45 ND vaccines were tested from the 9 vaccine brands (Nos 1-9), 35 of which belong to 5 different vaccine strains (Lasota, B1, Clone, Komarov and Hitcher) while 6 were unknown vaccine strain (Table 1). Thirty-five of the 45 (77.8%) ND vaccines tested had more than 4 HA titer (>64) and were therefore regarded as potent. All 15 ND

Table: Haemagglutination titre of Newcastle disease vaccines tested for potency

Brand designation	Vaccine brand	Strains	Haemagglutination titre	Score (%)	Elution before 30mins	Verdict
1	Vireo 116: (n=10)	Lasota	512, 128 and 1025	100	None Yes (1/2)	Very potent
		B ₁	64, 512	50		Fairly potent
		Lasota	512, 256, 512, 256 and 512	100		Very potent
2	ABIC: (n=5)	B ₁	2048, 128 and 128	100	Yes (1/3)	Potent
		Lasota	128 and 128	100		Potent
3	Biovac: (n=9)	B ₁	0, 2 and 256	33.3		Barely potent
		Clone	512, 256, 2048 and 64	75		Potent
		Lasota	128 and 128	100		Potent
4	Nobilis (n=3)	Hitchner	2	0		Not potent
		Lasota	256	100		Potent
		Clone	256	100		Potent
5	N.V.R.I (Vom): (n=12)	Komarov	256, 256, 640, 128, 256, 16, 64, 16, 512, and 512	70		Fairly potent
		Lasota	256 and 1024	100		Potent
6	R ₂ B: (n=2)	Not known	0 and 8	0	Yes	Not potent
7	BAL-ND: (n=2)	Not known	128 and 512	100		Potent
8	Fort dodge: (n=1)	Not known	512	100		Potent
9	Jovac: (n=1)	Not known	1024	100		Potent

n = number of vaccines tested

Lasota (100%), 7 out of 10 ND Komarov (70%), 4 out of 5 ND clone (80%) and 5 out of 8 ND B₁ strains (62.5%) were potent. None of the ND brand 'R₂B' vaccine as well as Hitchner from 'Nobilis' brand was potent, but all 5, 2, 1 and 1 vaccines tested from brands 'ABIC', 'BAL-ND', 'Fort dodge' and 'Jovac' respectively were potent. Similarly, 9 of 10, 6 of 9, 2 of 3 and 9 of 12 vaccines tested from brands 'Vireo 116', 'Biovac', 'Nobilis' and 'NVRI' were respectively potent (Table).

Discussion:

In Nigeria and elsewhere, live and inactivated vaccines are routinely used in the prevention of Newcastle disease (14). Newcastle disease vaccines are produced by different manufacturers which are sold to veterinarians under different brand names. The number of vaccines tested in this study is a reflection of the availability of each brand of vaccine and may also be a reflection of their popularity, the locally produced vaccine 'NVRI' (26.7%) being the most readily available, followed by 'Vireo 116' (22.2%) and 'Biovac' (20%) brands. The use of HA technique for evaluation of ND vaccine potency is well established (15,16). Ten of the 45 vaccines

(22.2%) tested were not potent, having 4 HA titer or less. The use of such vaccine flocks will result in vaccine failure with consequent risk of ND outbreak.

All ND vaccine Lasota and clone strains tested (100%), 8 of 10 (80%) ND vaccine Komarov strain and 5 of 8 (62.5%) ND vaccine B₁ strains were potent but none of two of the 'R₂B' brand of Komarov strain equivalent was potent. Therefore, in terms of potency reliability, ND vaccine Lasota and clone strains are most reliable. In view of the fact that some of the other strains are not potent, it will be advisable to subject these to potency test before use.

With regard to performance of brands, all strains of ND vaccines of brands 'Vireo 116' and 'ABIC' were potent and therefore are the best brands of choice followed by brand of 'NVRI', which is a local vaccine. The occurrence of ND vaccines that are not potent in this study is worrisome as this may be contributing to post vaccination failure as reported by earlier workers (6,14,17). Ramakrishnan et al., (1) and Okwor et al., (18) reported a gradual but sharp decline in the potency of ND Lasota vaccine when stored under conditions of irregular power supply. The vaccines that were not potent in this study

could have been partly caused by storage under irregular power supply.

Haemagglutination is a measure of the ability of a virus to attach to host cells. This is used to evaluate potency. Elution is an additional observatory phenomenon used to measure the weakness of the virus. Newcastle disease virus that elute earlier than 30mins is usually regarded as weak and therefore not capable of initiating immunological reaction. In this study, three of the vaccines tested were observed to elute before thirty minutes of haemagglutination.

Conclusion:

It is concluded from this study that some commercially available ND vaccines in Ibadan may not be potent as a result of very low HA titer and elution of the vaccine virus. It is therefore recommended that potency test should be carried out on representative ND vaccines before use to prevent vaccination failure.

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