



## **Overdose Administration of Thermostable Newcastle Disease Vaccines to Naïve Unvaccinated 6 Weeks Old Cockerels at NVRI, Vom Plateau state, Nigeria**

**A. N. Egbuji<sup>1</sup>, A. Chukwuedo<sup>1</sup>, L. N. Shedua<sup>1</sup>, J. K. Gyallak<sup>1</sup>, L. N. Swomen<sup>1</sup>,  
N. C. Obene<sup>1</sup>, U. K. Akachi<sup>1</sup>, H. C. Ngwu<sup>2</sup>, K. O. Idahor<sup>3</sup>, M. Muhammad<sup>1</sup>  
and L. U. Enurah<sup>4\*</sup>**

<sup>1</sup>National Veterinary Research Institute, Vom, Plateau State, Nigeria.

<sup>2</sup>Bauchi State Ministry of Agriculture and Natural Resources, Bauchi, Nigeria.

<sup>3</sup>Department of Animal Science, Nasarawa State University, Keffi, Nigeria.

<sup>4</sup>Department of Veterinary Microbiology and Pathology, Faculty of Veterinary Medicine, University of Jos, Nigeria.

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author ANE designed the study and wrote the first draft of the manuscript. Authors AC, LNS and JKG performed the statistical analysis while authors LNS, NCO and UKA managed the literature searches. Authors HCN, KOI and MM wrote the protocol. Author LUE managed the analyses of the study. All authors read and approved the final manuscript.*

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### **ABSTRACT**

One hundred and fifty (150) unvaccinated 6 weeks old cockerel were divided into six groups of 25 birds each. Pre-vaccination haemagglutination inhibition (HI) mean titers values of  $2^{1.4}$ ,  $<2^1$ ,  $2^1$ ,  $<2^1$ ,  $2^1$  and  $2^{1.6}$  were observed for groups A, B, C, D E and F respectively. Two batches of Newcastle

\*Corresponding author: E-mail: [enurahleonard@yahoo.com](mailto:enurahleonard@yahoo.com);

Disease vaccine (NDVI-2) of 50 and 200 doses produced at National Veterinary Research Institute Vom, with  $EID_{50}$  value of 7.7 and 7.6 per dose were administered to groups B and D at 10x (10 times the normal dose) of NDVI-2 via the intra-ocular and intranasal routes. The rest of the groups were administered normal doses of NDVI-2 through the intra-ocular route. Consequently, 40% of the vaccinated birds were randomly selected and sero-monitored twice at 2 and 4 weeks intervals. No observable ND clinical signs were seen in both groups B and D irrespective of the vaccine over dosage. Post-vaccination (HI) immune profiling of the vaccinated flock revealed steady increase in the HI mean titer value per group, while groups B and D which had 10x the normal dose showed high response in comparison to groups that had NDVI-2 standard dose. HI immune profile results 2 weeks post-vaccination revealed HI mean titer values of  $2^{3.0}$ ,  $2^{4.0}$ ,  $2^{3.2}$ ,  $2^{4.2}$ ,  $2^{3.6}$  and  $2^{4.4}$  for groups A, B, C, D, E and F respectively; week 4 post-vaccination haemagglutination inhibition mean titer values of  $2^{3.6}$ ,  $2^{5.4}$ ,  $2^{3.0}$ ,  $2^{5.7}$ ,  $2^{5.2}$  and  $2^{4.0}$  for the respective groups. Week 8 HI mean titer values of  $2^{2.2}$ ,  $2^{1.8}$ ,  $2^{2.4}$ ,  $2^{2.5}$ ,  $2^{3.2}$  and  $2^{1.8}$  were equally observed. While week 12 HI mean titer values of  $2^{2.0}$ ,  $2^{1.8}$ ,  $2^{2.0}$ ,  $2^{2.0}$ ,  $2^{1.8}$  and  $2^{1.8}$  were recorded. Post-vaccination HI mean titer values showed that groups B and D with 10x the normal dose had no observable adverse clinical signs but rather high ND antibody response was observed in groups where overdose of standard NDVI-2 vaccine were administered and evidence of ND antibody depletion was equally observed in all the vaccinated groups irrespective of the dose administered.

**Keywords:** *Overdose administration; thermostable newcastle disease vaccine; unvaccinated 6 weeks old cockerels.*

## 1. INTRODUCTION

Newcastle Disease (ND) is a highly contagious viral disease of both wild and domestic avian species of all ages [1,2]. The disease is caused by virulent strains of avian paramyxovirus type 1 (APMV-1) [3]. In unvaccinated poultry flock, morbidity and mortality rate of up to 100% have been reported [4]. The rate of morbidity and mortality vary greatly depending on the virulent nature of the infecting virus strain and susceptibility of the host bird or flock [2]. In endemic region especially in developing countries, outbreaks have been reported to have enormous economic consequences on backyard poultry flock [5], which are usually unvaccinated and free roaming [6].

Vaccination against Newcastle disease has been reported as the major control strategy against outbreaks especially in countries with endemic very virulent Newcastle Disease Virus (vvNDV) and highly deficient biosecurity measures [7,8,9]. Avirulent, NDVI-2 thermostable virus has been developed to control ND in rural poultry [10,11]. The vaccine was discovered after an extensive screening of ND virus isolates by scientists at the University of Queensland Australia; this ND vaccine strain has been extensively shown with capability to protect rural poultry in Asia and Africa [12,13,14].

In Nigeria, ND outbreak causes severe morbidity and mortality in susceptible poultry flocks with an

estimated 8.3 billion naira loss to poultry industry in 2008 [15]. It is estimated that with a population of about 183 million birds, village chickens account for 94% of the entire poultry population in Nigeria [15]. It contributes immensely to rural development by income generation for the owners and ensures household food security as it supplies high quality animal protein (meat and egg); provide petty cash derived from sales of poultry products, poverty alleviation and employment creation for rural dwellers [16,17]. Derivable benefit of rural poultry farming is constrained by occasional annual outbreaks of Newcastle disease as these birds are usually not vaccinated against ND [6] and are usually highly susceptible to ND infection. In tropical countries including Nigeria where poor cold storage facilities in field situation is a huge challenge, the use of thermostable vaccine such as NDVI-2 and NDV-4HR has been suggested and adopted for the control of ND outbreaks in rural poultry.

This study was carried out to determine the immune response of 6 weeks old cockerels given ten times the normal dose of NDVI-2 vaccine and to ascertain any adverse clinical signs compared to those given normal dose of NDVI-2. Since the vaccine is meant for rural poultry farmers who in most cases are illiterates and could administer excessive dose of the vaccine, it becomes imperative to check the effect of over dose on the birds. The results and implication of our findings are discussed.

## 2. MATERIALS AND METHODS

### 2.1 EID<sub>50</sub> Values Determination

The EID<sub>50</sub> values of batches of thermostable (NDVI-2) of 50 dose and 200 doses ND vaccine respectively produced by the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria were determined after inoculation in 10-day old chicken embryonated eggs (from specific antibody negative (SAN) birds) following OIE, [18] standard. The vaccine virus titer per dose (vaccine virus concentration per dose) were calculated and determined according to Kaaber method [19].

### 2.2 Cockerel

Two hundred and sixty (260) cockerels were hatched and brooded at NVRI Poultry Division. The birds were placed on *ad libitum* feeding and steady supply of water; with strict adherence to biosecurity and bio-exclusion practices at the rearing pen.

At week 4, 150 of the brooded birds were randomly selected and moved to Quality Control Experimental Unit, where they were divided into groups (A, B, C, D E and F) and each group was made up of 25 cockerels. The birds were allowed to stabilize and were equally placed on *ad libitum* feeding and water supply, with strict adherence to biosecurity and bioexclusion practices at the rearing pen. Sequel to stabilization at the rearing pen, blood samples were collected from the wing vein and harvested sera samples subjected to HI pre-vaccination antibody immune profiling.

Groups B and D were administered overdose at 10x (10 times the normal dose) for NDVI-2 of 50 and 200 doses respectively via the intra-ocular and intranasal routes. The rest of the other groups were administered normal doses for NDVI-2 using the intra-ocular route. Blood samples were collected via the wing vein twice at 2 and 4 weeks intervals for ND antibody immune profiling using the haemagglutination and haemagglutination inhibition test.

### 2.3 Haemagglutination Test

The serum samples collected from the birds were tested for ND virus antibodies by haemagglutination and haemagglutination inhibition tests. The HA titers of standard NDV antigen was determined as described by Allan and Gough [20]. The antigen one

haemagglutination unit (1HAU) of  $\log_2 2^9$  was used to determine the antigen four haemagglutination unit (4HAU). The determined 4HAU was used to carry out the haemagglutination inhibition tests thereafter.

### 2.4 Haemagglutination Inhibition Test

Haemagglutination inhibition (HI) test was carried out on serum samples collected pre- and post-vaccination. The HI mean titer for each screened serum sample was determined and expressed in  $\log$  base 2. The geometric mean antibody titer (GMTs) was also determined for each group pre- and post- vaccination. HI mean titre of  $\geq 2 \log_2$  was considered positive [20] while HI mean titers of  $\leq 2 \log_2$  were considered negative.

## 3. RESULTS

The EID<sub>50</sub> values of the vaccines used for the study were well above recommended EID<sub>50</sub> for NDVI-2 with  $\log 2.2$  and  $\log 2.1$ (Table 1) respectively. The extra EID<sub>50</sub> values as observed is recommended since it will carter for probable mean titer drop or loss in field situation that might result from poor handling and possible temperature variation during transit. The HI immune profiling showed that at week 2 and week 4 there were evidence of sero-conversion (detectible protective antibody production) in the vaccinated flock irrespective of the administered dose and absence of any observable clinical signs in the vaccinated flock (Table 2 and 3). The groups administered overdose showed higher titer values of up to 1 *logarithm* more than the groups that were administered normal NDVI-2 dose (Table 2). This observable antibody response persisted till week 4 (Table 2). However, significant ND HI mean antibody titer drop was observed in all the groups by week 8 post-vaccination (Table 2 and Table 3). Using Pedro Villegas conversion table of base-two *logarithmic* mean titer values to Geometric mean titer (GMT) values for all the vaccinated groups (Table 3), shows the GMT values progression for all the vaccinated groups, and also evidence of GMT reduction as the post-vaccination period advances (Table 3).

## 4. DISCUSSION

The findings of this study revealed that administration of 10 times the normal dose of wholesome, standard field fit NDVI-2 vaccine is not associated with any adverse observable

clinical consequences in young unvaccinated cockerels. Our findings have further demonstrated that in an event of NDVI-2 vaccine overdose, the resultant effect was rather an observable anamnestic or high HI antibody response by an unprimed vaccinated flock. This result is similar to the observation of [11,21], where it was reported that NDVI-2 vaccine virus, sequel to overdose administration produces no evidence of clinical respiratory signs, weight loss, and mortality in young chickens or egg production drop in laying birds. The NDVI-2 vaccine strain is innocuous to both birds and handlers and its overdose administration, is not associated with any known clinical pathological consequences [21]. Our findings and observations further support previous works on the consequent implication of wholesome, field fit NDVI-2 vaccine overdose administration to susceptible unvaccinated poultry flock, since birds were not vaccinated as shown by our pre-vaccination result.

Furthermore, our findings suggest that overdose administration of NDVI-2 vaccine to naïve unvaccinated flock will not hinder or unnecessarily prolong antibody depletion. The elicited antibodies formation from our study peaked between week 2 and week 4. Birds that received over dosage of NDVI-2 showed a *1logarithm* mean titer value higher than birds that received normal dose. Evidence of antibody waning or depletion was observed by week 8 in all the groups. This finding is equally in agreement with the finding of Wambura et al. [13] where it was suggested that NDVI-2 vaccine can confer protection for up to 8 weeks. Thus, as observed by Abdi et al. [22] it is recommended that repeated vaccinations induces progressively higher HI antibody titers that could correspond to high levels of protection; though repeated administration was not studied, our finding suggest a repeat administration of NDVI-2 vaccine between week 6 and week 8 post-vaccination.

**Table 1.  $EID_{50}$  values for NDVI-2 vaccines used for the study**

Vaccine type & dose	$EID_{50}$ values after production	log titer value above standard $EID_{50}$	Standard $EID_{50}$ Values
NDVI-2 50 doses	$\log_{10}$ 7.7 per dose	$\log$ 2.2,	$\log_{10}$ 5.5 per dose
NDVI-2 200 doses	$\log_{10}$ 7.6 per dose	$\log$ 2.1	$\log_{10}$ 5.5 per dose

**Table 2. Pre-vaccination and Post-vaccination haemagglutination inhibition immune profiling mean titers values**

Groups	Week 1 mean pre- vaccination HI titre value	Week 2 mean post- vaccination HI Titre value	Week 4 mean post- vaccination HI value	Week 8 mean post- vaccination HI Titre value	Week 12 mean post- vaccination HI titre value
A	$<2^{1.4}$	$2^{3.0}$	$2^{3.6}$	$2^{2.2}$	$2^{2.0}$
*B	$<2^1$	$2^{4.0}$	$2^{5.4}$	$2^{1.8}$	$2^{1.8}$
C	$<2^1$	$2^{3.2}$	$2^{3.0}$	$2^{2.4}$	$2^{2.0}$
*D	$<2^1$	$2^{4.2}$	$2^{5.7}$	$2^{2.5}$	$2^{2.0}$
E	$<2^1$	$2^{3.6}$	$2^{5.2}$	$2^{3.2}$	$2^{1.8}$
F	$<2^{1.6}$	$2^{4.4}$	$2^{4.0}$	$2^{1.8}$	$2^{1.8}$

\*Groups B and D showing high haemagglutination inhibition titre value at week 2 post-vaccination

**Table 3. Geometric (base two) mean titer values pre- and post-vaccinations for weeks 1,2,4,8 and 12**

Groups	Week 1 Mean Pre- vaccination HI titre value	Week 2 Mean Post- vaccination HI titre value	Week 4 Mean Post- vaccination HI titre value	Week 8 Mean Post- vaccination HI titre value	Week 12 Mean Post- vaccination HI titre value	Observable ND clinical signs/ symptoms
A	0	80	121	46	40	Nil
B	0	160	422	35	35	Nil
C	0	92	80	53	40	Nil
D	0	184	520	57	40	Nil
E	0	243	368	92	35	Nil
F	0	211	160	35	35	Nil

\*Conversion of base-two logarithmic mean titers values to Geometric mean titers (GMTs) (source, M Brugh[2])

Current conventional vaccination programs for ND virus include the use of either low-virulent live-virus vaccines or inactivated vaccines to induce protective immunity while producing minimal adverse effects in birds [23]. Vaccination using non-virulent NDV strains protects susceptible birds against ND disease, producing an antibody response either locally, systemically or both [23].

Therefore, for an effective and efficient ND control strategy especially in rural poultry flock which are usually not vaccinated [6], the use of wholesome, field fit NDVI-2 vaccine is recommended with revaccination in accordance with stipulated NDVI-2 vaccine schedule in regions where ND is endemic.

## 5. CONCLUSION

Vaccination against Newcastle disease outbreak remains the utmost viable control option for ND in domestic poultry flock. Our findings therefore concludes that in field situation where it is determined that the reconstituted NDVI-2 vaccine volume is more than number birds to be vaccinated, over dose administration of up to ten times (10x) of recommended dose can be administered via the intra ocular and intra nasal routes for certified wholesome, field fit NDVI-2 vaccine. This will curb vaccine wastages, since this study has shown and support other findings that over dose administration of NDVI-2, will trigger higher HI immune antibody response in susceptible vaccinated poultry flock..

## CONSENT

As per international standard or university standard written poultry farm owner consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

As per Animal ethic committee written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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