

## IMMUNE RESPONSE CAPABILITY OF 'SHUVRA' CHICKEN

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### ABSTRACT

The study was carried out to determine the humoral immune response to Newcastle Disease Virus (NDV) and Bangladesh Agricultural University Fowl cholera (BAUFC) vaccines in Shuvra chicken, a newly developed chicken strain by BLRI (Bangladesh Livestock Research Institute). Ten Shuvra chickens were vaccinated with Baby Chick Ranikhet Disease Vaccine (BCRDV) at day 7 through intra ocular route (i/o) and with Ranikhet Disease Vaccine (RDV) at day 35 through intramuscular (i/m) route. Vaccine induced serum Haemagglutination Inhibition (HI) antibodies were measured by HI test. Two weeks after final immunization all vaccinated and control Shuvra chickens were challenged with virulent field isolates of NDV where all the vaccinated birds survived without showing any typical signs of NDV during the period of ten days observation period and all the control chickens died. Another 10 Shuvra were vaccinated twice with BAUFC vaccine through intramuscular route at day 42 and 70, and 10 Shuvra chickens were kept as unvaccinated control. This vaccine also induced significantly higher level of antibody titre as determined by Passive Haemagglutination (PHA) test. Vaccinated chickens showed significantly higher survival (80%) following challenge with virulent fowl cholera isolate and all the control birds died within 10 days of observation period.

**Key words:** Shuvra, chick, vaccine, Passive Haemagglutination assay (PHA), antibody titre, fowl cholera

### INTRODUCTION

In Bangladesh chicken contributes 51% of the total meat production (FAO, 1999; BBS, 2009). Per capita annual consumption of meat in this country is 5.9 kg which is very low and only 7.98% of the universal standard (MoFL, 2006). The annual per capita egg consumption is only 23 although it should be 100 from a nutritional point of view. The predominant poultry breed in Bangladesh is local, kept in scavenging system. The productivity of a local hen is 40-60 eggs per year (Saleque and Mustafa, 1996). To develop a high yield layer strain of chicken, Bangladesh Livestock Research Institute (BLRI) has carried out a long strenuous research along with Japan International Cooperation Agency (JICA). Shuvra is the product of such research which is claimed to be very adaptable to rural condition. Shuvra start laying eggs within 19 weeks and an annual laying capacity of eggs is around 280 to 295 (BLRI).

Among infectious diseases of backyard poultry, Newcastle disease (ND) and fowl cholera (FC) are highly endemic (Siddique *et al.*, 1997; Samad, 2000) in Bangladesh. Although vaccination is being practiced to control NDV in Bangladesh but outbreaks of NDV infection has been reported by several investigators (Sarkar *et al.*, 2012, Rahman *et al.*, 2004).

Fowl Cholera (FC) is also a disease of economic importance, which occurs all over Bangladesh, causing 25-35% mortality in chickens and ducks (Chowdhury *et al.*, 1985). It is also known as avian cholera and avian haemorrhagic septicemia and is caused by *Pasteurella multocida* (Heddleston and Rhoades, 1978). As the BLRI claims that the newly developed chicken, Shuvra is capable to resist diseases and sustain at local environment. So it would be better to check the immune response and protective capability of Shuvra against common infections while dissemination of this newly developed breed of chickens for farming is advocated. So, this piece of research was undertaken to investigate the immune response capability of this strain of birds with Newcastle disease vaccines and Fowl cholera vaccines.

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## **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 Ranikhet/Newcastle Disease Virus (NDV) used for challenge was obtained from the repository of the Department of Microbiology and Hygiene, BAU, Mymensingh.

### **Fowl Cholera disease vaccine and bacteria**

FC vaccine called BAU Fowl cholera vaccine was collected from LPVRPC, and used according to the instruction provided by manufacturer. Virulent field isolate of Fowl cholera causing bacteria used for challenge was obtained from the repository of the Department of Microbiology and Hygiene, BAU, Mymensingh.

### **Experimental chicks**

A total of 40 day-old Shuvra chicks were obtained with the courtesy from BLRI, Savar, Dhaka. The chicks were then reared in the experimental poultry shed and were provided with sufficient nutritional diet and other management requirements with proper 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 and BAUFC vaccines**

Group A were vaccinated primarily with BCRDV through (i/o) route one drop per bird at 7 days of age followed by secondary vaccination with RDV through intramuscular (i/o) route at 35 days 1 mL per bird. Group B were kept as unvaccinated control. Sera were collected from all the chickens at 7, 21, 35, and 50 days, to determine Haemagglutination Inhibition (HI) antibody titre. Group C were vaccinated primarily with BAUFC vaccine at 42 days of age (i/m) 0.5mL per chicken followed by booster vaccination at 70 days of age with the same vaccine, dose and route. Serum was collected from all the chickens 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 samples collected from the chickens vaccinated with BCRDV and RDV against NDV. The HI titres of sera samples of control chicken were also analyzed to determine the maternal antibody titres. The test was conducted by using constant 4 HA unit antigen and increasing serum dilution method (Beta-procedure) following the method of Anon (1971). The HI antibody titer of each serum corresponded to reciprocal of highest original dilution of serum inhibiting agglutination of cRBC completely.

### **Protection test with NDV (Challenge experiment)**

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

### **Passive haemagglutination (PHA) test**

The PHA test was performed to determine antibody titres of chickens vaccinated with BAUFC (Group C) and of control (Group D) as per the method described by Tripathy *et al.* (1970).

**Protection test with FC (Challenge experiment)**

Sixteen days after booster vaccination with BAUFC vaccine the chickens of vaccinated (Group C) and control (Group D) were challenged intramuscularly (i/m) with virulent field isolate of FC @ 0.5mL per bird containing  $5.7 \times 10^9$  CFU/ mL (2.47 OD value at 550nm) as per the method described by Koly (2011).

**Statistical analysis**

Student's t-test was performed to determine significant differences in HI and PHA titres. Protection tests were analyzed by Mantel-Cox log rank test. A P value at  $\leq 0.05$  was considered statistically significant.

**RESULTS AND DISCUSSION**

**HI antibody titres of vaccinated and control chickens**

HI antibody titres in chickens of vaccinated (Group A) and control (Group B) against NDV vaccines at 7, 21, 35, and 50 days of age were converted to log<sub>2</sub> HI antibody titres. From this study, it was observed that the HI antibody titres of unvaccinated control (Group B) gradually decreased from day 7 to day 50. The pre-vaccination log<sub>2</sub> HI antibody titres of both vaccinated (Group A) and control (Group B) chickens were  $6.0 \pm 0.70$ ,  $6.00 \pm 0.73$ , respectively which gradually declined in control chicken (Group B) to  $3.20 \pm 0.44$  on day 50 (Fig. 1). On day 36 antibody titer were  $4.00 \pm 0.71$ . Rahman *et al.* (2004) reported a very high titre of maternally derived antibody in 4 day-old chick. Conversely, in vaccinated chicken (Group A), following primary vaccination with BCRDV HI titres were slightly increased at day 21 compared with the control birds (maternal antibody). Similarly, antibody titer of serum that was obtained at day 36 was also slightly ascended compared with day 21. In day 21, 35 and 50, the HI titre was recorded,  $6.20 \pm 0.51$ ,  $7.10 \pm 0.44$ ,  $8.8 \pm 0.70$  respectively (Group A). Similar findings were reported by several investigators (Chowdhury *et al.*, 1981; Kafi *et al.*, 2003; Sarkar *et al.*, 2012).

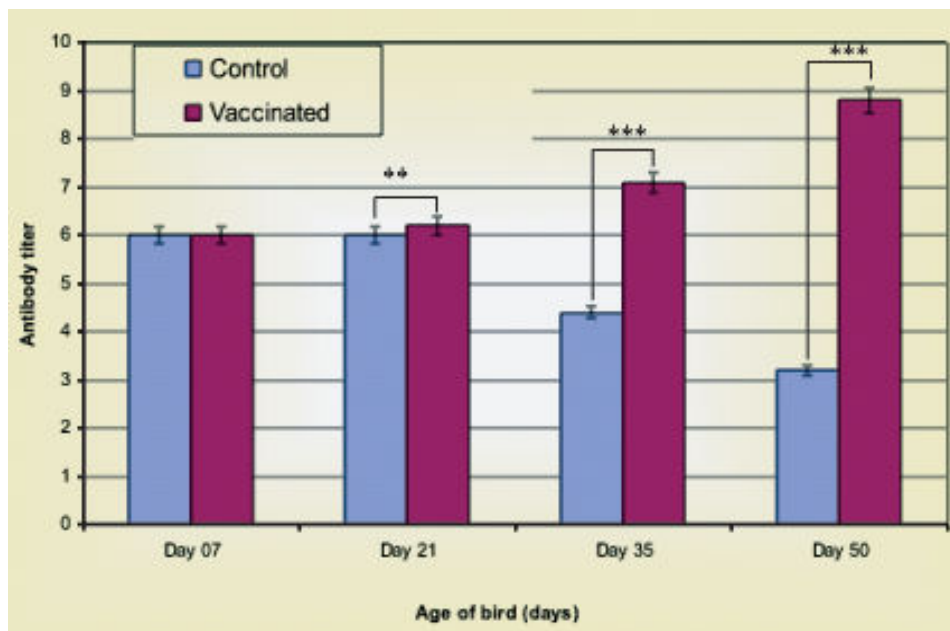


Figure 1. Serum antibody titer (log<sub>2</sub> base) against NDV antigen in chicken of vaccinated (Group A) and unvaccinated control (Group B). Vaccinated group were immunized with BCRDV through (i/o) route at 7 days of age and with RDV at 35 days of age through (i/m) route, while control group were not received no vaccine. Blood was drawn at 7, 21, 35, and 50 days of age of birds from both groups. Serum HI antibody titres against NDV antigen were determined by HI test. Results are shown in log<sub>2</sub> (Mean ± SD, where SD = Standard deviation, HI = Haemagglutination inhibition, \*\*p<0.01 and \*\*\*p <0.001).

**Protection test against NDV vaccines**

In case of vaccinated group all the 10 birds exhibited no signs of illness and survived after challenge infection throughout the period of observation of 10 days. Conversely, all the control birds started to show typical clinical signs of ND e.g. sneezing, gasping, greenish-watery diarrhoea and drowsiness from 3<sup>rd</sup> day of challenge infection. Control chickens started to die from 3<sup>th</sup> day after challenge infection and succumbed within 6 days. The birds also produced typical postmortem lesion for NDV (data not shown). It appeared that birds vaccinated with BCRDV and RDV conferred 100% protection following virulent challenge (Figure 2).

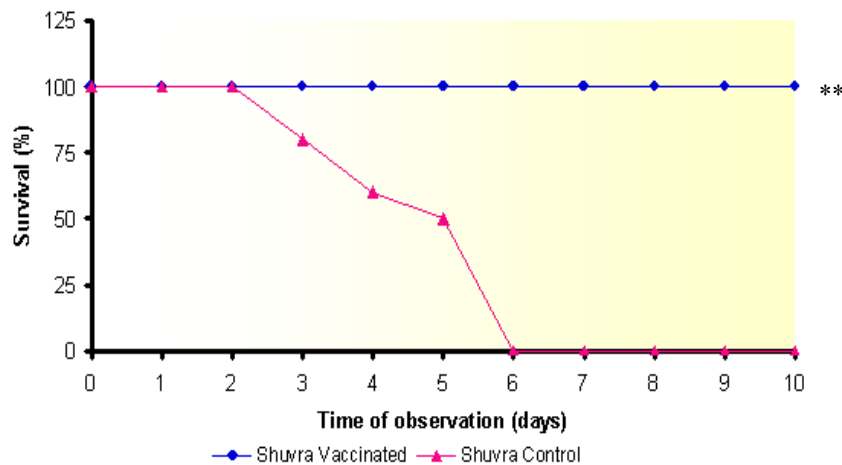


Figure 2. Survival rate of chicken following challenge infection intranasally with virulent field isolate of NDV. The figure show the survival rate of chicken was monitored following intranasal challenge with 2ELD<sub>50</sub> dose of NDV and was observed for subsequent 10 days where \*\*p < 0.01 by Mantal-Cox log rank test.

**PHA antibody titres of vaccinated and unvaccinated chickens**

Micro plate PHA test was conducted to determine the antibody titres of test sera collected from the vaccinated (Group C) and unvaccinated control chickens (Group D) used in this experiment and the titres are calculated and presented as mean of titre. Prevaccinated PHA titres of sera samples of all vaccinated and control birds was found to have a mean of <math>4.0 \pm 0.00</math>. The PHA antibody titre of vaccinated chickens was  $70.4 \pm 15.67$  after 15 days of primary vaccination and  $57.60 \pm 14.31$  after 28 days of primary vaccination (Figure 3).

After 15 days of booster vaccination the PHA antibody titre of sera of vaccinated chickens were also determined by microplate PHA test. The antibody titre of sera of Shuvra chickens revealed the mean antibody titre of  $179.2 \pm 66.09$ . The vaccination dose used for the production of immune response against fowl cholera was closely related with Khan *et al.* (1997). Booster dose was given with the similar dose and route at 28 days after primary vaccination. 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. Similar method to measure the antibody titres following administration of fowl cholera vaccine has been reported by several investigators (Chowdhury *et al.*, 1985; Mondal *et al.*, 1988; Sarker *et al.*, 1992).

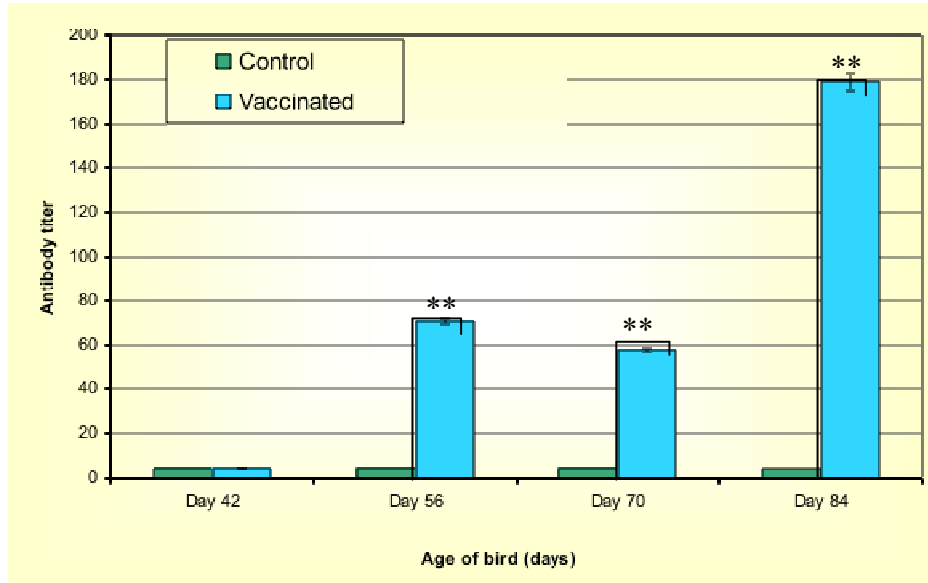


Figure 3. Serum antibody titre against Fowl cholera vaccine in chicken. Chicken was immunized at 6 weeks (42 days) of age while secondary vaccination was done at 10 weeks (70days) of age via I/M route at the dose rate of 0.5 ml ( $1.75 \times 10^8$  CFU/ ml) in each bird. Serum was obtained at 42 (6 wks), 56 (8 wks), 70 (10 wks) and 84 (12 wks) days of age. Serum antibody titre against vaccination was determined by PHA test. The graph depicts the mean  $\pm$ SE values (n = 5). \*\*p < 0.01 by student t-test

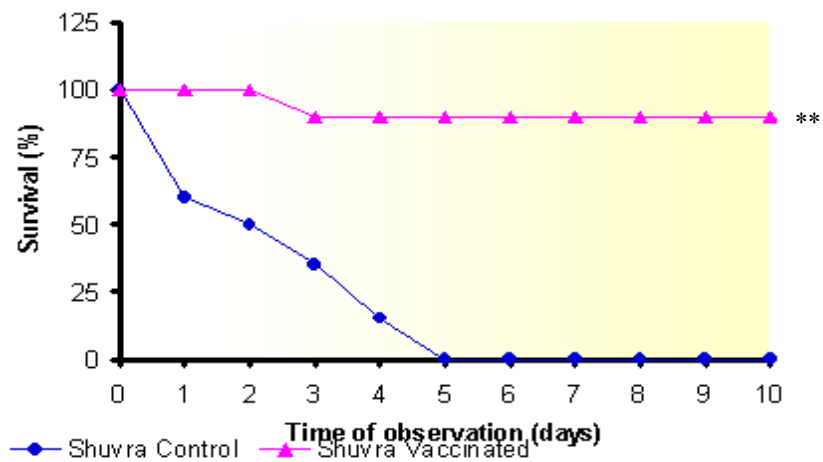


Figure 4. Survival rate of chicken after challenged with virulent *P. multocida* isolate. Chickens were immunized twice with FC vaccine at 6 weeks (42 days) of age and secondly at 10 week (70 days) of age through I/M route at the dose rate of 0.5 ml ( $1.75 \times 10^8$  CFU/ ml). Both vaccinated and control chickens were inoculated with 0.5 ml of fowl cholera culture (2.47 OD at 550nm) and the mortality was monitored for subsequent 10 days. \*\*p < 0.01 by Mantel–Cox log rank test.

#### **Protection test against fowl cholera vaccine**

Sixteen days after final immunization with FC vaccine the chickens were challenged (Figure 4) intramuscularly (i/m) with virulent field isolate of FC @ 0.5ml per bird containing  $5.7 \times 10^9$  CFU/ ml (2.47 OD value at 550nm).

In post challenge observations, control birds revealed characteristic clinical signs and symptoms of avian pasteurellosis like dullness, depression, anorexia, hyperthermia, laboured breathing, lameness, whitish (chalky) diarrhoea and ultimately death of all birds occurred within 5 days and similar was the observation of Avakian *et al.* (1989). Vaccinated birds protected themselves and did not show clinical signs except one birds died at 3<sup>rd</sup> day of observation. Super *et al.* (2002) observed 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%. In this experimental study BAU Fowl cholera vaccine protected 90% challenged Shuvra.

From this investigation, it may be concluded that-

- (i) Almost high level of antibody titre was observed in Shuvra following vaccination with either NDV or BAU fowl cholera vaccine.
- (ii) Shuvra were able to survive about 90% in a very hot, humid, congested and crowded environment following challenge.

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