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Immune response and protective potential following vaccination against Newcastle disease virus and fowl cholera in Naked neck chickens

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Abstract

Humoral immune responses to Newcastle Disease Virus (NDV) and Bangladesh Agricultural University (BAU) Fowl cholera (BAUFC) vaccines were evaluated in naked neck chickens (NNC). Ten birds were vaccinated with Baby Chick Ranikhet Disease Virus (BCRDV) at Day 7 through intra-ocular route and with Ranikhet Disease Virus (RDV) at day 35 of age through intramuscular route. Serum antibodies were measured by Haemagglutination Inhibition test. Two weeks after final immunization all birds were challenged with virulent field isolate of NDV where all vaccinated birds survived without illness during ten days, and all ten control birds died. Ten birds were vaccinated with BAUFC vaccine at Day 42 and 70 according to the Manufacturer's instruction, which induced detectable levels of antibody titre as determined by Passive Haemagglutination Assay (PHA) test. Eight vaccinated birds survived following challenge with virulent fowl cholera isolate two weeks after final vaccination and all ten control birds died. (*Bangl. vet*. 2012. Vol. 29, No. 2, 49 - 55)

Introduction

Backyard poultry contribute about 85% of the poultry population of Bangladesh, and Naked neck chicken (NNC) is the most important variety. The poultry industry is seriously affected by outbreaks of infectious diseases (Siddique, 1997; Zhuo *et al.,* 1998; Samad, 2000).

Newcastle disease (ND) is one of the major threats to the poultry industry, including backyard poultry. ND is caused by avian paramyxovirus serotype-1 (APMV-1) also known as Newcastle disease virus (NDV). ND may produce signs of depression, diarrhoea, prostration, oedema of the head and wattles. Velogenic viscerotropic Newcastle disease (vvND) often causes listlessness diarrhoea, increased rate of respiration, and death. Surviving birds may develop nervous signs such as muscular tremors, paralysis and torticollis (McFerran and McCracken, 1988). With extremely virulent viruses the disease may result in sudden death (Cheville *et al.,* 1972; Brown *et al.,* 1999).

Fowl cholera (FC) is also a disease of economic importance, which occurs all over Bangladesh, causing 25% to 35% mortality in chickens and ducks (Choudhury *et al.,*

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1985). It is also known as avian cholera and avian haemorrhagic septicemia and is caused by *Pasteurella multocida* (Heddleston and Rhoades, 1978). There are 16 sero-types: according to Choudhury *et al. (*1985), only two or three are present in Bangladesh. The clinical signs are anorexia, fever, ruffled feathers, mucus discharge from mouth, rapid respiration and diarrhoea.

As NNC is one of the important indigenous breeds in Bangladesh, this study was carried out to investigate their immune response to Newcastle Disease and Fowl cholera vaccines.

Materials and Methods

Newcastle disease vaccine and virus

Baby chick Ranikhet disease vaccine (BCRDV) and Ranikhet disease vaccine (RDV) were obtained from Livestock Research Institute (LRI), Mohakhali, Dhaka. Virulent field isolate of NDV was used for challenge test.

Fowl Cholera disease vaccine and bacteria

FC vaccine was collected from Livestock and Poultry Vaccine Research and Production Centre (LPVRPC) and used according to the instructions provided by the manufacturer. Virulent field isolate of bacteria causing Fowl cholera was used for challenge test.

Experimental chicks

A total of 40 day-old NNC chicks were obtained from Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka and reared in the experimental poultry shed providing sufficient nutrition with standard biosecurity.

Experimental immunization

The chicks were divided into four equal groups. Groups A and B were used for NDV vaccines while C and D were used for BAUFC vaccine.

Immunization with NDV vaccines

Group A were vaccinated primarily with BCRDV through intra-ocular (i/o) route one drop per bird at 7 days of age followed by secondary (booster) vaccination with RDV through intramuscular (i/m) route at 35 days 1 mL per bird. Group B were kept as unvaccinated control. Serum was collected from all birds at 7, 21, 35 and 50 days, to determine haemagglutination inhibition (HI) antibody titre.

Immunization with BAUFC vaccine

Group C were vaccinated primarily with BAUFC vaccine at 42 days of age i/m 0.5 mL per bird followed by secondary vaccination at 70 days with the same vaccine, dose and route. Serum was collected from all birds at 42, 56, 70 and 84 days, to determine PHA antibody titre.

Haemagglutination inhibition (HI) test

The HI test was performed to determine the HI antibody titres of the sera from the chickens vaccinated with BCRDV and RDV and of controls, to measure maternal antibody. The test used constant 4 HA unit antigen and increasing serum dilution method (Beta-procedure) following the methods of Anon (1971). The HI antibody titre of each serum corresponded to reciprocal of highest original dilution of serum inhibiting agglutination of cRBC completely.

Protection test with NDV against NDV vaccines

Sixteen days after secondary vaccination with RDV, Groups A and B were challenged with virulent field isolate of NDV intra-nasally (i/n) 0.1mL per bird containing $2ELD_{50}$ dose, which corresponded to about 100% mortality in chickens of 10 weeks of age (Sarkar *et al*., 2012).

Passive haemagglutination (PHA) test

The antibody titre of Group C following vaccination with FC vaccine and of control (Group D) was determined by PHA following the method described by Tripathy *et al.* (1970).

Protection test with Pasteurella multocida against FC vaccine

Sixteen days after final vaccination with BAU Fowl cholera vaccine the chickens of Group C and control (Group D) were challenged i/m with virulent field isolate of FC 0.5 mL per bird containing 5.7×10^9 CFU/mL (2.47 OD value at 550 nm) as described by Koly (2011).

Statistical analysis

Student's t-test was performed for significant differences of HI antibody titres and PHA antibody titres. Protection tests were analysed by Mantel-Cox log rank test. $P<0.05$ was considered statistically significant.

Results and Discussion

HI antibody titres of vaccinated and unvaccinated birds

The HI antibody titres (Mean ± SD) of vaccinated (Group A) and unvaccinated (Group B) birds against NDV vaccines at 7, 21, 35 and 50 days of age were converted into $\log_2$ HI antibody titres. The $\log_2$ HI antibody titres (Mean ± SD) of Group A were 6.0 ± 0.7, 6.4 ± 0.5, 7.2 ± 0.4 and 9.0 ± 0.7 at 7, 21, 35 and 50 days of age, respectively (Fig. 1). The $\log_2$ HI antibody (Maternal antibody) titres (Mean ± SD) of Group B were 6.0 ± 0.7, 6.2 ± 0.7, 4.4 ± 0.8 and 3.2 ± 0.4 at 7, 21, 35 and 50 days of age, respectively.

The log2 HI maternal antibody titres of Group B gradually declined (Fig. 1). Rahman *et al.* (2004) found a high titre of maternal antibody in 4 day-old chicks: the mean HI antibody titre was 8.3 ± 0.6. In Group A, following primary vaccination with BCRDV,

HI antibody titres were slightly higher at Day 21 than in control birds. Similarly, antibody titres at Day 35 were slightly higher compared with day 21. Antibody titre was sharply increased after primary vaccination with BCRDV and secondary vaccination with RDV. Similar findings were found by several investigators (Chowdhury *et al.,* 1981; Kafi *et al.,* 2003; Sarker *et al.,* 2012).

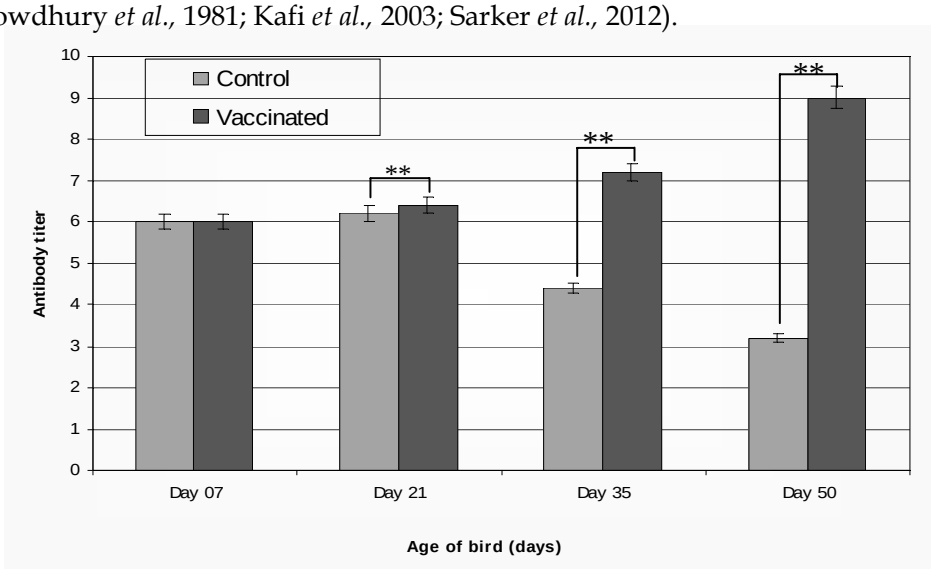

Fig 1. Serum HI antibody titre (log2 base) against NDV vaccines of unvaccinated control and vaccinated birds. The graph shows the mean ± SD values (n = 10 chickens/group). **P<0.01 by student's *t* test

Protection test against NDV vaccines

No birds of vaccinated group (A) showed clinical signs of illness (Fig. 2). Birds of control group (B) started to show clinical signs and to die from 3rd day following challenge and all succumbed within 7 days (Fig. 2) producing characteristic post-mortem lesions of NDV (Data not shown). Rahman *et al.* (2004); Chowdhury *et al.* (1981) had similar results.

PHA antibody titres of vaccinated and unvaccinated birds

The pre-vaccination PHA antibody titre against BAU fowl cholera was <4.0 ±0.0 (Fig. 3), similar to the results of Mondal *et al.* (1988).

The PHA antibody titre (Mean ± SE) of vaccinated chickens was 70.4 ± 15.7 at 15 days after primary vaccination, greater (*P*<0.01) than 28 days after primary vaccination (58.6 ± 14.3). The PHA antibody titre (Mean ± SE) increased sharply 15 days after secondary vaccination to 180.2 ± 66.1 (Fig. 3). The PHA antibody titre (Mean ± SE) of the control birds (Group D) was <4.0 ± 0.0 at 42, 56, 70 and 84 days of age (Fig. 3). Wu *et al.* (1986) suggested that two doses of FC vaccine were required for better immune response with an interval of two to four weeks after primary vaccination. Choudhury *et al.* (1985); Mondal *et al.* (1988); Sarker *et al.* (1992) used the same method to measure the serum antibody titres following administration of fowl cholera vaccine.

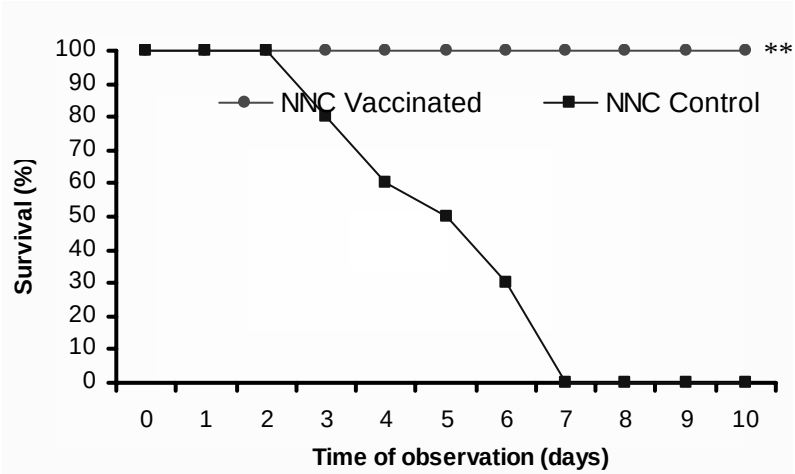

Fig 2. Survival rate of chicken following challenge infection intranasally with virulent field isolate of NDV P<0.01 by Mantel-Cox log rank test

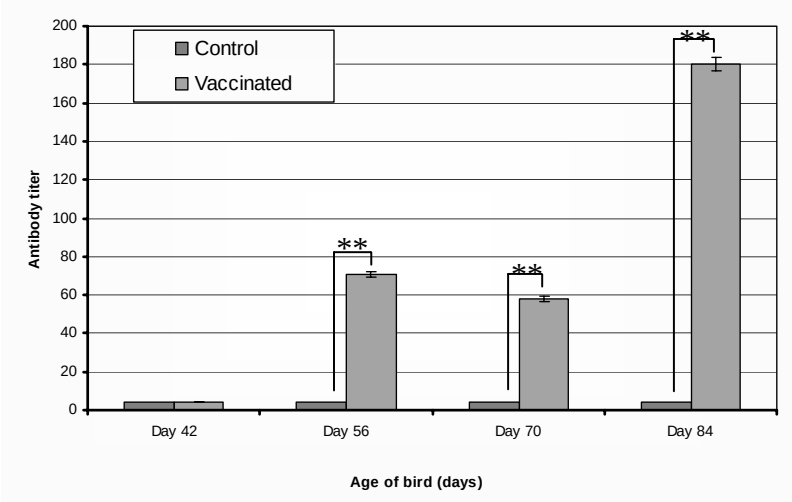

Fig 3. Serum PHA antibody titre against fowl cholera vaccine of the vaccinated and unvaccinated birds. The graph depicts the mean ± SE values (n = 10). **P<0.01 by Student's *t* test

Protection test against fowl cholera vaccine

The survival rate of vaccinated (Group C) and unvaccinated (Group D) birds was monitored for 10 days following challenge with *P. multocida* isolate. All the unvaccinated control (Group D) birds showed clinical signs of infection within one day and succumbed within 6 days (Fig. 4): a similar trend was observed by Avakian *et al.* (1989).

Two vaccinated birds died at 8th day, but the other 8 remained healthy throughout the 10-day period of observation (Fig. 4). Similar findings were observed by Super *et al.* (2002) who reported that the protection rate of the locally isolated *P. multocida* vaccine was 50-75% and Avakian *et al.* (1989) showed a survival rate of 86%.

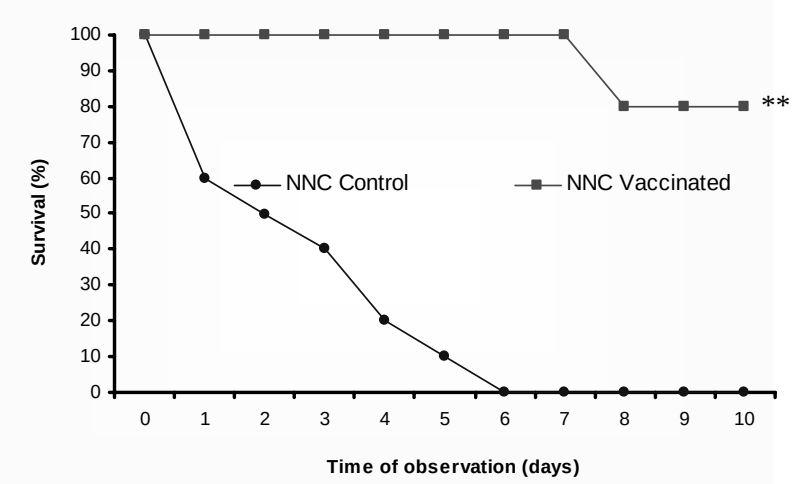

Fig 4. Survival rate of chicken after challenge with virulent *P. multocida* isolate. **P=<0.01 by Mantel-Cox log rank test

Conclusions

Naked neck chickens produced satisfactory levels of antibodies following vaccination with fowl cholera and NDV vaccines and 80-100% survived following challenge.

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