EFFECT OF POST-INFECTION VACCINATION ON IMMUNE STATUS OF NEWCASTLE DISEASE-INFECTED CHICKEN

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ABSTRACT

This study investigated the response of chicks infected with velogenic Newcastle disease virus (NDV) to Newcastle disease vaccine - NDV-I_2. A total of 90 day-old cockerel chicks were used for this study. At three weeks of age, the chicks were randomly separated into two equal groups of 45 chicks designated A and B. After separation, Group A chicks were vaccinated with NDV-I_2 vaccine intraocularly, while Group B chicks were not vaccinated. At six weeks of age, Groups A and B chicks were further randomly separated into sub groups A1, A2, A3 and B1, B2, B3 of 15 chicks each respectively. After separation, chicks in subgroups A2, A3, B2, and B3 were exposed to chicks infected with NDV. Upon manifestation of clinical infections, chicks in subgroups A2 and B2 were revaccinated with NDV-I_2 vaccine, while subgroups A3 and B3 were not vaccinated. Percentage morbidity was 42.86, 64.29, 100 and 92.86 % for subgroups A2, A3, B2 and B3 respectively, while percentage mortality was 14.29, 50, 100 and 71.43 % in subgroups A2, A3, B2 and B3 respectively. This study showed that vaccination of previously vaccinated chicks during Newcastle disease outbreak protects the chicks and reduces both morbidity and mortality significantly.

Keywords: Cockerels, Newcastle disease, Vaccination, Revaccination, Geometric mean titre, Morbidity, Mortality

INTRODUCTION

Newcastle disease (ND) is an acute and highly contagious viral disease that causes severe economic losses within the poultry industry. The disease is caused by Newcastle disease virus (NDV) which is a non-segmented, single-stranded, negative-sense RNA virus belonging to the genus *Orthoavulavirus* (Absalón *et al.*, 2019) of subfamily Avulavirinae within the family Paramyxoviridae, order Mononegavirales (Mayo, 2002; Lamb *et al.*, 2005).

Since the emergence of ND in 1926, there has been continuous emergence of new virulent genotypes from global epizootics with year to year changes in genome sequence of the virus (Diel *et al.*, 2012; Snoeck *et al.*, 2013). Newcastle disease is one of the most economically important viral diseases of poultry (Bankowski *et al.*, 1981; Alexander, 2000; Lamb and Parks, 2007; Miller *et al.*, 2010).

Over 250 species of chicks have been reported to be susceptible to NDV as a result of natural or experimental infections. Many more susceptible species exist but have not yet been identified (Kirkland, 2000). An accurate assessment of the distribution of NDV throughout the world is difficult to achieve due to the widespread use of live vaccines. However, studies have concluded that ND is endemic in many countries of Asia, Africa and the Americas (Alders *et al*., 2001; Westbury, 2001). In Africa and Asia, Newcastle disease is a major constraint to both commercial and village poultry production (Conan *et al*., 2012).

Infections with NDV can lead to a broad range of clinical signs, varying from asymptomatic enteric infections to systemic infections causing 100 % mortality. Vaccination is required to optimally protect chicks against economically devastating diseases of poultry such as ND in endemic regions. To date, a number of lentogenic NDV strains such as B1, F, LaSota, V4 and I_2 are extensively used as live attenuated vaccines for ND control (Rauw *et al*., 2009). All live attenuated ND vaccines are known to stimulate both mucosal and systemic immune responses similar to those of the natural infection. This is because of the ability of the viruses to replicate within the bird irrespective of the site of administration (Senne *et al.*, 2004). Other NDV strains used as popular live vaccines include the Komorov and Mukteswar strains, both of which are mesogenic and therefore suitable as booster vaccines following priming with lentogenic isolates (Roohani *et al*., 2015).

The strain I_2 of NDV is a thermostable vaccine which is becoming popular due to its many advantages such as thermostability, easy administration by various routes such as drinking, eye drop, and mix with food, providing good protection against virulent virus (Miller *et al*., 2009; Wambura, 2009), efficient ability to transmit to non-vaccinated sensitive chicks (Habibi *et al*., 2015) and good results in reducing serum concentration of acute phase proteins (Firouzi *et al*., 2014).

However, there have been reported cases of occasional outbreaks of ND even in vaccinated commercial chickens (Okoye *et al*., 2001). These have called for practices such as emergency vaccination of chicks post appearance of clinical signs. According to Okwor *et al.* (2012), post infection or emergency vaccination of chickens against some avian pathogens is commonly practiced in many developing countries of Africa and Asia. The authors also observed that post-exposure or post-infection vaccination can be used in the management of an outbreak of fowlpox in chickens.

The aim of this study was to investigate the effect of post ND infection vaccination with NDV-I_2 on the morbidity, mortality and immune responses of chicks challenged with viscerotropic velogenic strain of ND.

MATERIALS AND METHODS

Experimental Animals: A total of 90 white cockerels were sourced at day old from a local hatchery. The chicks were not vaccinated against Newcastle disease at day old as requested. They were brooded under the deep litter system with feed and water provided *ad libitum* as described by Sonaiya and Swan (2004).

Ethical Issues: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed (MRC, 2004).

Experimental Design: At three weeks of age, the chicks were laid out in a complete randomized design (CRD) of two equal groups of 45 chicks designated A and B. After separation, Group A chicks were vaccinated with NDV-I_2 vaccine by intraocular instillations in the eye using a standard dropper according to manufacturer's instruction, while Group B chicks were not vaccinated. The various subgroups were housed separately; feed and water were provided *ad libitum*. At 6 weeks of age, groups A and B chicks were further replicated thrice into replicates A1, A2, A3 and B1, B2, B3 of 15 chicks each respectively (Figure 1). After separation, chicks in subgroups A2, A3, B2 and B3 were exposed to chicks infected with NDV.

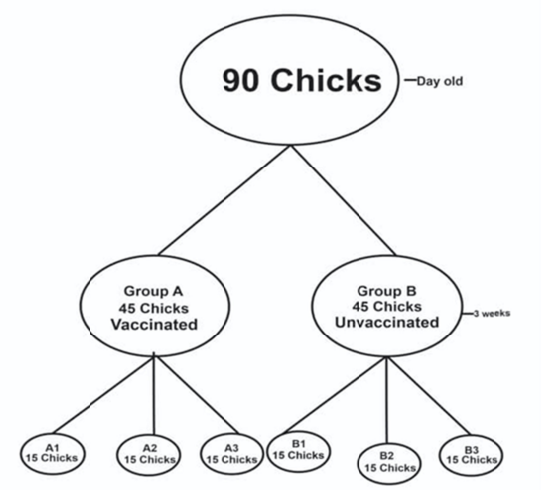

Figure 1: A diagrammatic representation of the experimental design

Upon manifestation of clinical infections, chicks in subgroups A2 and B2 were revaccinated again using the same vaccine, route and concentration while subgroups A3 and B3 were not vaccinated.

Eight chicks were intramuscularly challenged with 0.2 ml of the virus inoculums. Four days post challenge; the infected chicks were used to infect chicks in subgroups A2, A3, B2 and B3 by introducing two each of the intramuscularly infected chicks into their respective pens. Upon manifestation of clinical infections, chicks in subgroups A2 and B2 were revaccinated again using the same vaccine, route and concentration while subgroups A3 and B3 were not revaccinated.

The Vaccine: The EID_{50} value of the I_2 vaccine was determined after inoculation in 10-day old chicken embryonated eggs (from specific antibody negative (SAN) chicks) following OIE/WOAH (2012) standard.

Experimental Virus and Challenge: The NDV inoculum used was a velogenic strain of NDV. The viability and infective dose of the virus supplied was determined following the same protocol as stated for vaccine virus. The inoculums contained median embryo infective dose (EID_{50}) of $10^{6.5}$/ml. Eight chicks were intramuscularly challenged with 0.2 ml of the virus inoculums. Four days post challenge; the infected chicks were used to infect chicks in subgroups A2, A3, B2 and B3.

Morbidity and Mortality: Clinical signs and checks were observed for all the birds. Percentage morbidity was calculated by noting the number of chicks that were depressed and listless in a group and dividing by the total number of surviving chicks in the subgroup and multiplied by 100/day. Percentage mortality was assessed by noting the total number of dead chicks/day within the study period and this was also expressed in percentage.

Collection of Blood Samples: Three millilitres of blood each were collected from five chicks in each of the subgroups. The blood samples were allowed to clot under room temperature and the harvested sera were stored at -20^0C until used to determine the antibody titres of the chicks on days 42, 56, 63 and 70 days of age using the methods of Beard (1989) and Ruwaan *et al*. (2009).

Serology: The sera collected on 42, 56, 63 and 70 days of age were used to determine the antibody titres against ND (Beard, 1989). The geometric mean titre (GMT) was calculated and expressed as the reciprocal values of the highest dilutions that displayed hemagglutination-inhibition (HI) as described by Villegas and Purchase (1989).

Statistical Analysis: Descriptive statistics using percentages were adopted to compare morbidity and mortality rates of chicks in the subgroups, while GMT was used to measure the degree of immune responses.

RESULTS

Clinical Signs: Clinical signs of infection were first observed on day six post infection (PI) in subgroups B2 and B3 chicks and on day seven PI in subgroups A2 and A3 chicks. Signs of infections observed include depression, drop in feed and water consumption, listlessness, dullness, ruffled feathers, coughing and sneezing. Greenish-yellow diarrhea, torticollis and paralysis of the legs and sitting on the hock

were observed on day 8 PI in the chicks of B2 and B3. Clinical signs of Newcastle disease virus infection were more pronounced in subgroup A3 chicks than in subgroup A2 chicks.

Morbidity and Mortality Rates: Morbidity was 42.86 and 64.29 % for subgroups A2 and A3 respectively and 92.86 and 100 % in subgroups B3 and B2 respectively (Figure 2). Mortality was 14.29, 50, 71.43 and 100 % in subgroups A2, A3, B3 and B2 respectively (Figure 3).

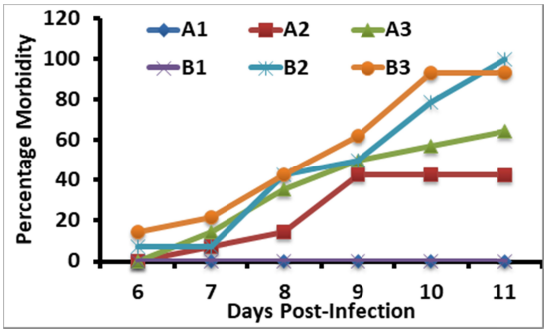

Figure 2: Morbidity profile of all groups following challenge with NDV. *Legend: A1= pre-vaccination only, A2 = pre-vaccinated + infection + post-vaccinated, A3 = pre-vaccinated + infection, B1= No vaccination, no infection, B2 = infection + post-vaccination, B3 = infection only*

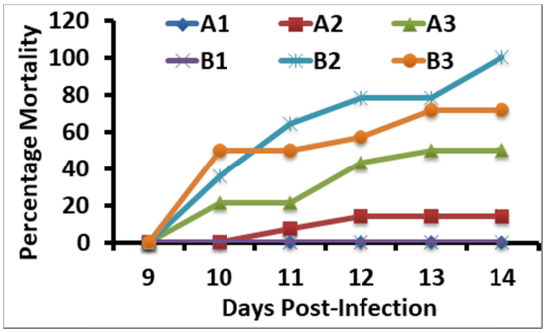

Figure 3: Mortality profile of all groups following challenge with NDV. *Legend: A1 = pre-vaccination only, A2 = pre-vaccinated + infection + post-vaccinated, A3 = pre-vaccinated + infection, B1= No vaccination, no infection, B2 = infection + post-vaccination, B3 = infection only*

Serology: The results of the mean antibody titres expressed as geometric mean titers (GMT) were presented in Figure 4. The antibody titres of chicks in subgroups A1, A2, A3, B1, B2 and B3 at 3 weeks of age prior to initial vaccination were all 0.00 GMT respectively.

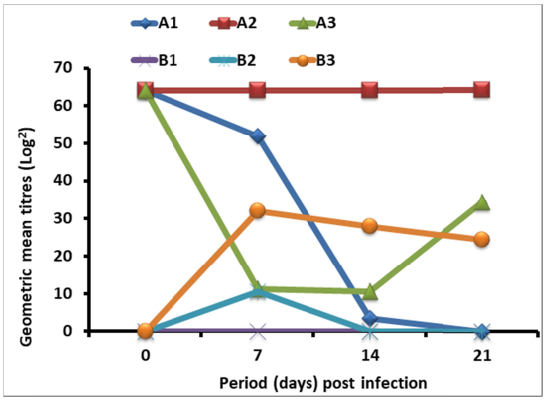

Figure 4: Geometric mean titer (GMT) in chickens of groups A and B following challenge and/or vaccination against NDV. *Legend: A1= pre-vaccination only, A2 = pre-vaccinated + infection + post-vaccinated, A3 = pre-vaccinated + infection, B1= No vaccination, no infection, B2 = infection + post-vaccination, B3 = infection only*

On day 42 (day 0) post infection (PI), the mean antibody titres of chicks in subgroups A1, A2, A3, B1, B2 and B3 were 64.0, 64.0, 64.0, 0.00, 0.00 and 0.00 respectively. It was consistently higher in vaccinated, infected and post-vaccinated subgroup A2, relative to other subgroups, throughout the experiment.

There was a gradual but steady decline in the mean antibody titres of chicks in subgroup A1. The vaccinated and infected chicks in subgroup A3 witnessed an initial decline and later an increase in mean antibody titre as follows; 64.0, 11.3, 10.6 and 34.3 on days 0, 7, 14 and 21 PI respectively.

Among the Chicks in Group B, the GMTs of chicks in subgroup B2 was 10.6 on day 7 PI, before the subgroup recorded 100 % mortality. The GMTs of B3 were 32, 27.9 and 24.3 on days 7, 14 and 21 PI respectively. The unvaccinated, uninfected subgroup B1 had zero GMT throughout the period.

DISCUSSION

The result of this experiment showed that post-exposure vaccination can be used to manage outbreaks of Newcastle disease in chickens. These findings were not in agreement with the reports of Okoye *et al.* (2001) whose findings did not encourage post infection vaccination of flocks in situations where most of the birds have started showing clinical signs of the disease in

ND infections. However, the findings of this study were in agreement with their recommendations that good results could be obtained when most of the chicks have not been infected prior to post-exposure vaccination. Our method of infection (in contact), led to a gradual spread of the infection in the flock, unlike in the intramuscular route as used by the above authors.

According to Okwor *et al.* (2012), post infection vaccination of chicks against some avian pathogens is commonly practiced in many developing countries of Africa and Asia and this has yielded differing results which may be due to certain conditions such as climatic factors, age, breed, sex of the chicks, and severity of infection at the time of vaccination. Other factors include level of immunity of the chicks before infection, number of animals infected before vaccination, type or nature of vaccine used for the vaccination and the nature or virulence of the pathogen itself.

The incubation periods obtained in the study was in agreement with those of Beard and Hanson (1984) and Hamid *et al*. (1991) who reported incubation periods of 2 – 16 days. The incubation period was longer than 3days as reported by Okoye *et al.* (2000). The intramuscular experimental route of infection used by these workers may have been responsible for short incubation period compared to infection via other routes such as by oral or inhalation. According to Ashraf and Shah (2014), the viral particles in oral or nasal infection would first assemble at the cell membranes where the first antigenic reaction takes place with some time lag or delay in intracellular invasion and subsequent viraemia.

It is noteworthy here that infection was established even among vaccinated flocks. The observation is also in agreement with the report of a similar work done by van Boven *et al.* (2008) who reported infection and clinical disease in vaccinated chicken with high circulating antibody (GMT $>3\log2_{10}$) but with limited mortalities.

A post-infection decrease in the mean antibody titre was observed in subgroups A2 and A3 that were vaccinated. This was in agreement with the findings reported by Tizard (1996), which showed that antibody titre decreased due to its use in the neutralization of the viral particles. Subgroup A2 chicks which were earlier vaccinated before and after ND infection had increased antibody titres after revaccination. This probably accounted for the decreased severity in disease manifestation. This was in agreement with the report by Allan *et al.* (1978) and Ezema *et al*. (2009) who observed that virulent ND strains may still replicate in vaccinated chicks, but the clinical signs would be greatly diminished in relationship to the antibody level achieved. The mean antibody titre in subgroup A3 decreased steadily due to neutralization of the virus until day 14PI when it started increasing. It was observed that the subgroup A3 had higher mortality than members of subgroup A2 indicating that revaccination boosted level of antibodies, hence, their protective ability.

There was a gradual increase in mean antibody titre in unvaccinated but infected subgroups B2 and B3 where the infecting virus served as the source of antigen. However, this increase in circulating antibody was not protective enough against the virus (Al-Garib *et al.*, 2003). Subgroup B2 chicks which were vaccinated upon the appearance of clinical infections had higher morbidity and mortality probably due to the combined effects of both vaccination stress and the infection.

Conclusion: Vaccination of previously vaccinated chicks during ND outbreak reduced both the morbidity and mortality rates and equally produced higher antibody titres against ND.

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