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Comparative efficacy of fowl cholera vaccines in ducks

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ABSTRACT

An experiment was conducted to determine the comparative efficacy of fowl cholera vaccines in ducks prepared at BAU, (FCV-BAU) Mymensingh with that of FC vaccine prepared at Livestock Research Institute, (FCV-LRI), Dhaka, using SC route of inoculation. For this 22 weeks aged ducks of Xinding breed was divided into three groups such as A, B, and C, of which group A was inoculated with FCV-BAU and group B with FCV-LRI, while group C was kept as unvaccinated control. Each bird received initially 1 ml of Fowl cholera vaccine administered SC followed by a booster dose given with the similar dose and route at 15 days interval. The birds were reared separately in duck shed of the department with recommended feed and other managemental requirements as well as maintenance of proper biosecurity. Pre-vaccination sera were collected from all the groups of ducks. Sera of the immunized and control ducks were collected at 15 days of primary vaccination and then 28, 35, 42, 49 and 56 days post vaccination. The degree of immunity produced in each vaccinated groups of ducks following primary and secondary vaccination were measured by using PHA test. In case of group A, the mean PHA antibody titers were 56±8.00, 112±16.00, 160±32.00, 96±18.47, 64±0.00 and 32±0.00, and in group B; 48±9.24, 96±18.47, 128±0.00, 80±16.00, 48±9.24, and 32±0.00 at 15, 28, 35, 42, 49 and 56 days of vaccination respectively.. In group C (control) the PHA titre was <4.0±0.00. The protective potential efficacy of fowl cholera vaccine was also measured by determining the rate of survivability of the birds of each vaccinated group by challenge exposure at 5 weeks post vaccination. Ducks of group A and group B conferred 100% protection while the unvaccinated controlled ducks succumbed to such infection. The PHA titres obtained from different groups of ducks were analyzed by T-test to determine the protective capacity of vaccinated ducks against challenge exposure. It was demonstrated that experimentally fowl cholera vaccine conferred 100% protection ($p<0.01$) against challenge infection. The study suggests Fowl cholera vaccines FCV-BAU and FCV-LRI are safe and effective for the vaccination of ducks against duck cholera.

Keywords: Fowl cholera vaccine, duck and efficacy.

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INTRODUCTION

Duck cholera also known as “avian cholera”, “avian pasteurellosis”, and “avian hemorrhagic septicemia” is one of the earliest reported bacterial disease caused by infection with *Pasteurella multocida (P. multocida).*

P. multocida is a Gram-negative non-motile, non-spore forming rod shaped organism occurs singly or in pairs and occasionally as chains or filaments. A capsule can be demonstrated in recently isolated cultures using of indirect methods of staining. Dextrose starch agar with 5% avian serum is an excellent medium for isolating and growing *P. multocida*. (Calnek et al., 1997).

The clinical signs of duck cholera are anorexia, fever, ruffled feathers, mucus discharge from mouth, rapid respiration and diarrhoea which is watery to yellowish initially and greenish with mucus finally (Rhoades and Rimler, 1990). The

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disease may appear as acute septicemic or chronic cases with localized infection and high morbidity and mortality (Rhoades, 1991).

Duck cholera is a disease of economic importance which occurs sporadically or enzootically all over Bangladesh causing about 25% to 35% of mortality in chickens and ducks (Choudhury et al., 1985). The disease primarily affects adult birds (less than 8 weeks of age) including chickens, ducks, turkeys and geese. This malady is encountered in most countries of the world including Bangladesh causing huge economic loss to the poultry raisers (Pande et al., 1981; Choudhury et al., 1985; Mustafa and Miah, 1988; Kamal et al., 1988; Baki et al., 1991).

Various vaccination programs have been reported 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. Vaccination along with strict biosecurity and proper nutrition can prevent the epornitics of diseases. Supar et al. (2001) prepared killed monovalent, bivalent and polyvalent aluminium hydroxide gel adjuvanted fowl cholera vaccines using local isolates of *P. multocida*. These authors reported that the local isolates of P. multocida would be useful for producing effective fowl cholera vaccine. Vaccination as a means of prevention of fowl cholera has been reviewed by (Collins, 1977).

Vaccines against Fowl cholera are being prepared in Bangladesh Agricultural University (BAU-FCV) and Livestock Research Institute (LRI-FCV) and made available in the local market for field use in ducks (Samad, 2000). However, limited research works on the efficacy particularly in ducks has been carried out. Therefore, the present study was undertaken to study the immune status of ducks following sub-cutaneous route of vaccination and to determine the comparative efficacy of locally prepared fowl cholera vaccines in ducks.

MATERIALS AND METHODS

Experimental ducks

A total of 10 twenty-two-weeks old Xinding breeds of ducks with history of no vaccination or infection with fowl cholera were purchased from Govt. poultry farm, Kishoregonj for this experiment. The birds were reared in the duck shed of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh. The birds were provided with recommended feed and other managemental requirements with maintenance of proper biosecurity.

Vaccines

Fowl Cholera vaccine produced by Poultry Biologics Unit (FCV-BAU, Mymensingh) and other vaccine (FCV-LRI) prepared by Livestock Research Institute, Mohakhali, Dhaka were used in this study.

Media

For cultivation of *P. multocida* Blood agar (BA), Nutrient Agar (NA) and Nutrient Broth (NB) are used. The yeast extract and beef extracts were also used with NB.

Stock culture of *P. multocida:*

A virulent local isolate of *P. multocida* (PM-38) serotype 1 (X-73) was collected from the stock culture slant of the laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh. This isolate was used for the preparation of fowl cholera vaccines.

Laboratory animals

Rabbit

Apparently healthy adult white rabbit was used for the collection of sera and used for PHA test. The animal was reared under recommended feed and other managemental requirements in the animal shed of the Department of Microbiology and Hygiene, BAU, Mymensingh.

Mice

The healthy suckling day-old mice were used in this study. The mice were kept in Animal shed of the Department of Microbiology and Hygiene.

The Experiment

The Xinding breeds ducks of 22 weeks aged were selected for this research. The ducks were divided into two groups such as vaccinated group and unvaccinated control group. The vaccinated group was again subdivided into two subgroups like group A and group B. Pre-vaccinated sera were collected to determine the status of ducks. The ducks of groups A were primarily vaccinated with the dose of 1ml 5×10^7 CFU / duck through SC route by FCV-BAU. The ducks of group B were vaccinated at the dose rate of 1 ml / duck through SC route by using FCV-LRI. Primary vaccinated sere were collected at 15 days of vaccination. Booster dose was given after 15 days of primary vaccination with similar dose and route in both Group A and Group B (Table 1). Post-vaccinated sera were collected at 28, 35, 42, 49 and 56 days of vaccination (DPV). The degrees of antibody level of pre-vaccination and post-vaccination sera were determined by passive haemagglutination (PHA) test. Protection test was carried out with vaccinated and control groups after 42 days of secondary vaccination.

Table 1
Experimental design for efficacy trial of Fowl Cholera Vaccine (FCV) in ducks.

Group of ducks	Name of vaccine	No. of ducks used	Dose of vaccine used per ducks	Vaccination			
				Primary vaccination		Booster vaccination	
				Route of vaccination	Age of ducks	Route of vaccination	Age of ducks
A	FCV, BAU	04	1 ml	SC	22 weeks	SC	24 weeks
B	FCV, LRI	04	1 ml	SC	22 weeks	SC	24 weeks
C	Control	02		Not done			

FCV- BAU = Fowl Cholera Vaccine prepared by Bangladesh Agricultural University
FCV- LRI = Fowl Cholera Vaccine prepared by Livestock Research Institute
SC = Sub-cutaneous

Vaccination of the ducks

Fowl cholera vaccine was administered at the dose rate of 1 ml through SC route in each selected groups of ducks of groups A and B. Booster dose was administered with the same dose and via same route after 15 days of primary vaccination in both the groups A, and B respectively.

Collection of serum

Duck serum was collected from the jugular vein of vaccinated ducks of each group according to the procedure performed by Siddique et al. (1997). Rabbit serum was collected was collected either from marginal ear vein or central ear artery as referred by Hudson and Hay (1989).

Inactivation of serum

Normal rabbit serum was first inactivated at 56^0C for 30 minutes in hot water bath and then one ml of the inactivated serum was added with 99 ml of PBS (p^H 7.2) in a conical flask to obtain 1% solution. The serum solution was also kept in refrigerator at 4^0C to 8^0C until used. This was carried out according the procedure of Siddique (1997). The stored serum samples were inactivated at 56^0C for half an hour in a hot water bath before using in PHA test following the procedure of Siddique (1997).

Inactivation of complement

Inactivation of complements was made according to Choudhury et al. (1985). The sera collected from experimentally immunized chickens with formalin killed fowl cholera vaccine and were heated at 56^0C for an hour in water bath in order to inactivate the collected sera.

Estimation of colony forming unit (CFU)

Colony forming unit was calculated according to the method described by Michael et al. (1979). For this purpose, a serial 10 fold dilutions ranging

from 10^{-3} to 10^{-7} of 1 ml NB culture of *P. multocida* were prepared in sterilized PBS solution. Five BA plates were inoculated with 0.1 ml of each dilution. The inoculum was spread along the entire surface of the plates so that the edge of the plates remained free. The plates were incubated at 37^0C in bacteriological incubation for 24 hours. The colonies were counted after incubation. The average of the colonies from five plates were calculated and expressed as CFU per ml of inoculum.

Preparation of capsular antigen

For performing the PHA test the capsular antigen of *P. multocida* was prepared according to the method suggested by Choudhury et al. (1987). The fresh subculture of *P. multocida* was diluted with PBS and heated at 56^0C for 30 minutes in hot water bath to assist the removal of the capsular antigen. After heating, the suspension was centrifuged at 4500-6000 rpm for 20 minutes by using coarse stone bids. The supernatant was considered as a source of capsular antigen.

Challenge infection

Both the vaccinated and control group of ducks were subjected to challenge by IM administration of a virulent field isolate of *P. multocida* (PM-38) serotype 1 (X-73) following the procedure of Choudhury et al. (1987). The challenge inoculum contain 5×10^7 colony forming unit (CFU) and the infection was done in each group after 42 days of boostering and at the same time in unvaccinated control group. In view of contagious nature of *P. multocida*, strict biosecurity was followed until end of protection test.

Post-challenge observation of birds

Birds after challenge infection were observed daily upto one week for any clinical signs and symptoms of fowl cholera. The clinical findings of both the vaccinated and unvaccinated chickens were observed and recorded.

Determination of humoral immune response (HIR) in ducks

PHA test was used to determine titres of antibodies in duck serum following vaccination and comparative efficacy of locally prepared fowl cholera vaccines in ducks. PHA test was performed according to the method described by Tripathy et al. (1970) and Siddique et al. (1997) with modification.

Horse red blood cells (2.5% HRBC) suspension was prepared according to the method described by Siddique et al. (1997). Tannic acid treatment of HRBC was accomplished according to the method described by Tripathy et al. (1970a and 1970b). Horse red blood cells (HRBC) were tanned by an equal volume of 1: 20,000 dilution of tannic acid and 2.5% packed horse red blood cells (10 ml of 2.5% HRBC and 10 ml of 1: 20,000 dilution of tannic acid) were mixed thoroughly and the mixture was then incubated at 37^0C for 10-15 minutes in water bath. Afterwards the cells were centrifuged at 1500 rpm for 10 minutes, the sediment was then washed with PBS. Washed tanned HRBC so obtained was again diluted to make 2.5% suspension with PBS and used for the test.

Sensitization of capsular antigen with tannic acid treated horse red blood cells (coating) for performing PHA test were carried out according to the method described by Tripathy et al. (1970a and 1970b). About 3ml (2.5%) of sensitized horse red blood cells, 1 ml of a 1: 10 dilution of capsular antigen and 8ml PBS were mixed thoroughly. This mixture was then incubated at 37^0C for 20-30 minutes. After sensitization, the cells were centrifuged for 10 minutes at 1500 rpm, then the supernatant fluid was decanted to discard and the sediment HRBC were collected and diluted with 1% normal rabbit serum diluent (NRSD) at the ratio of 1: 4. This was then mixed thoroughly and was kept at room temperature for an hour and then centrifuged for 10 minutes. The cells were resuspended in 1% NRSD to make 0.5% sensitized cells for use in microtitre plate method and stored at 4^0C until used.

RESULTS AND DISCUSSION

In the present study, duck cholera vaccine (DCV) was administered at the dose rate of 1 ml of 5×10^7 CFU through SC at the neck region in each group

of A and B. Booster dose was given with the same dose via same route at 15 days interval of primary vaccination. OIE Manual (2000) and Choudhury et al. (1985) suggested that fowl cholera vaccine should be given through IM or SC route. Derieux (1978) and WU et al. (1986) suggested that for production of effective immunity, two doses of vaccine were required with an interval of two to four weeks. They also reported that inactivated vaccines could not produce full immunity until approximately two weeks after the second dose of a primary vaccination. Leonchuk and Tsimokh (1977) administered dual dose of emulsified fowl cholera vaccine through IM or SC route at weekly interval and recorded that a schedule resulted in increase of serum gamma-globulins and long lasting strong immunity in IM dosing compared to SC.

Passive haemagglutination assay (PHA) test

Passive haemagglutination test was conducted to determine the humoral immune response of Xinding ducks having been inoculated at 22 weeks of age as per the method described by Carter (1955) and Chang (1987) but slight modification was done as suggested by Mondal et al. (1988a), Sarker et al. (1992) and Siddique (1997).

Two groups such as A and B were vaccinated while group C was maintained as unvaccinated control. Pre vaccination sera samples were obtained from ducks of all the groups of ducks. Post vaccination sera samples were collected from ducks of each group at 15, 28, 35, 42, 49 and 56 days of vaccination.

The pre-vaccination PHA titres of sera samples showed a mean of <4.0±0.00 (Table 2). After primary vaccination, there was a slight rise of serum PHA titres in each group which ranged from 64 to 128 (Table 2). Secondary dose triggered the production of PHA titres in each groups and was found to be ranging from 128-256 (Table 2). Thus, the mean PHA antibody titers in group A were 56±8.00, 112±16.00, 160±32.00, 96±18.47, 64±0.00 and 32±0.00 and in group B there; 48±9.24, 96±18.47, 128±0.00, 80±16.00, 48±9.24, and 32±0.00 (Figure 1) while in group C served as control birds the PHA titre was <4.0±0.00 at 15 DPV, 28 DPV, 35 DPV, 42 DPV, 49 DPV, and 56 DPV respectively (Table 3). The bar diagram and linear representation of the antibody titers of the vaccinated ducks are also presented in figure 9 and 10 respectively.

Table 2
PHA titres of sera of duck vaccinated primary and secondary dose of vaccines.

Groups	Type of Vaccines	Route of inoculation	Tag no.	Pre-vaccination serum titre	Post vaccination serum titre					
					Primary dose	Secondary dose				
				0 day	15 DPV	28 DPV	35 DPV	42 DPV	49 DPV	56 DPV
A	FCV-BAU	SC	01	< 4	64	128	128	64	64	32
			02	<4	64	128	256	128	64	32
			03	<4	64	128	128	128	64	32
			04	<4	32	64	128	64	64	32
B	FCV-LRI	SC	01	< 4	64	64	128	128	64	32
			02	<4	32	128	128	64	32	32
			03	<4	64	128	128	64	64	32
			04	<4	32	64	128	64	32	32
C	Nil	Not done	01	< 4	<4	<4	<4	<4	<4	<4
			02	<4	<4	<4	<4	<4	<4	<4

SC= Subcutaneously; PHA = Passive Haemagglutination Assay; DPV= Days Post Vaccination

Table 3
Mean PHA titers of sera of Ducks vaccinated with BAU and LRI Fowl Cholera vaccine as determined by T-test.

Groups	Secondary Vaccination Intervals	Mean PHA±SE						
		0 day	15 DPV	28 DPV	35 DPV	42 DPV	49 DPV	56 DPV
A	15	<4±0.00	56±8.00	112±16.00	160±32.00	96±18.47	64±0.00	32±0.00
B	15	<4±0.00	48±9.24	96±18.47	128±0.00	80±16.00	48±9.24	32±0.00
C	_	<4±0.00	<4±0.00	<4±0.00	<4±0.00	<4±0.00	<4±0.00	<4±0.00

PHA= Passive Haemagglutination Assay; DPV= Days Post Vaccination; PVS=Pre-vaccination sera, SE= Standard error.

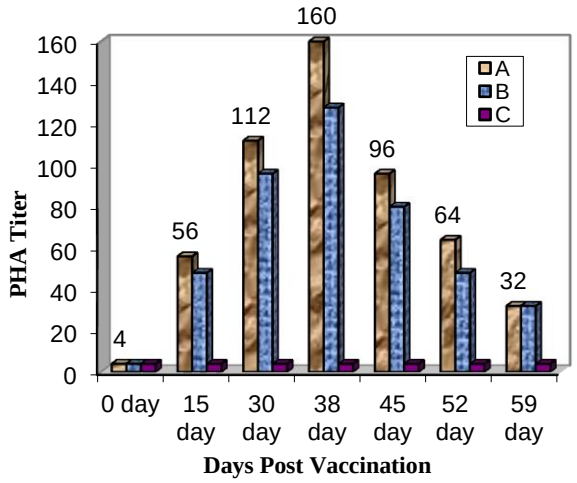

Figure 1
Comparison of pre and post vaccination PHA titres at different durations in group A, B and C.

The pre-vaccination PHA titres of sera samples of birds both the groups were found to be a mean of <4.0±0.00 which was closely related with Mondal et al. (1988).

The primary vaccination induced slight rise of PHA titres of 64 and in some cases a titre of 128 at 15 days of post-vaccination in birds of both the group of A and B (Table 2). The findings were closely related with Coates et al. (1977) and Mondal et al. (1988).

In group A birds vaccinated SC with FCV-BAU, secondary dose was given after 15 days of primary vaccination. The antibody titres of 128-256 was found to be highest which sustained up to 35 days and then the titre started to decline gradually but continued to maintain dependable immunity up to 2 month of post vaccination (Table 2) shown in figure 1.

In group B vaccinated SC with FCV-LRI secondary dose was also given after 15 days of primary vaccination. The PHA titres gradually increased and reached up to 64. Boostering at this stage elucidated a rapid increase of PHA titres of 128 and continued to remain at a dependable immunity up to two month of post vaccination (Table 2).

From the present study it is observed that 15 days boostering interval produced dependable immune response in ducks vaccinated with fowl cholera vaccine of either BAU or LRI in group A and B respectively shown in figure 1.

The findings of this study in respect of impetuous production of PHA titres were similar with the observation of Collins (1977), Dua and Maheswaran (1978), Kodama et al. (1983) and Mondal et al. (1988). These authors reported that inoculation of single dose of fowl cholera vaccine resulted in slight detectable rise of antibody titres and introduction of second dose of vaccine seven days later brought about an increase in such titers. In this respect, Choudhury et al. (1987) observed that immune response of birds following single and dual vaccination indicated that dual vaccination at two weeks interval were more effective than single vaccination.

Mondal et al. (1988) reported that sera possessing PHA titre of 32-64 at the end of 2nd weeks of primary vaccination had a triggering effect at that stage following another dose of vaccine which was found to be highest ranging from 128-256 at the

end of 7th week post vaccination. Thereafter, PHA titre started to decline gradually from the 9th week and continued up to 25 weeks post vaccination.

Sarker et al. (1992) observed that PHA titre of sera of birds following alum-precipitated fowl cholera vaccinated chickens remained at 128 or above up to 18 weeks post vaccination and there after the level decreased spontaneously and the titre was 128 and 64 in 83% and 17% of the sera tested at 22 weeks post vaccination respectively.

It was observed that there was a slight variation in the pattern of antibody production in group A and group B where the titre was slightly higher in group A (Table 3). The reason for such happening might be due to individual antigenic variation among the vaccine strain.

Protection test

Both vaccinated and control group of ducks were subjected to challenge by IM rout with a virulent field isolate of *P. multocida* (PM-38) serotype 1 (X-73), following the procedure of Choudhury et al. (1987). The challenge inoculum contains 5×10^6 colony forming unit (CFU)/ml. The challenge infection was done in each group after 15 days of boostering and at the same time in unvaccinated control group. The experimental vaccine of fowl cholera conferred 100% protection of ducks while all the unvaccinated control birds succumbed to such infection (Table 4).

The PHA titres were analyzed by T-test to determine the protective capacity of vaccinated birds against challenge exposure. It was demonstrated that experimental duck cholera vaccine conferred 100% protection ($p<0.01$) against challenge infection after 42 days of secondary vaccination when unvaccinated control ducks were found to be affected with infection.

Results of challenge exposure (Table 4) demonstrated that the alum-precipitated duck cholera vaccine conferred 100% protection ($p<0.01$) against challenge infection when none of the unvaccinated control birds survived. Similar observation was recorded by Coates (1972), Bhasin and Biberstein (1968) and Mondal et al. (1988). Coates (1972) found 100 percent protection against challenge infection at 3 weeks of post vaccination following administration of fowl cholera vaccine at the dose of 1.2×10^7 CFU. Bhasin and Biberstein (1968) found 60 percent survivability against challenge infection of alum-precipitated fowl cholera vaccinated birds at 16 week post vaccination. However, Mondal et at. (1988) found 100% protection against challenge infection of an alum-precipitated fowl cholera vaccinated birds at 5th week post vaccination.

Clinical findings of post challenge in ducks

Control birds showed characteristic clinical signs of acute pasteurellosis. The clinical signs included dullness, depression, hyperthermia, and rapid respiration, followed by lameness and whitish diarrhoea with mucus and ultimately death but vaccinated flock did not show such abnormalities.

The stimulation of a high degree of immunity induced by the fowl cholera vaccine under investigation might be due to higher immunogenic characters of the isolate (PM-38) as reported by Choudhury et al. (1988). Addition of alum as adjuvant might have further increased the immunogenicity of the vaccine. Bhasin and Biberstein (1968) reported that aluminum hydroxide was more effective as adjuvant inducing immunity against fowl cholera.

In post challenge observations, control birds showed characteristic clinical signs and symptoms of avian pasteurellosis like dullness, depression, anorexia, hyperthermia, labored breathing followed by lameness, whitish (chalky) diarrhoea and ultimately death occurred. Vaccinated birds protected themselves and did not show clinical signs except dullness, depression and drowsiness. Similar findings was observed by Sharma et al. (1974), Gordon and Jordan (1985), Rhoades and Rimler (1990).

Table 4
Rate of survivability at challenge infection.

Groups	Booster intervals	Challenge at post booster days	Total birds	No. of birds survived	No. of birds died	Percentage of survivability	Percentage of death
A	15	42	03	03	0	100	0
B	15	42	04	04	0	100	0
C	-	-	02	0	02	0	100

Sharma et al. (1974) described that hyperthermia, dullness, incordination of movements, greenish yellow diarrhoea, laboured and painful breathing and unusual sitting posture were the most prominent clinical symptoms in case of experimentally produced avian pasteurellosis.

Gordon and Jordan also (1985) observed that death of birds in good body condition was the only indication in some outbreaks of fowl cholera. The author observed that the ducks before the death remained in normal posture by keeping the weight of the body on the keel none and the beak. This posture has been considered as a characteristics sign of duck cholera (Baki et al. 1991).

Necropsy lesion of fowl cholera recorded in this study included blood vascular congestion, hemorrhagic enteritis, increased amount of pericardial and peritoneal fluid, hemorrhage in lungs and intestinal mucosa, swollen and congested liver and sometimes multiple necrotic foci were found on the parietal surface of the liver. These gross findings of fowl cholera recorded in ducks are in conformity with Ali (1974), Sharma et al. (1974), Matsumoto and Helfer (1978), Partadiredja et al. (1979), Park (1982), Choudhury et al. (1985), Wickramasighe and Peiris (1985), Fraser (1986), Kamal et al. (1988), Chakraborty et al. (1989), Baki et al. (1991), Khan (1994), Rimler and Glisson (1997) and Calnek et al. (1997). The isolation and identification of *P. multocida* organism from the heart blood and liver of dead ducks caused by challenged infection also confirmed that the challenged inoculum contained highly virulent organisms.

CONCLUSION

Fowl cholera vaccines FCV-BAU and FCV-LRI were safe and effective for the vaccination of ducks against duck cholera. Both the vaccines induced significant protection ($P<0.01$) in dual vaccinated birds following the same SC route at 15 days interval.

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