

## Specific antibody response of village chickens to single or combined Newcastle disease and infectious bursal disease vaccines

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### ABSTRACT

This study was conducted to assess the interaction of specific immune responses produced after vaccination using live attenuated Newcastle disease (ND) LaSota and infectious bursal disease (IBD) vaccines in village chickens of Nigeria. After immunization with the vaccines (individually or in different combinations), specific antibody levels in the chickens were measured using hemagglutination inhibition (HI) and agar gel immunodiffusion (AGID) tests. The cases of administration of ND LaSota alone, ND LaSota followed by IBD vaccine after one week, and simultaneous use of ND LaSota and IBD vaccines were seroconverted against ND virus. Interference of antibody production against NDV or IBDV was observed when primary vaccination was done by using any one of the two and the remaining one was given after one week. However, simultaneous administration of the vaccines did not interfere with each other in terms of antibody responses. In all the vaccination trials, elicited immunity conferred protection to the chickens challenged with virulent NDV and IBDV. Individual vaccination with ND LaSota followed by IBD vaccines or vice versa giving an interval of more than one week, or simultaneous use of both vaccines are recommended to confer protective antibody levels against NDV and IBDV in village chickens.

### Keywords:

AGID test, antibodies, HI test, IBD vaccine, ND LaSota vaccine, village chickens

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### INTRODUCTION

Newcastle disease (ND) and infectious bursal disease (IBD) have remained as two most important infectious diseases threatening the village chicken and commercial poultry production in most parts of the world (El-Yuguda et al., 2005; El-Yuguda et al., 2009). The epidemiology of these two diseases is usually influenced by certain factors like host's immune status, wide host range, thermo-stability and variation in strains of the causative viruses. Although vaccination of chickens has remained as the principal method to control these diseases (Okwor et al., 2013; Susan et al., 2013); outbreaks have continued to be reported in both commercial and village poultry (El-Yuguda and Baba, 2004). Some important factors determining the success of vaccination include the time of vaccination, combined or simultaneous vaccination, vaccine type, maternal antibodies in the chicks and pathogenicity of the offending virus (Hair-Bejo et al., 2004). Farmers administer ND and IBD vaccines simultaneously with the aim to reduce stress by catching of individual birds and to minimize labor cost (Okwor et al., 2013). However, some viruses that are immunosuppressive in nature may interfere with chicken's immune responses to other vaccine viruses which may lead to vaccine breaks (Phong et al., 2003; Hair-Bejo et al., 2004).

This study was designed to investigate the effect of combined administration of ND and IBD vaccines in village chickens in terms of their specific antibody responses.

## MATERIALS AND METHODS

**Experimental chickens:** A total of 120 unvaccinated village chicks of 3 weeks of age were used in this study. The chicks were reared in a single cage provided with chick mash (Livestock Feeds, Nigeria) and water *ad libitum* and allowed to acclimatize for one week before vaccination.

**Vaccines and challenge viruses:** The ND LaSota and IBD vaccines each supplied in 1 ml (200 doses) vials ( $10^3\text{EID}_{50}/\text{ml}$  and  $10^{2.5}\text{EID}_{50}/\text{ml}$  of ND LaSota and IBD vaccines, respectively) were purchased from the National Veterinary Research Institute (NVRI), Vom, Nigeria. These two vaccines are live attenuated in nature commonly used for routine vaccination against ND and IBD in Nigeria.

The viruses used for challenge infections were kindly supplied by NVRI, Vom, Nigeria. The challenge viruses were the Nigerian field isolates of NDV and IBDV. For each bird, 0.1 ml of  $10^6\text{EID}_{50}/\text{ml}$  NDV and/or 0.1 ml of  $10^5\text{EID}_{50}/\text{ml}$  IBDV were given.

**Experimental procedure:** At four weeks of age, the experimental chickens ( $n=120$ ) were divided into six equal groups (A to E and X), and the birds were housed in separate cages. The chickens of group A were vaccinated only with ND LaSota; group B were vaccinated with ND LaSota followed by IBD after one week; group C were vaccinated with IBD followed by ND LaSota after one week; group D were vaccinated simultaneously with ND LaSota & IBD; group E were vaccinated only with IBD and group X were considered as unvaccinated control. All the bird groups were kept in separate cages. During feeding, vaccination and bleeding, chickens of unvaccinated group were attended first followed by vaccinated groups throughout the study. Both vaccines were reconstituted in chlorine free water and administered orally to the birds of respective groups except group X which were given water without any vaccine. At day 21 post vaccination (PV), ten chickens from each group were challenged as follows: 5 chickens from group A, B, C, D and X and 5 chickens from group B, C, D, E and X were challenged orally with 0.1 ml (per bird) of  $10^6\text{EID}_{50}/\text{ml}$  NDV and 0.1 ml of  $10^5\text{EID}_{50}/\text{ml}$  IBDV, respectively. All the experimental chickens were bled through the wing

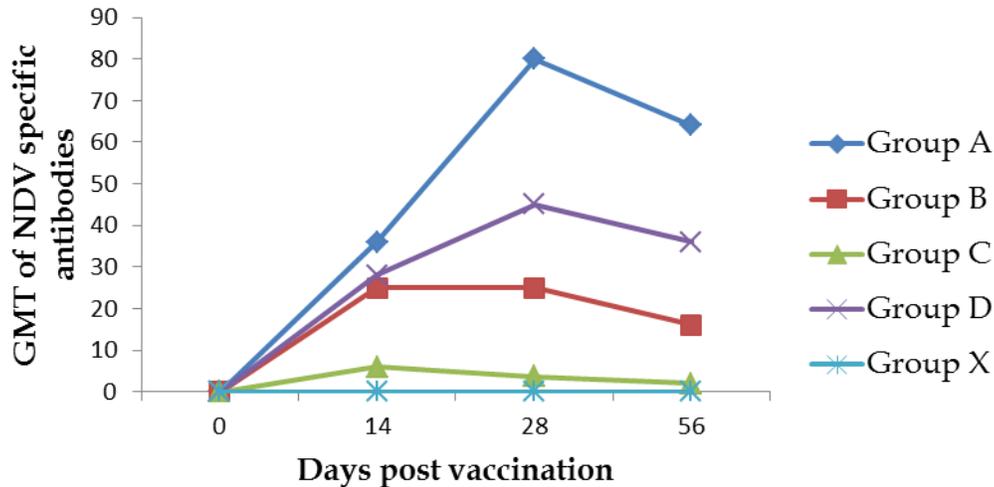
vein on day 0, 14, 28 and 56 PV. The challenged birds were bled additionally on day 21 and 31 of PV.

**Serology:** Serum was prepared from the collected blood following the procedure described previously (El-Yuguda and Baba, 2004). The serum samples were assayed to detect ND and IBD specific antibodies using hemagglutination inhibition (HI) and agar gel immunodiffusion (AGID) tests respectively as previously described by Baba et al. (1998) and El-Yuguda and Baba (2004).

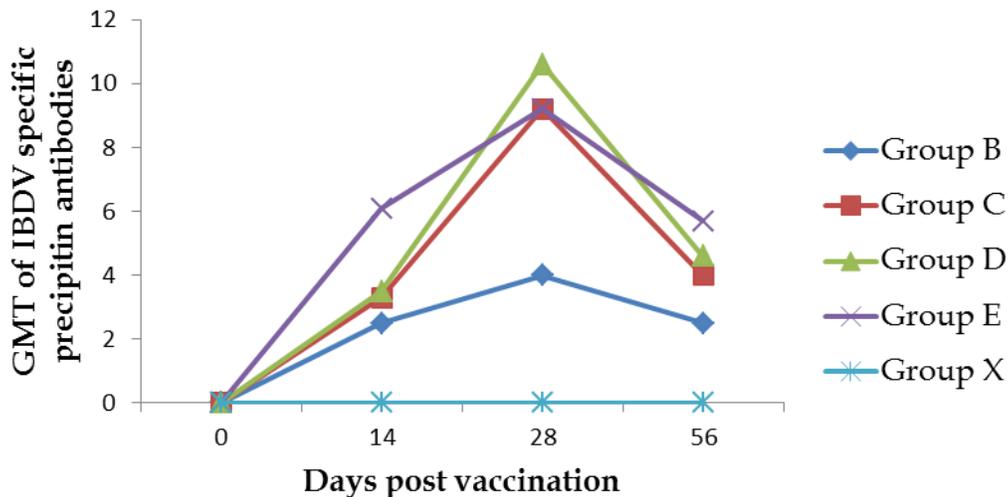
**Statistical analysis:** The data obtained in this study on different variables were calculated and converted to geometric mean titer (GMT) values using the formula-  $X_{\text{geo}} = \text{antilog}_{10} \{1/n (\sum f_i \log_{10} X_i)\}$ , where  $f_i$ =frequency and  $X_i$ =reciprocal of dilution and  $f_i$ =frequency (CDC Atlanta Georgia, 1988). The GMT values were analyzed with analysis of variance (ANOVA) using *Statgraphic plus Version 5.0*, November 2000 (Statistical Graphics Corp.). The level of statistical significance was set at  $p$ -value less than or equal to 0.05.

## RESULT AND DISCUSSION

Levels of NDV (LaSota) and IBDV specific antibodies in vaccinated chickens are shown in Figure 1 and 2, respectively. It was found that the ND LaSota Vaccine alone (Group A), ND LaSota and IBD vaccine used after one week (Group B) and ND LaSota and IBD vaccines used simultaneously (Group D) were seroconverted to the protective antibody titers ( $\geq 1:10$ ) against NDV. A significant difference ( $p < 0.05$ ) in antibody response was noted among chicks of groups A, C and D (Figure 1). A significantly lower ( $p < 0.05$ ) GMT value of NDV antibodies was exhibited by the chicks vaccinated with IBD vaccine followed by ND LaSota Vaccine given on a week interval (Group C) (Figure 1). These findings revealed that IBD vaccine may interfere with the proper antibody response by the village chicks against NDV. This observation supports the findings of El-Yuguda et al. (2007) who reported a lowered antibody response to ND LaSota vaccine among guinea fowls that were infected with IBDV or vaccinated with IBD vaccine. Similar results were demonstrated by Ali et al. (2007) in chickens. However, in contrast, Tabidi et al. (2004) and Okwor et al. (2013) observed no effect in the response of chickens to mixed ND and IBD vaccines. This difference may possibly be due to the different type of vaccines and the chicken breeds used. The possible reason of our observation might be due to- (i) the immunosuppressive effect of the live attenuated IBD vaccine virus on the immune



**Figure 1. Geometric mean titer (GMT) of Newcastle disease virus (NDV) specific antibodies of village chickens following vaccination with ND LaSota and/or IBD vaccines.** Group A- ND LaSota Vaccine only; Group B- ND LaSota vaccine followed by IBD vaccine after one week; Group C- IBD vaccine followed by ND LaSota vaccine after one week; Group D- ND LaSota and IBD vaccines simultaneously, Group X- unvaccinated control.



**Figure 2. Geometric mean titer (GMT) of infectious bursal disease virus (IBDV) specific antibodies of village chickens following vaccination with ND LaSota and/or IBD vaccines.** Group B- ND LaSota vaccine followed by IBD vaccine after one week; Group C- IBD vaccine followed by ND LaSota vaccine after one week; Group D- IBD and ND LaSota vaccines simultaneously, Group E- IBD vaccine only; Group X- unvaccinated control.

system of the birds, (ii) the effect of interferons induced by the IBDV, (iii) the relatively slow replication of ND LaSota vaccine virus as compared to that of IBD vaccine virus.

Bursal repair is reported to take one to several weeks for complete regeneration following IBDV infection (Abdu et al., 1988) leading to a severe and prolonged immunosuppression that can result in concurrent viral and bacterial infections along with vaccination failure (Bhatla et al., 2003).

The antibody response to the IBD vaccine by the experimental chicks is presented in Figure 2. A significant difference ( $p < 0.05$ ) was noted between the group B and E (Figure 2). A significant decrease in the antibody response to IBD vaccine was observed in our study when ND LaSota vaccine was given after one week of IBD vaccination (Group C). However, this finding does not support the findings of Ali et al. (2007) who reported no adverse effect of ND LaSota vaccine on IBD vaccination in chickens. The poor response could be attributed to the effect of NDV on the

lymphoid organs and/or interferon induced by the NDV as reported by McFerran and McCracken (1987).

In our study, all the vaccinated birds survived a challenge infection with virulent NDV or IBDV given on day 21 of PV. However, in terms of antibody production, the birds of group B and C showed about four fold rise of antibody production against IBDV and NDV, respectively. In contrast, no significant difference of antibody production was observed in the birds of control group challenged with virulent IBDV. These findings proved the involvement of the vaccine in antibody production in the birds. However, it was difficult to predict whether the insignificant rise of antibody titer observed in this study was due to the replication of respective virus or not. No clinical sign of either ND or IBD was observed in vaccinated groups although a low antibody titer was observed. This might be reflected that protection of the birds is not only due to the presence of antibodies alone.

The unvaccinated birds (Group X) did not show any noticeable antibody titer against either of the viruses (Figure 1 and 2). All the challenged control birds were died showing typical clinical signs and symptoms. The control birds challenged with NDV were died between days 5 and 8 of post challenge, whereas 3 among 5 were died after challenging with IBDV, and the remaining 2 recovered. In post-mortem findings, the birds died of NDV revealed inflamed lungs, thick mucus on congested trachea, inflamed cecal tonsils and hyperemic intestine. On the other hand, IBDV challenged birds showed urate deposition in the ureters, inflamed and congested bursa and hemorrhages at gizzard-proventriculus junction and in the thigh and breast muscles.

## CONCLUSIONS

The administration of both ND and IBD vaccines at one week interval could interfere with the antibody response of village chickens to the respective vaccines. It is therefore recommended that in case of vaccination with both ND LaSota and IBD vaccines, it is better to give either the vaccines individually within a time frame of more than one week or they should be given simultaneously.

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