sciendo

DOI: 10.2478/fv-2023-0039

FOLIA VETERINARIA, 67, 4: 61—66, 2023

COMPARISON OF NDVI-2 VACCINE EID_{50} TITRE VALUES AFTER THREE WEEKS OF FREIGHT UNDER COLD MONITORED STORAGE CONDITION

Anthony N. Egbuji, James Gyallak, Fatima Mukaila, Henry E. Nnabuife, Anthony A. Chukwuedo
National Veterinary Research Institute (NVRI), Vom Plateau State
Nigeria

wisemenvet@yahoo.com

ABSTRACT

The EID_{50} titres per dose values of three batches of NDVI-2 were determined as log_{10} EID_{50} 7.7, 7.6 and 6.8 after production, against reference EID_{50} 5.5 per dose. These values were log_{10} EID_{50} 2.2, 2.1 and 1.3 above the reference EID_{50} for NDVI-2. These vaccines were re-evaluated at PANVAC laboratory Debre-Zeit, prior to release. The initial EID_{50} values were compared with EID_{50} values obtained by the certifying laboratory, after three weeks of cold monitored freight. The packaged vaccine cold storage temperature of +1.9 °C prior to the shipment, was recorded with the aid of a temperature logger. The temperature logger was set to record and retain packaged vaccine cold storage temperature at hourly intervals during the freight. At the end of three-week freight, the temperature logger readings were analysed, and temperature range of -2.48 °C to 7.7 °C was observed throughout the freight. However, sudden rise in packaged vaccine storage temperature from 7.7 °C to 22.28 °C was noticed on the last day of freight. The initial EID_{50} titre per dose values, when compared with EID_{50} log_{10} 6.5, 6.8, and 6.6 per dose obtained by the certifying laboratory after the cold monitored freight and re-evaluation, showed EID_{50} titre loss of log_{10} EID_{50} 1.2, 0.8 and 0.6, respectively. The observed EID_{50} loss could not invalidate these vaccine batches, considering that these had an initial EID_{50} titre per dose values that were well above the reference EID_{50} titre for NDVI-2. In Nigeria, and to our knowledge, this is the first recorded monitored cold storage temperature transit for NDVI-2.

Key words: monitored cold storage conditions; NDVI-2 compared EID_{50} potency value; three weeks of freight

INTRODUCTION

Newcastle disease (ND) aetiologic agent is the Newcastle disease virus (NDV), also known as type 1 avian paramyxovirus, a member of the genus *Avulavirus* of the *Paramyxoviridae* family [5, 8, 13]. This is a single stranded, enveloped, negative sense RNA virus [8, 13]. Newcastle disease affects all avian species, however, outbreak of ND in unvaccinated poultry flock results in severe losses [4, 6]. Mortality rate of 75 % to 100 % in unvaccinated poultry flock has been reported [16].

Due to poor cold keeping conditions in developing countries such as Nigeria, as a result of inadequate electricity supply especially in the rural communities across

the country, the NDVI-2 vaccine was developed to bridge this gap [9, 17]. The avirulent thermostable Newcastle disease vaccine virus strain (NDVI-2) was tested in several laboratories across the world and has proven to be efficient against the local virulent strains of the ND virus [2,14]. Thus, it has been recommended for use in developing countries for the protection of rural backyard poultry flock against severe consequences of Newcastle disease infection [9]. Vaccination against ND has been shown to be an efficient control strategy against ND outbreak in susceptible poultry flocks [3, 7, 14, 16]. Strategic Newcastle disease control through vaccination require the use of wholesome, safe, efficacious and potent ND vaccine [10]. Therefore, careful handling of vaccine according to the required standard method in regards to cold storage condition of vaccine after production till the time of use, is critical in effective ND vaccination control strategy, especially in Newcastle disease endemic regions. The strategic Newcastle disease control programme in endemic regions will involve series of vaccinations and re-vaccinations in accordance with stipulated NDVI-2 vaccination schedule [10]. Thus, vaccine cold storage and cold keeping condition during transit, till the time of use is critical for an efficient Newcastle disease eradication programme. Poor vaccine cold storage and handling can significantly contribute to vaccine EID_{50} titre loss. Therefore, this study was carried out to examine the potential EID_{50} titre loss for NDVI-2 vaccine after an extended transportation under monitored cold keeping conditions with minimal alterations in packaged vaccine cold storage temperature.

Our study revealed that within a monitored cold storage transportation with evidence of alterations in vaccine cold storage condition as recorded by the temperature logger, EID_{50} titre drop or loss is possible for the NDVI-2 thermostable vaccine. Thus, cold storage condition at all time is critical and essential in vaccine EID_{50} titre longevity irrespective of vaccine thermostable and thermolabile nature.

MATERIALS AND METHODS

Vaccine source

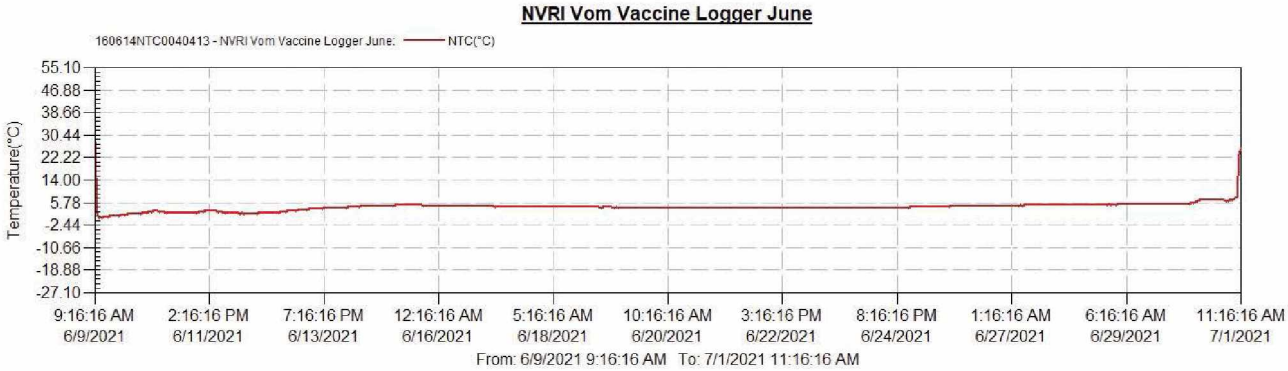
Samples of three batches of NDVI-2 vaccine produced at the National Veterinary Research Institute, Vom, Nigeria, were titrated to determine their EID_{50} titre per dose values after production. The vaccine is lyophilized live attenuated, packaged in 50 doses per vial and administered through the intra ocular route.

Determination of EID_{50} values

The EID_{50} titre per dose values of three batches of Newcastle disease (NDVI-2) vaccine of 50 dose produced by the National Veterinary Research Institute (NVRI), Vom, were determined after titration and inoculation into 10-day old embryonated chicken eggs. The inoculated eggs were incubated in a humidified incubator at 37 °C following the World Organization of Animal Health (WOAH) Newcastle disease live vaccine titration protocol. The NDVI-2 vaccine EID_{50} titre per dose values (virus concentration per dose) were determined according to Karber method [12]. The original vaccine EID_{50} titre per dose values obtained after production were consequently compared to EID_{50} titre per dose values obtained after re-evaluation or re-validation by the Pan African Vaccine Centre (PANVAC) laboratory, Debre-Zeit, Ethiopia, which is the certifying laboratory, prior to release for field veterinary deployment.

RESULTS

The temperature monitor logger was set to monitor, record and store the packaged vaccine cold storage temperature at an hourly interval during the vaccine freight to the certifying laboratory. The temperature logger readings showed steady temperature maintenance throughout the vaccine three-week transit, with minor temperature variations. The temperature logger recorded temperature of 27.2 °C (Fig. 1) (the laboratory room temperature) prior to insertion into the cold storage packed vaccine shipment box. The vaccine cold storage condition from the temperature logger reading range between -2.48 °C to 7.7 °C throughout the duration of the vaccine three-week freight (Fig. 1); from June 9, 2021 to July 1, 2021. The cold storage temperature of the vaccine during the freight was not adversely altered. At the time of the initial temperature reading on the day of freight (June 9), the temperature logger recorded +1.9 °C and the final reading on July 1 was +7.7 °C. However, a sudden rise in temperature on July 1 (last day of the vaccine transit) was equally observed with the packaged vaccine temperature rising from 7.7 °C to 22.28 °C (Fig. 1). This sudden rise in temperature was



Temperature in °C monitored during the freight is indicated vertically while, freight time duration is indicated horizontally

Fig. 1. Three-week monitored temperature logger chart from June 9 to July 1

attributed to possible custom clearance handling at the port of entry, and probably the vaccine transportation from the airport at Addis Ababa to Debre Zeit. Though the full event at this stage that triggered the significant rise in temperature from 7.7 °C to 22.28 °C was not understood or studied. Thus, the use of temperature logger, should where possible be incorporated in vaccine storage and transport as it will reveal true vaccine cold temperature keeping condition prior to NDVI-2 vaccine use.

The NDVI-2 vaccine batches showed EID_{50} titre per dose value of $\log_{10}$ EID_{50} 7.7; 7.6; and 6.8 per dose after production (Table 1); which is 2.2, 2.1 and 1.3 logarithm above the recommended standard reference EID_{50} 5.5 titre per dose value for NDVI-2 vaccine. However, logarithm titre loss of 1.2, 0.8 and 0.6 titres were equally observed after the extended monitored transit (Table 2). The temperature logger showed evidence of alteration or variations in the vaccine cold keeping temperature (Fig. 1). Furthermore, despite the obvious titre loss of $\log_{10}$ EID_{50} 1.2, 0.8 and 0.6 respectively (Table 2) after comparison of EID_{50} per dose titre values obtained by both laboratories, the vaccine batches EID_{50} titre per dose value after transit, were well above the minimum reference $\log_{10}$ EID_{50} 5.5 titre value for NDVI-2 intended for field veterinary use. Thus, the EID_{50} titre per dose value loss was not enough to invalidate these NDVI-2 vaccines batches, after the extended transit and re-testing for certification. This finding further demonstrates that a naturally thermostable vaccine is prone to EID_{50} titre value drop, with evident minimal alterations in temperature.

Table 1. NDVI-2 vaccine EID_{50} titre per dose value after vaccine production

EID_{50} titre per dose value after vaccine production	EID_{50} titre per dose value above recommended standard reference value after vaccine production	Reference EID_{50} titre per dose value for NDVI-2
$\log_{10}$ 7.7 per dose	$\log_{10}$ EID_{50} 2.2 per dose	$\log_{10}$ EID_{50} 5.5 per dose
$\log_{10}$ 7.6 per dose	$\log_{10}$ EID_{50} 2.1 per dose	$\log_{10}$ EID_{50} 5.5 per dose
$\log_{10}$ 6.8 per dose	$\log_{10}$ EID_{50} 1.3 per dose	$\log_{10}$ EID_{50} 5.5 per dose

Table 2. Vaccine *EID_{50}* titre per dose value after three-week freight and re-evaluation by the certifying laboratory

EID_{50} titre per dose value after vaccine re-evaluation	EID_{50} titre per dose value loss after cold monitored freight and re-evaluation	Reference EID_{50} titre per dose value for NDVI-2
$\log_{10}$ 6.5 per dose	$\log_{10}$ 1.2 per dose	$\log_{10}$ EID_{50} 5.5 per dose
$\log_{10}$ 6.8 per dose	$\log_{10}$ 0.8 per dose	$\log_{10}$ EID_{50} 5.5 per dose
$\log_{10}$ 6.2 per dose	$\log_{10}$ 0.6 per dose	$\log_{10}$ EID_{50} 5.5 per dose

DISCUSSION

The vaccination of immunodeficient poultry flock with Newcastle disease vaccines especially NDVI-2 has been established and demonstrated to be a major control strategy against ND pillage in poultry population in endemic region [3, 7, 14, 15, 16]. Thus, the use of various forms of ND vaccines at various stage of poultry flock growth has been inculcated towards achieving this strategic Newcastle disease control objective.

The thermostable nature of the NDVI-2 vaccine has an additional edge over other Newcastle disease thermolabile vaccines such as the intra-ocular, Komarov and Lasota. Thus, the use of ND thermostable NDVI-2 vaccine has

been suggested by [9, 11] in situations where ND vaccine cold chain maintenance is difficult. The use of the NDVI-2 thermostable vaccine under poor cold storage conditions does not entail or encourage complete or absolute negligence of standard vaccine cold storage condition; despite the NDVI-2 vaccine thermostable nature. This is also in agreement with the findings of [10] where it was reported that the thermostable nature of the NDVI-2 vaccine does not exempt it from EID_{50} titre value drop. It is therefore important to note that a thermostable vaccine must be handled with same care accorded to other thermo-sensitive biological products; thus, thermostable vaccine such as NDVI-2 cannot be expose to sunlight and frequent alterations in temperature and still be expected to remain potent [1]. It has also been observed and reported that variation in temperature can alter NDVI-2 vaccine viability especially if the vaccine cold storage temperature is altered [1]. Thermostable and thermolabile vaccines require good vaccine cold storage condition from the time of production to the period of vaccine veterinary use. In many cases this cold keeping conditions are not strictly adhered to; especially in regions of the world where electricity supply is grossly inadequate; for appropriate vaccine storage especially in rural settings.

Furthermore, efficaciousness of NDVI-2 vaccine in field situation depends on its wholesomeness, safety, potency, with standard, and acceptable EID_{50} titre per dose value, thus, vaccine cold keeping condition and handling from the time of production to the time of veterinary field application; especially where the vaccine use is geared toward Newcastle disease strategic control, and eradication initiative cannot be overemphasized.

The findings of our study revealed that at a temperature range of +1.9 to 22.28 °C under monitored cold storage condition and with limited observable temperature alterations, the EID_{50} titre drop is most likely; based on the readings of the temperature data logger (Fig. 1) and the compared EID_{50} titre values obtained by both laboratories. Table 1 shows the NDVI-2 vaccine EID_{50} titre value after production while (Table 2) shows the NDVI-2 vaccine EID_{50} titre value after extended transit and re-evaluation by the certifying laboratory. Table 2 shows observable EID_{50} titre value loss of $\log_{10}$ 1.2, 0.8 and 0.6 EID_{50} per dose respectively, after three weeks of freight. The record of the temperature logger analysis, showed that the vaccine cold storage condition was fairly maintained throughout the duration of the freight with minimal temperature variations, but a significant rise in temperature was equally observed on the day the vaccine was moved from the port of entry to the certifying laboratory (Fig. 1).

The re-validated vaccine final EID_{50} titre per dose values were well within the acceptable EID_{50} potency titre value for NDVI-2 (Table 2), despite the observable EID_{50} titre loss of $\log_{10}$ 1.2, 0.8 and 0.6 respectively. The vaccine potency EID_{50} per dose titre values were well above the recommended $\log_{10}$ 5.5 EID_{50} titre per dose reference for NDVI-2. The event of July 7, 2021, showed a sudden and significant rise in temperature based on the temperature logger data, although, the full event that led to this rise in temperature was not studied; however, it was attributed to possible clearance delay by customs service at the point of entry and probably to delay during the road trip from Addis Ababa to Debre Zeit.

The extra EID_{50} titres per dose value of $\log_{10}$ 2.2, 2.1 and 1.3 per dose (Table 1), incooperated into these NDVI-2 vaccines batches while being compounded as observed in this study, were able to carter for the vaccine EID_{50} titre loss observed (Table 2). Our finding and that of [10], is a further confirmation of the robustness, and integrity NDVI-2 vaccine, produced by the National Veterinary Research Institute Vom, Nigeria. It is thus suggested that for all vaccines, be it thermolabile or thermostable, that extra EID_{50} value of about 1 to 2 EID_{50} logarithm values be in-cooperated to carter for possible EID_{50} titre loss that might occur due to poor cold vaccine storage conditions, especially in regions of the world with limited, or poor electricity supply.

CONCLUSIONS AND RECOMMENDATIONS

Our study revealed that irrespective of the Newcastle disease vaccine thermostable nature, its cold storage condition from the time of production till time of use, should be accorded recommended vaccine cold storage condition. Therefore, having demonstrated EID_{50} titre loss in thermostable NDVI-2 vaccine under limited variations in vaccine cold storage conditions; the need for in-cooperation of extra EID_{50} titre per dose value during vaccine production cannot be overemphasized, since vaccine poor handling, and possible temperature alterations during transit in the field situation, cannot be ignored, or treated with levity

by the Newcastle disease vaccine end users. The vaccine producers must insist on strict adherence to vaccine cold keeping conditions at the point of purchase.

Based on our findings, being the first reported NDVI-2 vaccine transportation under cold storage monitored condition in Nigeria; we recommend the use of temperature logger for vaccine cold storage monitoring prior to NDVI-2 vaccine veterinary field deployment and use.

ACKNOWLEDGEMENTS

We appreciate the contribution of Mr. Sati Lokason who helped with the vaccine sterility check and Miss. Helen Gyan who screened the vaccine for molecular identity assay. We also thank the staff of AU PANVAC for screening the vaccine and providing us with the temperature logger readings.

COMPETING INTEREST

Authors declare no competing interest.

REFERENCES

1. **Alders, R. G., Spradbrow, P. B., 2001a:** *Controlling Newcastle Disease in Village Chickens: A Field Manual.* Canberra, Australian Centre for International Agricultural Research. Monograph 82, 112 pp.
2. **Alders, R. G., Spradbrow, P. B., 2001b:** SADC planning workshop on Newcastle disease Control in village chickens. In *Proceedings of an International Workshop,* Maputo, Mozambique, 6–9 March, 2000. ACIAR Proceedings No. 103, 158 pp.
3. **Aini, I., Ibrahim, A. L., Spradbrow, P. B., 1990:** Field trials of a food-based vaccine to protect village chickens against Newcastle disease. *Res. Vet. Sci.,* 49, 216–219.
4. **Alexander, D. J., Bell, J. G., Alders, R. G., 2004:** *A technology Review: Newcastle Disease, with Special Emphasis on its effect on village Chickens,* FAO Animal production and health book No. 161, Food and Agriculture Organization of United Nations, Rome, 23–63.
5. **Alexander, D. J., Senne, D., 2008:** Newcastle disease and other avian paramyxoviruses. In Dufour-Zavala, L. (Ed.): A Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens. 5th edn., Athens, American Association of Avian Pathologists, 135–141. DOI: 10.20506/rst.19.2.1231.
6. **Alexander, D. J., 2000:** Newcastle disease and other avian paramyxoviruses. *Rev. Sci. Tech. Oie.*, 19, 443–455.
7. **Allan, W. H., Lancaster, J. E., Toth, B., 1978**: *Newcastle Disease Vaccines-their Production and Use.* FAO Animal Production Series No. 10. FAO, Rome, Food and Agricultural Organization, 54–94.
8. **Barbezange, C., Jestin, V., 2005:** Molecular study of the quasi-species evolution of a typical pigeon paramyxovirus type 1 after serial passages in pigeons by contact. *Avian Pathology*, 34, 111–122.
9. **Bensink, Z., Spradbrow, P., 1999:** Newcastle disease virus strain I-2, a prospective thermostable vaccine for use in developing countries. *Vet. Microbiol.*, 68, 131–139.
10. **Egbuji, A. N., Chukwuedo, A. A., Shedua, L. N., Gyallak, J. K., Swomen, L. N., Muhammad, M., et al., 2020:** Effect of extended transit and temperature variations on EID_{50} values of thermostable NDVI-2 vaccines. *Europ. J. Biol. Biotechnol.,* 1, 5. DOI: 10.24018/ejbio.
11. **Ideris, A., Ibrahim, A. L., Spradbrow, P. B., Hung, Seng., C., 1987:** Development of food pellet Newcastle disease vaccine. In **Copland, J. W.** (Ed.): *Newcastle Disease in Poultry: A New Food Pellet Vaccine. Implication for Diagnosis and Control.* Book of proceedings, Canberra, ACIAR, 20–23.
12. **Karber, G., 1931:** 50 % end point calculation. *Arch. Exper. Pathol. Pharmakol.,* 162, 480–483.
13. **Mayo, M. A., 2002:** Summary of taxonomic changes recently approved by ICTV. *Arch. Virol.*, 147, 8, 1655–1656. DOI: 10.1007/s007050200039.
14. **Musa, U., Abdul, P. A., Emmenna, P. E., Ahmed, M. S., 2010:** Vaccination with Newcastle disease vaccine strain I2 and LaSota in commercial and local chickens in Plateau state Nigeria. *Nig. Vet. J.,* 31, 1, 46–55.
15. **Orajaka, L. J. E., Adene, D. F., Anene, B. M., Onuoha, E. A., 1999:** Seroprevalence of Newcastle disease in local chickens from southeast derived savannah zone of Nigeria. *Rev. Elev. Med. Vet. Pays. Trop.,* 52, 3–4, 185–188.
16. **Palya, V., 1991:** Manual for the production of Marek's disease, Gumboro disease and inactivated Newcastle disease vaccines. Technical Centre for Agricultural and Rural Cooperation. *FAO Anim. Prod. Health Pap.,* 89, 1–25.
17. **Spradbrow, P., 1992b:** A review of the use of food carriers for the delivery of oral Newcastle disease vaccine. In **Sprad-**

brow, P. B. (Ed.): *Proceedings of an International Workshop Kuala Lumpur,* Canberra Australian Centre for International Agricultural Research, No. 39, 18–20.

18. **Spradbrow, P. B., Sabine, M., 1995:** Australian studies on Newcastle disease virus. The French heritage. *Vet. Microbiol.*, 46, 1–3, 15–19.

19. **WOAH 2021: Office of International Epizootics:** *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, 12th edn., 2023, 16.

Received July 10, 2023
Accepted October 25, 2023