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COMPARATIVE EFFICACY OF BAU-FOWL CHOLERA AND DLS-FOWL CHOLERA VACCINES IN INDIGENOUS CHICKEN

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ABSTRACT

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The present study was conducted to determine the immune response induced in indigenous chicken produced against BAU-FC and DLS-FC vaccines with their efficacy study against *Pasteurella multocida*. A total of forty (40) chickens were selected and divided into Group A (15), Group B (15) and Group C (10). Group A and B were vaccinated with BAU-FCV and DLS-FCV, respectively at the dose rate of 0.5 ml through SC at six weeks of age followed by boosting at 10 weeks of age while Group C was kept as unvaccinated control. Sera samples were collected after primary and booster vaccination and antibody titre was determined by Passive hemagglutination (PHA) test. The mean PHA titres recorded at 4 weeks after primary vaccination was 51.20 ± 7.84 in birds of group A and 38.40 ± 6.40 in birds of Group B. After booster vaccination, mean PHA titer was found 140.80 ± 31.35 at 16 weeks of age in case of BAU-FC vaccinated group and 115.20 ± 12.80 in case of DLS-FC vaccinated group. The mean PHA titer was 204.80 ± 31.35 and 179.20 ± 31.35 at 19 weeks of age in birds of BAU-FC and DLS-FC vaccinated group, respectively. Birds of all groups were challenged with virulent *P. multocida* at 17 weeks of age. It was observed that vaccinated chickens showed maximal resistance (100%) following challenge with virulent whereas unvaccinated control birds failed to resist the challenge infection. It can be assumed from the findings of present research work that both BAU-FCV and DLS-FCV are able to protect indigenous chicken from the outbreak of avian pasteurellosis and BAU-FV vaccine showed relatively higher immuno-protective titre than that of DLS-FC vaccine.

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INTRODUCTION

In Bangladesh, poultry keeping is an integral part of the rural farming system that provides family income and creates employment opportunity for rural people particularly small, marginal and landless poor farmers (Khan et al., 1999). The current approximate poultry population is 300 million including 50 million ducks and 250 million chickens (DLS, 2013). The villagers prefer indigenous chicken to rear because of their sustainability and rearing cost than other commercial breeds of poultry in traditional rearing system. As indigenous birds are relatively resistant compare to commercial chicken and no capital is invested, the women in villages mainly prefer to rear them. These are also a source of earning money for the poor people. Nevertheless, outbreaks of various types of infectious diseases are considered as the most leading causes of economic loss discouraging poultry rearing in this country (Das et al., 2005). Among the bacterial diseases, fowl cholera is one of the major threats to poultry rearing in villages. Fowl cholera (FC) caused by *Pasteurella multocida* is a disease of poultry occurring sporadically or enzootically in most countries of the world including Bangladesh. It is contagious bacterial diseases that affects domestic and wild avian species including chicken and hamper the profitable poultry production (OIE, 2008). It usually appears as a septicemic disease associated with high morbidity and mortality. About 25% to 35% mortality in chicken is due to fowl cholera in Bangladesh (Chowdhury et al., 1985). In order to control fowl cholera, strict bio-security, improvement of sanitary conditions as well as vaccination are essential.

A number of research programme has been carried out on the immunogenic performance of both DLS-FC and BAU-FC vaccines by Chowdhury et al. (1987), Khan et al. (1997), Islam et al. (2004), Sukul et al. (2004), Modak et al. (2012) and Sultana et al. (2013). But, till now no research has been conducted to check the immune response and protective potential of these two vaccines targeting the indigenous chicken reared in traditional system of village. Therefore, the present study was conducted to determine the immuno-protective potential and comparative efficacy of BAU-FC and DLS-FC vaccines in indigenous chicken reared in villages.

MATERIALS AND METHODS

Experimental chickens

A total number of 40 six-week aged indigenous chicken of either sex reared in free ranging system at Lakhipur village, Gouripur, Mymensingh were selected and divided into three groups namely, Group-A, B and C contained 15,15 and 10 birds, respectively.

Vaccines

Two types of vaccine were used to immunize the chicken namely, BAU-Fowl cholera vaccine produced at Livestock and Poultry Vaccine Research and Production Center (LPVRPC), Bangladesh Agricultural University, Mymensingh and DLS-Fowl cholera vaccine produced at Livestock Research Institute (LRI), Mohakhali, Dhaka.

Experimental immunization with Fowl cholera (FC) vaccines

The Experimental immunization of indigenous chicken was performed with BAU- FC and DLS-FC vaccines. Primary vaccination was carried out to birds of Group-A and B at 6 weeks of age with BAU-FC and DLS-FC vaccines, respectively @ 0.5 ml through SC route. Similarly, birds of both the vaccinated groups were boosted at 8 weeks of age with same vaccine, dose and route. Chicken of Group-C were kept as unvaccinated control throughout the study period.

Collection of serum from immunized and non-immunized chicken

The sera samples were collected on 6 weeks of age as pre-vaccinated sera. The vaccinated sera samples were obtained at 8 and 10 weeks of age (after primary vaccination), and at 12, 16 and 19 weeks of age (after booster vaccination) from chicken. Sera samples from control chickens were also obtained at 6, 8, 10, 12 and 16 weeks of age. All the collected sera samples were heat inactivated and stored for further study.

Challenge test

Five randomly selected birds of each vaccinated and control group were taken from the experimental area to the experimental shed of Department of Microbiology and Hygiene, BAU, Mymensingh and challenged with 1 ml containing 3.6×10^6 CFU/ml of virulent *P. multocida* through oral route. Chickens after challenge infection were observed daily up to 14 days for any clinical signs and symptoms of FC. The clinical findings of both the vaccinated and unvaccinated birds were observed and recorded.

Passive haemagglutination (PHA) test

This test was used to determine the antibody titres in chickens of both vaccinated and unvaccinated groups as per the method of Tripathy et al. (1970), Akter et al. (2004), Hossain et al. (2005) and Rana et al. (2010).

RESULTS AND DISCUSSION

Pre-vaccination PHA titres of all vaccinated and control chicken were found to have a mean of $\leq 4.0 \pm 00$ (Table 1) which are in agreement with the findings of Mondal et al. (1988), Islam et al. (2004) and Sultana et al. (2013). The mean PHA titer was found 44.80 ± 7.84 and 51.20 ± 7.84 in birds at 8 weeks and 10 weeks of age after primary vaccination (Table 1, Fig. 2) through SC route with BAU-FC vaccine, respectively. Similarly, mean PHA titer was found 38.40 ± 6.40 and 38.40 ± 6.40 in birds at 8 and 10 weeks of age after primary vaccination (Table 1, Fig. 3) with DLS-FC vaccine. After booster vaccination, mean PHA titer was found 102.40 ± 15.68 and 140.80 ± 31.35 at 12 and 16 weeks of age in case of BAU-FC vaccinated group and 89.60 ± 15.68 and 115.20 ± 12.80 in case of DLS-FC vaccinated group (Table 1, Fig. 1, Fig. 2, Fig. 3). Similar types of findings were described by Wu et al. (1986), Sultana et al. (2013) and Islam et al. (2017) after primary and booster vaccination with BAU-FC or DLS-FC vaccines. The mean PHA titer was 204.80 ± 31.35 and 179.20 ± 31.35 in birds of BAU-FC and DLS-FC vaccinated group, respectively at 19 weeks of age after challenge infection (Table 1). The increase in titer after challenge was also observed by Sultana et al. (2013). In our study, we found that the PHA titre was higher in the birds of BAU-FC vaccinated group compare to DLS-FC vaccinated group. Both the vaccine conferred 100% protection against challenge where all birds of control group died within 3 to 5 days post infection. Rahman et al. (2004) also observed the similar pattern in challenge experiment. Khan et al. (1997) reported 80% protection of chickens vaccinated with LRI-FCV against challenged with 1 ID (4.8×10^5 CFU/ml) of virulent *P. multocida*. Most of the challenge experiments were performed through IM route. In the present study, we gave challenge through oral route considering (4.8×10^5 CFU/ml) that in the field condition, indigenous chicken usually get infection by oral or nasal route and for this reason, the challenge dose was 3.6×10^6 CFU/ml as 1 ID. In post challenge observations, control birds showed characteristic clinical signs and symptoms of avian pasteurellosis like dullness, depression, anorexia, hyperthermia, labored breathing, lameness, whitish (chalky) diarrhea and ultimately death occurred within 3-5 days post challenge, while vaccinated birds protected themselves and did not show any clinical signs after challenge (Fig. 4). Postmortem samples were collected from dead control chicken for the re-isolation of *P. multocida*. During postmortem examination, lung was enlarged and hemorrhagic, liver was swollen and somewhere congested, and heart was enlarged and congested (Fig. 5). Cultural (Table 2), staining and biochemical findings (Fig. 6) indicated that the re-isolated bacteria was *P. multocida*.

The survival rate of both vaccinated (Group A and Group B) and control birds indicates that there is a significant change in the rate of survivability between these groups and revealed that both the vaccines have the potentiality to protect the chicken following booster vaccination (Fig. 2). The result of survivability in the present research work is in agreement with the findings of Tirumurugaan et al. (2004).

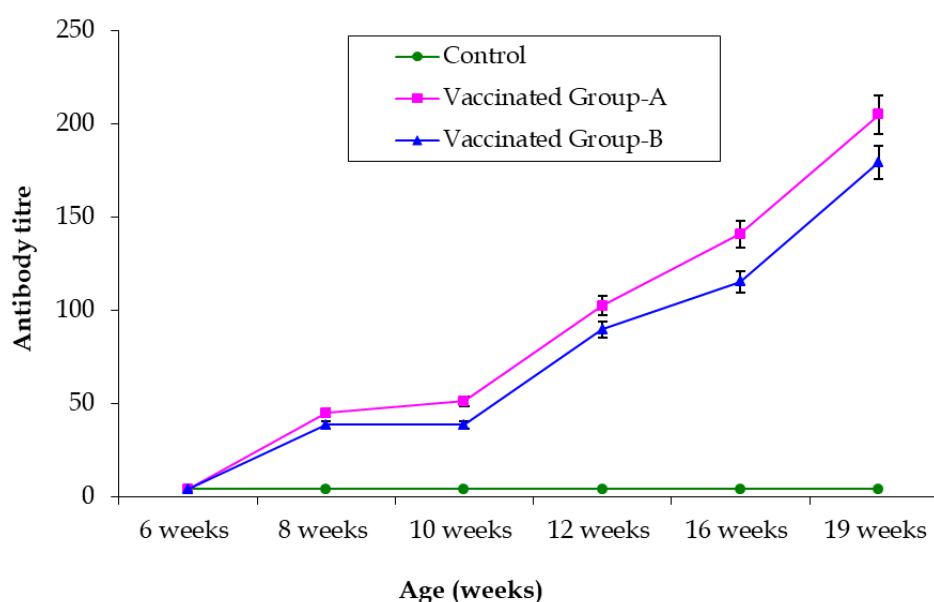
Table 1. Comparison between mean PHA titres with standard error in sera of indigenous chickens vaccinated with BAU-FC and DLS-FC vaccines

Gr.	Vaccine used	Dose and Route	Serum Antibody titre					
			Pre-vac.	After 2 weeks of primary vaccination	After 4 weeks of primary vac.	after 2 weeks of booster vaccination	After 6 weeks of booster vaccination	After 2 weeks of challenge
A	BAU-FC	0.5ml/SC	$\leq 4.0 \pm 0.0$	44.80 \pm 7.84	51.20 \pm 7.84	102.40 \pm 15.68	140.80 \pm 31.35	204.80 \pm 31.35
B	DLS-FC	0.5ml/SC	$\leq 4.0 \pm 0.0$	38.40 \pm 6.40	38.40 \pm 6.40	89.60 \pm 15.68	115.20 \pm 12.80	179.20 \pm 31.35
C	Control	Unvac.	$\leq 4.0 \pm 0.0$	$\leq 4.0 \pm 0.0$	$\leq 4.0 \pm 0.0$	$\leq 4.0 \pm 0.0$	$\leq 4.0 \pm 0.0$	$\leq 4.0 \pm 0.0$

N.B.: Gr. = Group, vac. = vaccination, Unvac. = unvaccinated, pre-vac. = pre-vaccination

Table 2. Cultural characteristics of *P. multocida* re-isolated from dead chicken after challenge test

Sources of isolates	Colony characteristics on different media					
	Blood agar	Nutrient agar	MacConkey's agar	EMB agar	S-S agar	Nutrient broth
Suspected dead chicken	Whitish, opaque, circular, translucent in appearance and no hemolysis	Whitish, opaque, circular, translucent appearance	No colony appear	No colony appear	No colony appear	Diffuse turbidity

**Figure 1.** Comparison of PHA titres (mean \pm SE) between the vaccinated indigenous chicken of Group-A (BAU-FCV) and Group-B (DLS-FCV)

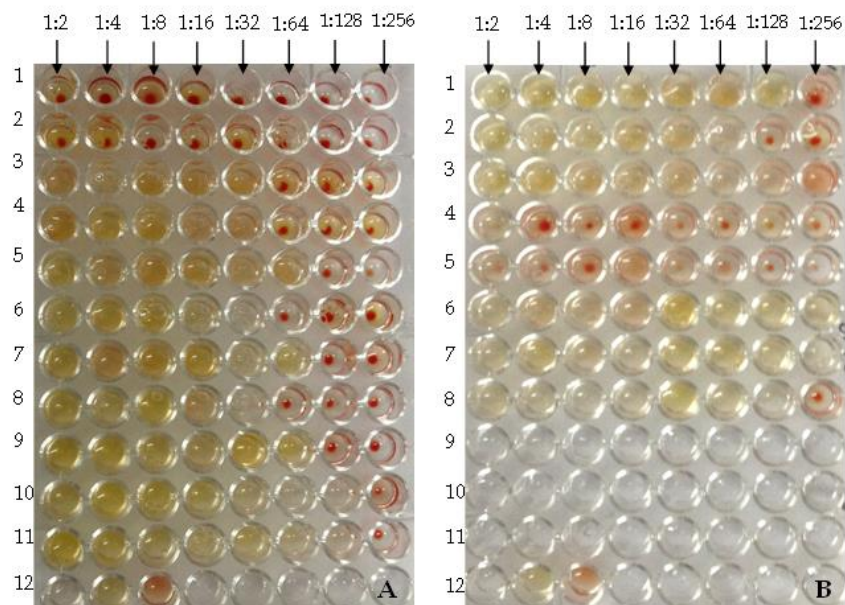


Figure 2. Microtitre plate showing PHA titres in indigenous chickens vaccinated with BAU-FC vaccine where (A) row 1, 2 for pre-vaccination titres; row 3, 4, 5 for 14 DPPV; row 6, 7, 8 for 28 DPPV; row 9, 10, 11 for 14 DPSV; row 12 for control; (B) row 1, 2, 3 for 42 DPSV; row 4, 5 for control titre; row 6, 7, 8 for post challenge and row 12 for control.

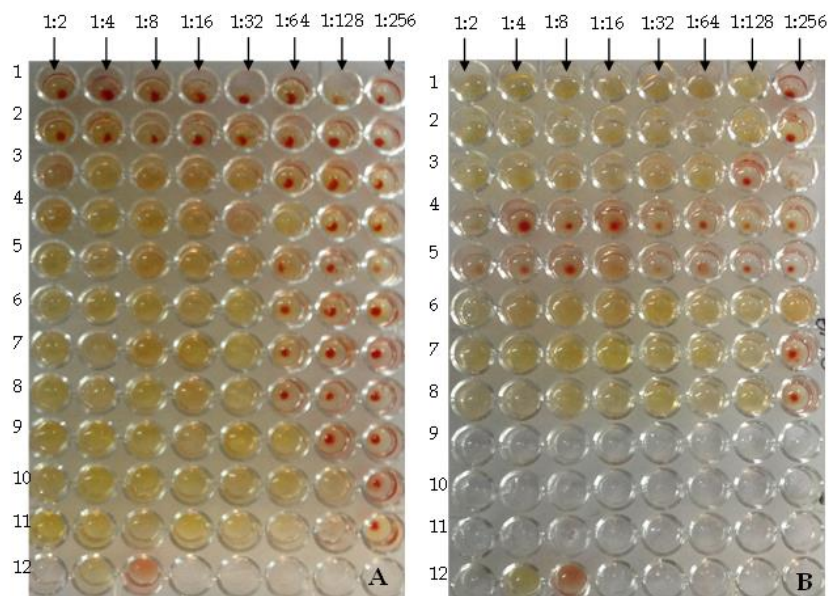


Figure 3. Microtitre plate showing PHA titres in indigenous chickens vaccinated with DLS-FC vaccine where (A) row 1, 2 for pre-vaccination titres; row 3, 4, 5 for 14 DPPV; row 6, 7, 8 for 28 DPPV; row 9, 10, 11 for 14 DPSV; row 12 for control, (B) row 1, 2, 3 for 42 DPSV; row 4, 5 for control titre; row 6, 7, 8 for post challenge and row 12 for control.

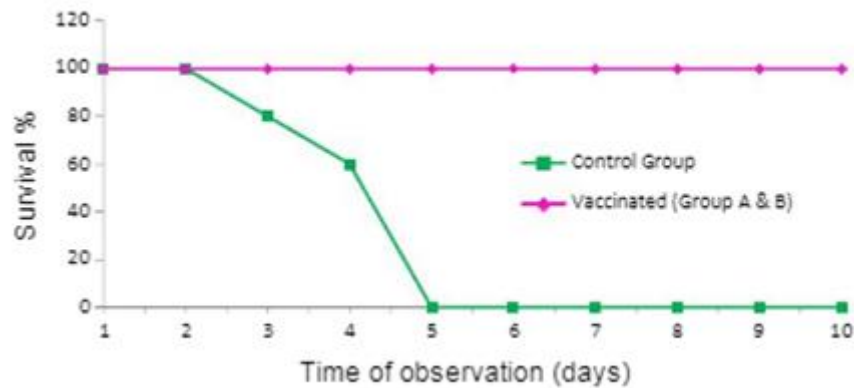


Figure 4. Survivability of vaccinated and unvaccinated chicken (5 of each group) after challenge infection

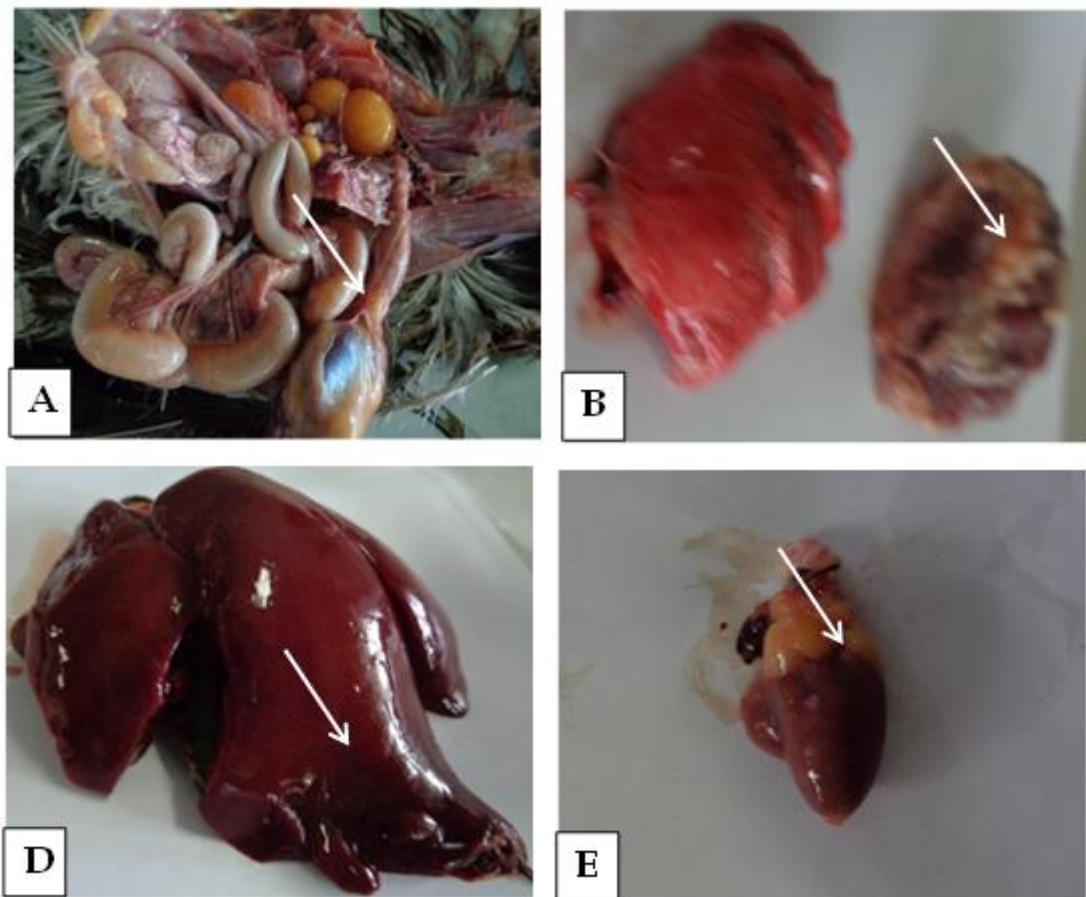


Figure 5. (A-D) Post-mortem examination of dead bird of control group (Group-C) was performed as per the procedures described in materials and methods section. (A) Blood vascular congestion in intestine. Death of egg laying chickens, (B) Congestion in one portion of lung, (C) Congestion and Necrotic foci observed on the surface of enlarged liver, (D) Petechial hemorrhage at the base of the heart

CONCLUSIONS

It might be assumed from the findings of present research work that BAU-FC vaccine induces relatively better immune response compare to DLS-FC vaccine. Both BAU-FCV and DLS-FCV are able to protect indigenous chicken from the outbreak of avian pasteurellosis and will help in the loss of farmers by decreasing the mortality rate.

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