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Comparative immunogenicity of fowl cholera vaccine in Jinding ducks

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Abstract

This study compared the immunogenicity of alum-precipitated formalin-killed fowl cholera vaccines (BAU-FCV and LRI-FCV) in Jinding ducks. The ducks were divided into three groups (A = 14, B = 14, C = 12). Group A was inoculated with BAU- FCV 0.5 mL and group B with LRI- FCV 1.0 mL intramuscularly (im) at the age of six weeks and group C served as unvaccinated control. Booster vaccination was administered similarly at 11 weeks of age in groups A and B. Challenge infection was given to all birds two weeks after booster vaccination. Passive Haemagglutination Assay (PHA) antibody titres in group A were 59.4 ± 4.6 21 days after primary vaccination, 137.1 ± 21.8 15 days after booster vaccination, 100.6 ± 12.9 21 days after booster vaccination, and 256.0 ± 48.4 15 days after challenge. In group B, titres were 50.3 ± 6.5, 118.9 ± 9.1, 91.4 ± 12.9, 237.7 ± 51.7, respectively, whereas titres in group C remained at ≤4.0 ± 0.0. The antibody titres were insignificant when compared between pre-vaccination and 21 days after primary vaccination in both vaccinated groups (A and B). PHA antibody titres of groups A were significantly ($P < 0.0001$) increased at 15 days after booster and in case of group B the antibody titres were insignificant. At 15 days after challenge the antibody titres were highly significant in both groups (A and B). There was no significant difference between the two vaccinated groups. Following challenge infection with virulent *Pasteurella multocida* 88.9% of birds vaccinated with BAU-FCV, and 77.8% of birds vaccinated with LRI-FCV survived, while all unvaccinated birds died. Both vaccines were safe and effective. (*Bangl. vet*. 2013. Vol. 30, No. 2, 41 – 45)

Introduction

Ducks comprise about 10% of the total poultry population and occupy second place to chicken in the production of table eggs in Bangladesh (Khan *et al.,* 1999). Diseases constitute the major constraints causing economic loss (Das *et al.,* 2005). Fowl cholera is a major threat to poultry industry. This is caused by *Pasteurella multocida* and occurs sporadically or enzootically all over Bangladesh causing 25% to 35% mortality in chickens and ducks (Choudhury *et al*., 1985). Baki *et al*. (1991) observed that 11% of mortality of domestic ducks was due to fowl cholera. In order to control it, strict biosecurity and vaccination are essential. Vaccines are produced by Livestock Research Institute (LRI) and Bangladesh Agricultural University (BAU) to control fowl cholera in chickens and ducks. Field studies on such vaccines have been reported (Islam *et al*., 2004; Sukul *et al*., 2008; Rana *et al*., 2010). Jinding ducks were immunized by LRI-FCV subcutaneously (sc) and BAU-FCV intramuscularly (im), (Islam *et al*.,

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2004).

This study was conducted to evaluate the humoral immune response in Jinding ducks following im vaccination with Fowl Cholera Vaccine (FCV) prepared by Bangladesh Agricultural University (BAU), Mymensingh and Livestock Research Institute (LRI), Mohakhali, Dhaka.

Materials and Methods

Experimental ducks

A total of 40 day-old ducklings of either sex of Jinding breed were collected from a hatchery at Brahmanbari. The birds were reared in a small shed (Huque, 1991) with biosecurity and were divided into three groups, A, B and C consisting of 14, 14 and 12 ducks, respectively.

Immunization of ducks

Birds of group A were vaccinated with 0.5 mL (1.75×10^8 CFU/ mL) BAU-FCV, group B with 1 mL LRI-FCV and group C were unvaccinated controls. Booster dose was provided using same dose and route five weeks after primary vaccination.

Collection of blood and preparation of sera

Blood serum was collected as described by Siddique *et al.* (1997). About three mL of blood without anticoagulant was collected from the right wing vein of all ducks and the syringes were held in slanted position and blood was allowed to clot at room temperature for an hour; clots were detached from the wall of the syringe by pressing the piston and were kept overnight at 4°C for separation of the serum. Then serum was carefully removed and centrifuged at 2000 rpm for 10 minutes and stored at -20°C.

Determination of Passive Haemagglutination Assay (PHA) titres

The antibody titres were determined by PHA as described by Tripathy *et al.* (1970).

Challenge to the experimental ducks

After 28 days of booster immunization, all ducks were challenged with virulent field isolate of *P. multocida,* 10 LD_{50} of mice (0.25 mL containing 5.7×10^9 CFU/mL) injected into thigh muscle. These were observed daily at three-hour intervals for ten days for any clinical signs. In case of fatality post-mortem examinations were performed. For re-isolation of *P. multocida*, swabs from affected tissues were taken from ducks that died and streaked on to blood agar plates. The plates were examined after 24-48 hours of incubation at 37°C (Matsumoto and Helfer, 1978) and positive cases were further confirmed by standard procedures (Cowan and Steel, 1985).

Statistical analyses

Statistical analyses were performed using SAS (2008) statistical package programme to evaluate differences in PHA titre between the three groups of ducks, and the survival rates were evaluated using Mantel-Cox log rank test. A P value of <0.001 was considered significant.

Results and Discussions

Antibody titres were calculated and presented as Mean ± Standard error. The pre-vaccination PHA titres of sera samples of all ducks were a mean of <4.0 ± 0.0, in agreement with Mondal *et al.* (1988). The mean antibody titre of group A was 59.4 ± 4.6, 21 days after primary vaccination, 137.1 ± 21.8 15 days after booster vaccination, 100.6 ± 12.9 28 days after booster vaccination, and 256.0 ± 48.4 at 15 days after challenge. In group B titres were 50.3 ± 6.5, 118.9 ± 9.1, 91.4 ± 12.9, 237.7 ± 51.7, respectively.

Table 1. Mean PHA titres with standard error of sera of ducks vaccinated with BAU-FCV and LRI-FCV. Ducks were immunized at 6 weeks of age and boosted at 11 weeks of age im 0.5 mL/ duck (1.75×10^8 CFU/ mL) in group A and @ 1 mL/ duck in group B. Serum was obtained at 6, 9, 13, 15 and 17 weeks of age. Serum antibody titre against vaccination was determined by PHA test. Means bearing dissimilar superscript in a row differ significantly (P<0.0001).

Groups	Vaccines	Dose and route	Pre-immuniza-tion	Post-immunization PHA titres			Post-challenge
				Post-primary	Post-secondary		
				21 days	15 days	28 days	15 days
A	BAU-FCV	0.5 mL im	≤4.0 ± 0.0^{d}	59.4 ± 4.6^{cd}	137.1 ± 21.8^{b}	100.6 ± 12.9^{bc}	256.0 ± 48.4 ^{a}
B	LRI-FCV	1 mL im	≤4.0 ± 0.0^{c}	50.3 ± 6.5^{bc}	118.9 ± 9.1^{b}	91.4 ± 12.9^{b}	237.7 ± 51.7^{a}
C	Control	-	≤4.0 ± 0.0	≤4.0 ± 0.0	≤4.0 ± 0.0	≤4.0 ± 0.0	≤4.0 ± 0.0

The antibody titres were insignificant when compared between pre-vaccination and 21 days after primary vaccination in both vaccinated groups. When compared between 21 days of primary vaccination and 15 days of booster vaccination the antibody titres were highly significant (P < 0.0001) in group A and in case of group B the antibody titres were insignificant. The antibody titres were insignificant when compared between 15 days of booster vaccination and 28 days of booster vaccination in both groups. However, the antibody titres were highly significant (P< 0.0001) when compared between 28 days of booster vaccination and 15 days following challenge but when compared between two groups (A and B) there was no significant variation.

At 21 days after primary vaccination the antibody titres were significantly increased (P < 0.01) in both vaccinated groups, but there was no significant difference between these groups. At 15 days after booster vaccination, the antibody titres in groups A and B had increased significantly (P < 0.01) compared with 21 days after primary vaccination, but was not significantly different compared with that at 15 and 28 days after booster vaccination. However, the antibody titres were significant (P < 0.01) when compared with those obtained at 28 days of booster vaccination and also those

obtained at 15 days following challenge but when compared between two groups, there was no significant variation.

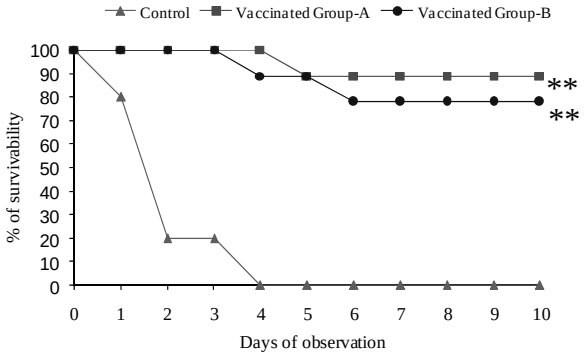

Fig. 1. Survival of ducks following challenge with virulent field isolate of *Pasteurella multocida* in Group A (BAU-FCV), Group B (LRI-FCV) and Group C (Control). ** ($P < 0.01$) by Mantel-cox log rank test when compared with control group.

After challenge with a virulent isolate of *P. multocid*a 88.9% of ducks immunized with BAU-FCV, and 77.8% immunized with LRI-FCV survived, whereas none of the unvaccinated control ducks survived. Islam *et al.* (2004) immunized ducks with LRI-FCV and BAU-FCV and showed 95% and 90% survived challenge infection three weeks after vaccination. The present results support the report of Ali and Sorwar (1975); Khan *et al*. (1994) who recorded 80% protection of chickens vaccinated with LRI-FCV.

Conclusions

It may be concluded that both the fowl cholera vaccines were safe and effective providing satisfactory protection against duck cholera in Jinding ducks.

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