# COMPARATIVE STUDY OF ANTIBODY TITRES IN LAYERS BOOSTED WITH INACTIVATED NEWCASTLE DISEASE VACCINE AND THE THERMOSTABLE ND I-2 VACCINE

**[1]AMEMOR, Esther, [1]NYARKU, Rejoice, [1]ADARKWAH, Frederick, [1]OPOKU-AGYEMAN, Tony, [1]FOLITSE, Raphael, [1]EMIKPE, Benjamin Obukowho, [2]AGBEHADZI, Richard Koblah and [3]AMPONSAH, Patrick Mensah**

[1]School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[2]Regional Centre of Excellence in Avian Science (CERSA), University of Lome, BP 1515, Togo.
[3]Kumasi Veterinary Laboratory, Kumasi, Ghana.

**Corresponding Author:** Emikpe, B. O. School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology. Kumasi, Ghana. **Email:** banabis2001@gmail.com **Phone:** +233 549410841

***Received*** *February 15, 2022;* ***Revised*** *March 26, 2022;* ***Accepted*** *April 03, 2022*

**ABSTRACT**

***Despite effective vaccination schedules employed in the control of Newcastle disease (ND) in Ghana, the preponderance of ND outbreaks is still high. This study was therefore carried out to compare booster effects of inactivated ND vaccine and thermostable ND I-2 vaccine in layers and its impact on egg production at a private farm in Kumasi, Ghana. Thirty thousand layers (10,000 per group) of 36 weeks old were used for the study. Group C was vaccinated with inactivated ND vaccine (Nobilis Newcavac, South Africa), Group D with ND I-2 vaccine and Group F was the control group, which was not vaccinated. Antibody titres of the birds were determined 10 days and a day before vaccination and from day 4 to day 28 after vaccination using haemagglutination-inhibition (HI) test and the mean titres were calculated. Both inactivated ND vaccine and thermostable ND I-2 vaccine produced booster responses. The inactivated ND vaccine produced a higher average titre increase of 37.20 % compared to ND I-2 vaccine, 33.33 %. Average titres of the control population reduced by 24.26 %. Egg production reduced by 8 % and 3 % in populations vaccinated with inactivated ND vaccine and ND I-2 vaccine respectively. In conclusion, inactivated ND vaccine and the thermostable ND I-2 vaccine produced booster effect with ND I-2 causing minimal reduction in egg production, indicating that the use of easily applicable ND I-2 vaccine should be encouraged.***

**Keywords:** Newcastle disease, Inactivated Newcastle disease vaccine, Thermostable ND I-2 vaccine, Haemagglutination-inhibition test, Antibody titres

## INTRODUCTION

Newcastle Disease (ND) is a highly contagious viral disease of birds caused by virus in the family Paramyxoviridae, genus *Avulavirus* and species avian paramyxovirus type 1 (APMV-1) (Murcia *et al*., 2009). Based on pathogenicity, four strains exist: velogenic, mesogenic, lentogenic and apathogenic strains (Creelan *et al*., 2002). Transmission is mainly by inhalation or ingestion of the virus, or by contact with mucous membranes, especially the conjunctiva of infected birds. Infected birds shed virus in aerosol, respiratory discharge and faeces (Capua and Alexander, 2009). Depending on the predilection site, clinical signs and symptoms from the respiratory tract, digestive system, nervous system and reproductive tract may be present (Perttula, 2010).

Vaccination against ND is routinely practiced in most endemic countries as the main control strategy against the disease at various stages of the bird developmental life (Senne *et al*., 2004). Although vaccination protocol depends on the endemicity of the disease in a specific area, live vaccines are usually used at the early stages of life up to about 10 weeks of age, where the inactivated vaccines are used as booster (OIE Terrestrial Manual, 2021).

ND has been identified as a major impediment to poultry production (Boakye *et al*., 2016). The inactivated ND vaccine is the commonly used booster in Ghana to control Newcastle disease. The inactivated ND vaccine has a lower thermostability accounting for a number of vaccine failures, laborious to apply and with a number of vaccination complications (Bell, 2001). However, the ND I-2 vaccine is thermostable, less laborious to apply and pose minimal vaccination complications (Alders *et al*., 2001). There is limited literature comparing the immunological responses induced by these two vaccines, hence the aim of this study was to compare changes in egg production and the antibody titres in layers boosted with inactivated ND vaccine (Nobilis Newcavac) and the thermostable ND I-2 vaccine.

## MATERIALS AND METHODS

**Study Area:** The study was conducted at Akate Farms, Nwamase, in the Kwabre East district, Ashanti region, Ghana. Akate Farms was selected because it is one of the largest poultry farms in Ghana.

**Study Design:** Field experimental study was carried out using 30,000 layers which were purposively selected for the study. The total population was divided into three groups of 10,000 birds each: A, B and C. Each group consisted of ten (10) pens, with one thousand (1000) birds per pen. The birds were 36 weeks of age, kept on the deep litter and had earlier been vaccinated with HB1, Lasota and inactivated Newcastle vaccine at various stages of their life. All the birds were subjected to the same management practices and protocols. Blood samples were randomly collected from two hundred birds per group.

**Experimental Design:** Each bird in group A was vaccinated with 0.5 ml inactivated ND vaccine (Nobilis Newcavac, South Africa), intramuscular. Group B was vaccinated with ND I-2 vaccine at 10 ml per 1000 birds in drinking water. Group C was not vaccinated and served as the control treatment. Haemagglutination test (HA) and the haemagglutination-inhibition (HI) were used to determine the antibody titer. Each group was monitored daily and egg production was recorded from day 1 to day 28. Egg production reduction rate was calculated as egg production before vaccination minus the production on the $28^{th}$ day after vaccination divided by the total number of birds in the group at vaccination.

**Sample Collection and Assay:** Blood samples were taken 10 days and a day before vaccination and on the $4^{th}$, $14^{th}$ and $28^{th}$ day after vaccination. About 1 – 3 ml of blood was collected from the wing vein of each bird into a plain tube and made to stand for serum formation. Serum was collected into Eppendorf tubes and stored in the freezer and later analysed for ND antibody titres using haemagglutination-inhibition test (Pedersen, 2008).

**Data Analysis:** Data obtained on the antibody titre values were subjected to descriptive analysis to obtain the Geometric mean titre (GMT) of each sample group. Analysis of variance (ANOVA) was used to compare the difference between geometric mean titres and the egg production reduction rate of the three groups at 5 % significant level ($p = 0.05$) using SPSS version 20. Results were presented in tables.

## RESULTS AND DISCUSSION

Results on ND antibody titres at 10 days and a day before vaccination, as well as 4, 14 and 28 days after vaccination were as shown in Table 1. Despite intensive vaccination programs implemented, Newcastle disease is endemic in poultry in Ghana. This is a constraint to the development of the poultry sector with annual losses, partly due to the disease and cost-related preventive measures, hence the need to study vaccine efficacy against NDV.

**Table 1: Geometric mean titres after haemagglutination-inhibition test at 10 days and a day before vaccination, as well as 4, 14 and 28 days post vaccination**

| Groups | Geometric Mean Titre (2^) | | | | |
|---|---|---|---|---|---|
| | Before vaccination | | After vaccination | | |
| | 10 days | 1 day | Day 4 | Day 14 | Day 28 |
| **ND I-2** | 8.40 ± 0.81$^{b}$ | 7.55 ± 1.21$^{a}$ | 7.90 ± 0.63$^{a12}$ | 10.00 ± 1.11$^{c}$ | 10.07 ± 1.18$^{c2}$ |
| **Inactivated** | 8.37 ± 0.93 | 7.50 ± 1.04 | 8.10 ± 0.95$^{2}$ | 10.10 ± 0.93 | 10.29 ± 0.77$^{2}$ |
| **Control** | 8.38 ± 0.89 | 7.52 ± 0.93 | 7.40 ± 1.03$^{1}$ | 7.14 ± 2.3 | 6.33 ± 2.03$^{1}$ |

*Means with different letter (row) or number (column) superscripts are significantly different (p<0.05)*

This study was carried out to compare the antibody titres produced in primed layers boosted with inactivated ND vaccine and the thermostable ND I-2 vaccine.

Antibody titres of samples collected were recorded 10 days before vaccination. When samples were analysed a day before vaccination, antibody titres reduced in all studied populations. This implied that the antibodies were waning in the birds.

Both inactivated Newcastle disease vaccine and the thermostable ND I-2 vaccine produced inappreciable immune response in the birds within the first four days post vaccination. At four days post vaccination, the inactivated Newcastle disease vaccine produced a higher booster effect than the ND I-2 vaccine. Though it is known that antibody response occurs within 10-14 days after vaccination; changes is probably due to individual changes in antibody profile. Antibody titres of the control group reduced from $2^{7.52}$ ± 0.93 to $2^{7.40}$ ± 1.03. However, there was no statistically significant difference (p>0.05) in antibody titres among the three groups.

Statistically significant difference (p<0.05) in antibody titres existed among the groups when samples were collected on the 28$^{th}$ day post vaccination. This may be due to immune system response following continuous virus replication and shedding (Sarcheshmei *et al.*, 2016). This finding agrees with the fact that antibodies are detected in the blood beginning at six days after infection or live virus vaccination and peaks 21 – 28 days after infection (Al-Garib *et al.*, 2003).

Inactivated Newcastle disease vaccine produced a higher immune response (37.20 %) than ND I-2 (33.33 %) which corroborated with a similar work done in village chicken (Bell, 2001). Although this study evaluated humoral immunity, other studies have shown that birds vaccinated with inactivated vaccines tend to have higher humoral antibody levels, they do not develop a strong cell mediated response, while live vaccines provide both mucosal and humoral immunity (Schijns *et al.*, 2008), while live vaccines provide both mucosal and humoral immunity (Dimitrov *et al.*, 2017). There was a slight decrease in the vaccinated flock possibly due to stress associated with vaccination especially using parenteral application route. The inactivated vaccine with higher stress of handling recorded higher egg production reduction (Table 2).

**Table 2: Egg production reduction rate from day 1 to day 28 post-vaccination with inactivated Newcastle disease vaccine and ND I-2 vaccine**

| Group | Egg production reduction rate (%) |
|---|---|
| **ND I-2** | 7800 to 7500 (3 %, reduction)$^{b}$ |
| **Inactivated** | 7600 to 6800 (8 % reduction)$^{c}$ |
| **Control** | 7500 to 7522 (0.0022 increase)$^{a}$ |

*Means with different letter (column) superscripts are significantly different (p<0.05)*

The average titres in the control population decreased by 24.28 %. However, the egg production slightly increased; this may be due to the fact that the group was not stressed.

**Conclusion:** In conclusion, both inactivated Newcastle disease vaccine and the ND I-2 vaccine produced booster immune response in the birds. ND I-2 can be used as booster vaccine in commercial farms as it produces protective levels of antibody titres with ease in vaccine application and minimal egg production losses. Commercial poultry farmers should be encouraged to use ND I-2 as booster vaccines

as it is less laborious and produces sufficient immune response. Further studies should be done to know the protection duration of both vaccines and the rate of decay of the antibodies after it peaks.

**ACKNOWLEDGEMENTS**

We acknowledge the management and staff of Akate Farms, Nwamase, Kwabre East district, Ashanti region, Ghana for providing the research team access to their facility.

**REFERENCES**

AL-GARIB, S. O., GIELKENS, A. L. J., GRUYS, E., HARTOG, L. and KOCH, G. (2003). Immunoglobulin class distribution of systemic and mucosal antibody responses to Newcastle disease in chickens. *Avian Diseases*, 47(1): 32 – 40.

ALDERS, R., DOS ANJOS, F., BAGNOL, B., FUMO, A., MATA, B. and YOUNG, M. (2001). *Controlling Newcastle Disease in Village Chickens: A Training Manual for Trainers of Community Vaccinators*. Monograph Number 86, Australian Centre for International Agricultural Research (ACIAR), Canberra.

BELL, J. G. (2001). A comparison of the different vaccine available for the control of Newcastle disease in village chickens. Pages 56 – 60. *In:* ALDERS, R. G. and SPRADBROW, P. B. (Eds.). *SADC Planning Workshop on Newcastle Disease Control in Village Chickens*. Proceedings of an International Workshop by Australian Centre for International Agricultural Research (ACIAR), Proceedings Number 103, Maputo, Mozambique, 6 – 9 March, 2000.

BOAKYE, O. D., EMIKPE, B. O., FOLITSE, R. D., BONNAH, S. G., ADUSEI, K., OWUSU, M. and OYEBANJI, V. O. (2016). Serological detection of Newcastle disease virus antibodies in local chickens and guinea fowls in the area of Kumasi, Ghana. *Brazilian Journal of Poultry Science*, 18(1): 87 – 92.

CAPUA, I. and ALEXANDER, D. J. (2009). *Avian Influenza and Newcastle Disease: A Field and Laboratory Manual*. Springer-Verlag, Milan, Italy.

CREELAN, J. L., GRAHAM, D. A. and MCCULLOUGH, S. J. (2002). Detection and differentiation of pathogenicity of avian paramyxovirus serotype 1 from field cases using one-step reverse transcriptase-polymerase chain reaction. *Avian Pathology*, 31(5): 493 – 499.

DIMITROV, K. M., AFONSO, C. L., YU, Q. and MILLER, P. J. (2017). Newcastle disease vaccines – a solved problem or a continuous challenge? *Veterinary Microbiology,* 206: 126 – 136.

MURCIA, P., DONACHIE, W. and PALMARINI, M. (2009). Viral pathogens of domestic animals and their impact on biology, medicine and agriculture. *Encyclopedia of Microbiology*, 2009: 805 – 819.

OIE TERRESTRIAL MANUAL (2021). *Newcastle Disease (Infection with Newcastle Disease Virus). Chapter 3.3.14. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2021*. OIE-WAHIS (OIE World Animal Health Information System) Terrestrial Manual Online Access. https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/ Accessed December 12, 2021.

PEDERSEN, J. C. (2008). Hemagglutination-inhibition test for avian influenza virus subtype identification and the detection and quantitation of serum antibodies to the avian influenza virus. Pages 53 – 66. *In*: SPACKMAN, E. (Ed.). *Avian Influenza Virus.* Humana Press, Totowa, New Jersey, USA.

PERTTULA, L. (2010). *Epidemiology and Characterization of Newcastle Disease in Smallholder Poultry in Mozambique*. Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Sweden.

SARCHESHMEI, M., DADRAS, H., MOSLEH, N. and MEHRABANPOUR, M. J. (2016).

Comparative evaluation of the protective efficacy of different vaccination programs against a virulent field strain of the Newcastle disease virus in broilers. *Brazilian Journal of Poultry Science*, 18(3): 363 – 370.

SCHIJNS, V. E., VAN DE ZANDE, S., LUPIANI, B. and REDDY, S. M. (2008). Practical aspects of poultry vaccination. Pages 345 – 362. *In:* DAVISON F., KASPERS, B. and SCHAT, K. A. (Eds.). *Avian Immunology.* Academic Press, Elsevier, Amsterdam.

SENNE, D. A., KING, D. and KAPCZYNSKI, D. (2004). Control of Newcastle disease by vaccination. *Developments in Biologicals (Basel),* 119: 165 – 170.