Original Article

**In vitro** assessment of the potency of some Newcastle disease vaccine brands in Ibadan, Nigeria

Okanlawon, A. A., Ameen, S. A., Kadir, R. A., Ambali, H. M., Baba, Y. A., Azeez, O. M. and Owoade, A. A.

**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), ‘R/B’ (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 Hitchner 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
Abstrait:

Contexte: La maladie de Newcastle (ND) est une maladie très courante et économiquement importante des volailles. Il n’existe aucun médicamente pour le traitement de la maladie lors d’une épidémie dans des troupées 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 troupées 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), ‘Fort 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-
oral 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), 50µl 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 50µl of PBS and 50µl 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. 50µl 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
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

---

Table: Haemagglutination titre of Newcastle disease vaccines tested for potency

| Brand designation | Vaccine brand | Strains | Haemagglutination titre | Score (%) | Verdict | Elution before 30mins |
|-------------------|--------------|---------|-------------------------|-----------|---------|---------------------|
| 1                 | Vireo 116:   | Lasota  | 512, 128 and 1025       | 100       | Potent  | None                |
|                   | (n=10)       | B1      | 64, 512                 | 50        | Very potent | Yes (1/2)         |
|                   |              | Lasota  | 512, 256, 512 and 512   | 100       | Very potent | Fairly potent      |
| 2                 | ABIC:        | B1      | 2048, 128 and 128       | 100       | Potent  | Potent              |
|                   | (n=5)        | Lasota  | 128 and 128             | 100       | Potent  | Potent              |
| 3                 | Biovac:      | B1      | 0, 2 and 256            | 33.3      | Barely potent | Not potent |
|                   | (n=9)        | Lasota  | 128 and 128             | 75        | Potent  | Potent              |
|                   |              | Clone   | 512, 256, 2048 and 64   | 100       | Potent  | Potent              |
| 4                 | Nobilis:     | Hitchner| 2                       | 0         | Not potent | Not potent |
|                   | (n=3)        | Lasota  | 256                     | 100       | Potent  | Potent              |
|                   |              | Clone   | 256                     | 100       | Potent  | Potent              |
| 5                 | N.V.R.I (Vom): | Komarov| 256, 256, 640, 128, 256, 16, 64, 16, 512, and 512 | 70        | Potent  | Potent              |
|                   | (n=12)       | Lasota  | 256 and 1024            | 100       | Potent  | Potent              |
| 6                 | R.B:         | Not known| 0 and 8                 | 0         | Not potent | Not potent |
|                   | (n=2)        |         |                         |           |         |                     |
| 7                 | BAL-ND:      | Not known| 128 and 512             | 100       | Potent  | Potent              |
|                   | (n=2)        |         |                         |           |         |                     |
| 8                 | Fort dodge:  | Not known| 512                     | 100       | Potent  | Potent              |
|                   | (n=1)        |         |                         |           |         |                     |
| 9                 | Jovac:       | Not known| 1024                    | 100       | Potent  | Potent              |
|                   | (n=1)        |         |                         |           |         |                     |

n = number of vaccines tested

---

"Invitro potency of Newcastle disease vaccine" Afr. J. Clin. Exper. Microbiol. 2020; 21 (4): 328-332
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.

**References:**

1. Grimes, S. E. A basic laboratory manual for the small-scale production and testing of 1-2 Newcastle disease vaccine. Food and Agriculture Organization of the United Nations (FAO)-Animal Production and Health Commission for Asia and the Pacific (APHCA). 2002.

2. Alexander, D. J. Newcastle disease, other avian Paramyxoviruses, and Pneumovirus infection. In: Saf, Y. M., Barnes, H. J., Glisson, H. J. R., Fadly, A. M., McDougald, L. R., and Swayne, D. E. (eds). Diseases of Poultry. 11th ed. Iowa State Press, Iowa, USA. 2003: 63-99.

3. Okwor, E. C., Okoye, J. O. A., and Eze, D. C. Studies on the time of detection of Newcastle disease virus in the brain in relation to other organs. J Anim Vet Adv. 2010; 9 (5): 946-948.

4. Couceiro, E. S. S., Couceiro, J. N. S. S., and Cabral, M. V. Hemagglutination and fusogenic activities of Newcastle disease virus: studies on receptor binding specificity and pH-induced conformational changes. Men Inst Oswaldo Cruz, Rio de aneiror. 1995; 90 (4):515-520.

5. Chauhan, H. V. S., and Roy, S. Newcastle disease (ND) or Ranikhet disease (RD). In: Poultry Disease Diagnosis and Treatment. 3rd ed. New Delhi: New Age Int., 2007; 556-562.

6. Abbas, T., Muneer, M. A., Ahmed, M. D., Khan, M. A., Younus, M. and Khan, I. Comparative efficacy of five different brands of commercial Newcastle disease Lasota virus vaccines in broilers. Pakistan Vet J. 2006; 26 (2): 55-58.

7. Spadbrow, P. B. Thermostable vaccines in the control of Newcastle disease in village chickens: a history. N: Village chickens, poverty alleviation and the sustainable control of Newcastle disease. Proceeding of International Conference held in Dar es Salaam, Tanzania, 5-7 October 2005. Alders R. G., Spadbrow P. B., and Young, M. P. (eds.) Australian Centre for International Agricultural Research (ACIAR) Proceeding. 131 (235): 27-34.

8. Okoh, A. E. J. Newcastle disease in falcons. J Wildlife Dis. 1979; 15 (3): 479-480. https://doi.org/10.7589/0090-3558-15.3.479

9. Nwanta, J. A., Umoh, J. U., Ajogi, I., and Adeiza, A. A. Field trial of Malaysian thermostable vaccine in village chickens in Kaduna State, Nigeria. Livestock Res. Rural Dev. 18. 5. 2006. http://www/1rrd1855/Swanim1860.htm

10. FAO. Assistance for the control of Newcastle disease (Phase II of TCP/ZIM/4553). Consultant report, Food and Agricultural Organization.1998. http://www/foa.org/docrep/field/38297.htm

11. FAO- Animal Production and Health Commission for Asia and the Pacific (APHCA), 2002

12. Nsien, M. A. S., and Adene, D. F. Thermostability of reconstituted Newcastle disease virus strains at 360C temperature. Afr J Biomed Res. 2002; 5: 87-89.

13. Anon. Health management disease control and vaccination. In: ROSS 308 Parent Stock Management Manual. 2006. www.aviagen.com

14. Ezema, W. W., Okoye, J. O., and Nwanta, J. A. Lasota vaccination may not protect against the lesions of velogenic Newcastle Disease in chickens. Trop Anim Hith Prod. 2008. (Online) www.thepoultry.net/enarticles/Lasota%20vaccinat ion%20.

15. Amin, M. A., Amin, M. M., Khan, M. S. R., Choudry, K. A., Siddiky, M. A., and Sarker, A. J. Characterization of Newcastle disease virus isolates from caged birds in Bangladesh. Bangladesh J Vet Med. 2004; 2 (2): 113-116.

16. Panda, A. Role of hemagglutinin-neuraminidase protein in Newcastle disease virus pathogenesis. PhD. Thesis. Faculty of the Graduate School. University of Maryland. 2003 http://en.scientificcommonsorg/aruna panda.

17. Abu, F. D., Oyeyide, O., and Ikede, B. O. Characterization of Nigerian strains of Newcastle disease virus. Avian Dis. 1985; 29 (3): 829-931. http://www.ijor.org/abstract152054.

18. Ramakrishnan, M. A., Velayudhan, B. T., Anantharaman, S., Noll, S. L., Halvorson, A. K. V., and Goyal, S. M. Effects of Temperature and Stabilizer on the viability of a Live Attenuated Avian Metapneumovirus Vaccine. Avian Dis. 2007; 51 (4): 979 - 981. www.bioone.org/doi/pdf/10.1637/7962-030107_1