Bangl. J. Vet. Med. (2018). 16 (2): 131-136

Received: 26-08-2018, Accepted: 11-11-2018

DOI:https://doi.org/10.33109/bjvmjd1801

### Comparative efficacy of BAU-Fowl cholera and DLS-Fowl cholera vaccines in indigenous ducks

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# Abstract

#### Background

Duck cholera is an acute, fatal, septicemic disease of domestic ducks which is responsible for significant loss in duck population. The present study was conducted to compare the immunogenicity of two formalin killed fowl cholera vaccines (BAU-FCV and DLS-FCV) in indigenous ducks.

# Methods

The experimental ducks were divided into three groups (A=15, B=15 and C =10 ducks) of which birds of Group A and Group B were inoculated with 0.5 ml of BAU-FCV and DLS-FCV, respectively through subcutaneous route at the age of 10 weeks whereas ducks of group C were kept as unvaccinated control. Booster vaccination was done with same dose and route at 14 weeks of age. Challenge infection was conducted after 2 weeks of booster vaccination.

## Results

The mean PHA antibody titres on 15 days post vaccination (DPPV), 28 DPPV, 15 days postsecondary vaccination (DPSV), 28 DPSV and 15 days post challenge were  $25.60 \pm 3.92$ ,  $51.20 \pm 7.84$ ,  $89.60 \pm 15.68$ ,  $166.40 \pm 38.40$  and  $204.80 \pm 31.35$ , respectively in ducks of Group A whereas, the mean antibody titres in ducks of Group B were  $25.60 \pm 3.92$ ,  $44.80 \pm 7.84$ ,  $64.00 \pm 7.53,102.40 \pm 15.68$  and  $179.20 \pm 31.35$  at 15 DPPV, 28 DPPV, 15 DPSV, 28 DPSV and 15 days after challenge, respectively. In this investigation, slightly higher immune responses were observed in ducks of Group A vaccinated with BAU-FCV compare to ducks of Group B vaccinated with DLS-FCV. Birds of both vaccinated groups conferred 100% protection against challenge infection with virulent *Pasteurella multocida* whereas, 100% mortality was observed in control ducks after challenge.

## Conclusion

Both vaccines were found to be safe and effective for the vaccination of indigenous ducks against duck cholera.

**Key words:** Duck cholera, DLS-FCV, BAU-FCV, Indigenous ducks, Comparative efficacy, Formalin killed vaccine

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#### Introduction

Bangladesh is one of the least developed countries having large population and small land area. More than 31% of its people still live below poverty line. Duck comprises about 16% (42.68 million) of the total poultry population (270.71 million), occupying second place next to chicken in the production of table eggs in this country (BER, 2010). Among the Asian countries, Bangladesh is in 11<sup>th</sup> and 4<sup>th</sup> position with respect to duck meat and egg production, respectively (Pingel, 2011). Duck provides hard-cash income and creates employment opportunities for the rural farmers and landless women. Indigenous poultry has already proved itself as a source of potential income generation and poverty alleviation, improvement of a human nutrition through the supply of meat and eggs and also contributes 2.73% of GDP (Economic Survey, 2009) in Bangladesh. There are many rivers, lakes, bills, ponds and other lowlands facilities for duck rearing in Bangladesh. The farmers live in the sides of rivers, canals, haors and bills prefer to rear ducks due to fact that the cost of feeding are very cheap. Ducks are also found relatively resistant to infectious disease compared to the chicken (Ahmed et al., 1986: Hossain et al., 2005; Hoque et al., 2011). Duck cholera, caused by Pasteurella multocida is one of the important duck diseases responsible for mortality (Akter et al., 2004) and is mainly prevented by vaccination in Bangladesh (Islam et al., 2005: Rana et al., 2010). A number of vaccination programme have so far been undertaken with bacterium to control this disease. Khan et al. (1997) reported that a safe and sterile vaccine could protect 40% in single vaccinated and 80% in double vaccinated birds when challenged with one infective dose. At present, fowl cholera vaccine are being prepared at Bangladesh Agricultural University, Mymensingh (BAU-FCV) and Livestock Research Institute (LRI-FCV), and made available in the local market for field use in ducks (Samad, 2000). Considering the above points, the whole research work was conducted to determine the antibody titres and protective efficacy of BAU fowl cholera and DLS fowl cholera vaccines in indigenous ducks reared in traditional system.

#### **Materials and Methods**

#### **Experimental ducks**

A total number of forty 10 weeks of aged indigenous ducks reared in free ranging system by the village women of Lakkhipur, Gouripur, Mymensingh were selected for the present study. Ducks were divided into Group A, B contained 15 birds each and C where contained 10 birds. Indigenous ducks were reared in the village up to booster vaccination and brought to the experimental shed of Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh for challenge experiment. The research protocol was approved by the ethical committee of Faculty of Veterinary Science, BAU, Mymensingh-2202.

#### Vaccines

Two types of vaccine namely BAU-fowl cholera vaccine (BAU-FCV) produced at Livestock and poultry Vaccine Research and Production Centre (LPVRPC), BAU, Mymensingh obtained from department of Microbiology and Hygiene, BAU, Mymensingh and Department of Livestock Services fowl cholera vaccine (DLS-FCV) produced at Livestock Research Institute, Mohakhali, Dhaka purchased from Upazila livestock office, Mymensingh were used in this experiment.

# Experimental immunization of indigenous ducks with fowl cholera vaccines

Ducks of Group-A were vaccinated with 0.5 ml of BAU fowl cholera vaccine through subcutaneous route, Group-B with 0.5 ml LRI fowl cholera vaccine through subcutaneous route, while ducks of Group-C were kept as unvaccinated control. All the ducks of both vaccinated groups were boosted with same vaccine, dose and route at 14 weeks of age.

# Challenge to the vaccinated and unvaccinated ducks

Five ducks each of vaccinated and control groups were challenged with virulent *P. multocida*  $(3.6 \times 10^6 \text{ cfu/ml})$  through oral route. Ducks were observed daily up to 10 days for any clinical signs and symptoms of FC.

#### Collection of serum from immunized and nonimmunized ducks

Pre-vaccinated sera samples were collected at 10 weeks of age. The vaccinated sera samples were obtained at 12 and 14 weeks of age (after primary vaccination), on 16, 18 and 21 weeks of age (after booster vaccination) of ducks. Sera samples from control ducks were also obtained at the same time. Each serum was transferred into sterile eppendorf tube and preserved at -20°C until used.

#### Post-challenge isolation of bacteria

Within 5 days of challenge exposure, the control ducks were died and vaccinated ducks survived. Tissue samples were taken from liver, lung, heart and spleen from dead birds and inoculated in nutrient broth. The broth containing organisms were streaked on to blood agar plates after overnight incubation at 37°C for overnight. The plates were examined after 24 hours of incubation at 37°C for the growth of *P. multocida*. The positive cases were confirmed by the usual standard procedure described by Marchant and Packer (1967).

#### Passive haemagglutination (PHA) test

Sera samples collected from birds of all groups were tested by microplate PHA test after 2 and 4 weeks of primary and booster vaccination to determine the antibody titres in vaccinated indigenous ducks as per the method of Hossain *et al.* (2005) and Rana *et al.* (2010).

#### **Results and Discussion**

The mean PHA titre was  $\leq 4.00 \pm 0.00$  in all vaccinated and control ducks throughout the study period which is similar to the findings of Mondal *et al.* (1988) and Sultana *et al.* (2013). The mean antibody titres were  $25.60 \pm 3.92$ ,  $51.20 \pm 7.84$ ,  $89.60 \pm 15.68$ ,  $166.40 \pm 38.40$  and  $204.80 \pm 31.35$  at 15 DPPV, 28 DPPV, 15 DPBV, 28 DPBV and 15 days after challenge, respectively in ducks of Group A (Table 1, Fig. 1) whereas, the mean antibody titres in ducks of Group B were  $25.60 \pm 3.92$ ,  $44.80 \pm 7.84$ ,  $64.00 \pm 7.53$ ,  $102.40 \pm 15.68$  and  $179.20 \pm 31.35$  at 15 DPPV, 28 DPPV, 28 DPPV, 15 DPBV and 15 days after challenge, respectively in ducks of Group A (Table 1, Fig. 2). The increases in PHA titres after primary

vaccination following booster vaccination up to challenge were also observed by Wu *et al.* (1986) and Sultana *et al.* (2013). Highest PHA titre (166.40  $\pm$  38.40) was observed after 4 weeks of booster vaccination with BAU-FCV compare to DLS-FCV (102.40  $\pm$  15.68) (Table 1). This indicates that BAU-FCV induces better immune response compare to DLS-FCV.

In previous study, most of the challenges were done through intramuscular or subcutaneous routes. But in the present study, all the selected indigenous ducks of vaccinated and nonvaccinated groups were challenged with virulent P. multocida isolate through oral route considering the point that in the field condition ducks usually get the infection through oral route. Both groups of vaccine conferred 100% protection to vaccinated ducks while all the unvaccinated control ducks became infected and died following challenge infection (Table 2). The clinical signs were first observed at 6 hours PI that included dullness and depression. At 12 hours PI was dullness, depression, slight rise of body temperature. The manifested clinical signs at 24 hours PI consisted of severe weakness, drowsiness, anorexia, rise of body temperature, increased respiratory rate, lameness, whitish (chalky) diarrhea with mucus. At 96 hours the clinical signs were related with signs of chronic infection. The other signs included anorexia, lameness, and greenish diarrhea with mucus, subnormal temperature, decreased respiratory labored breathing, emaciation rates. and dehydration. Similar type of findings was also observed by Sharma et al. (1974), Gordon and Jordan (1985) and Rhoades and Rimler (1990). Finally all the control ducks died at 5 days PI (Table 2). Liver and blood from heart of dead ducks were collected as sample followed by reselective agar media isolation on and identification by staining cultural, and biochemical properties. All the findings were similar with the findings of Kamruzzaman et al. (2016) which confirmed the re-isolate as P. multocida.

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Table 1. Comparison of mean PHA antibody titres of sera collected from indigenous ducks vaccinated with BAU-FCV and DLS-FCV

Group	Vaccine	Dose and	Mean PHA titres					
	used	Route of vaccination		After 4 weeks	After 2 weeks of booster	After 4 weeks of booster	After 2 weeks of challenge	
		vaccination	of primary vaccination	of primary vaccination	vaccination	vaccination	experiment	
А	BAU-FCV	0.5 ml/SC	25.60 ±3.92	51.20±7.84	89.60±15.68	166.40±38.40	204.80±31.35	
В	DLS-FCV	0.5 ml/SC	25.60±3.92	44.80±7.84	64.00±7.53	102.40±15.68	179.20±31.35	
C	Unvaccinated control		≤4.0±0.00	≤4.0±0.00	≤4.0±0.00	≤4.0±0.00	≤4.0±0.00	

PHA: Passive hemagglutination, DLS-FCV: Department of Livestock Services fowl cholera vaccine, BAU-FCV: Bangladesh Agricultural University fowl cholera vaccine

Table 2. Results of challenge experiment

Group	Route of	Total	Number of birds	Number of	Percentages of	Percentages of
	challenge	birds	of survivability	birds of dead	survivability	dead birds
А	Oral	5	5	0	100%	0%
В	Oral	5	5	0	100%	0%
С	Oral	5	0	5	0%	100%

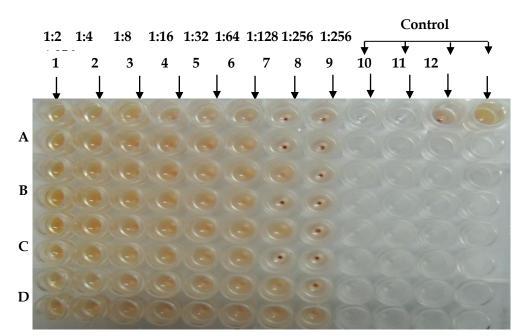


Figure 1. Passive hemagglutination test for the detection of antibody titres in ducks of Group-A vaccinated with BAU-FCV. Row A, B, C and D: dilution of serum collected after 2 weeks of booster vaccination; E, F, G and H: dilution of serum collected after 4 weeks of booster vaccination.

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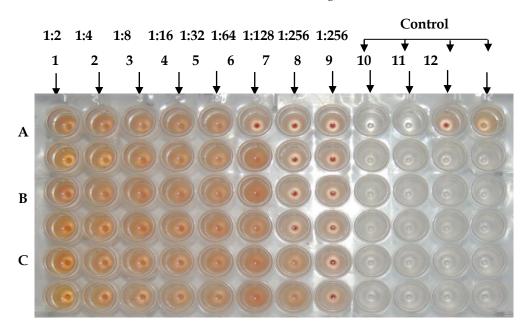


Figure 2. Passive hemagglutination test for the detection of antibody titres in ducks of Group-B vaccinated with DLS-FCV. Row A, B and C dilution of serum collected after 2 weeks of booster vaccination; D, E and F dilution of serum collected after 4 weeks of booster vaccination.

#### Conclusions

The study indicated that both BAU-FCV and DLS-FCV revealed good protection to indigenous ducks reared in free ranging system. Vaccinating ducks with fowl cholera vaccine will reduce mortality rate and economic loss of the farmers.

#### Acknowledgement

Not Applicable

#### **Competing Interest**

The authors declare that they have no competing interests

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