

Determination of immune response against alum-precipitated fowl cholera vaccine in the quail, *Coturnix japonica*

M.T. Iqbal¹, M.H. Haque*, S. Sarker², M.A. Islam¹ and K.A. Choudhury¹

¹Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh,

²Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi-6205, Bangladesh,

*Corresponding Author: DR. Md. Hakimul Haque, Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi-6205, Bangladesh; E-mail: hakimul80@gmail.com

Abstract: The immune response of a formalin-inactivated alum-precipitated fowl cholera vaccine (FCV) was evaluated in quails, *Coturnix japonica* (Order: Galliformes and Family: Mimidae). All quails, irrespective of sex and 8-weeks-old were immunized with 5×10^7 CFU/ml/quail subcutaneously (SC) and intramuscularly (IM). A Booster immunization was given with similar dose and routes at 15 days after primary immunization in groups A and B while group C served as unimmunized control. Pre-immunized sera were collected from all groups of birds to assay the primary antibody levels in them. Sera of the immunized and control quails were collected at 15 and 30 days post immunization. The degree of immunity produced in each group of quails following primary and secondary immunizations were determined by measuring their serum antibody titres using passive haemagglutination assay (PHA) test. The level of antibody was significantly increased both primary and booster immunization in immunized quails as determined by PHA titres. Two weeks after final immunization, the quails were challenged with a virulent isolate of fowl cholera and immunized quails conferred 100% protection while all the control quails were dead within 10 days post challenge.

Key words: Fowl cholera, alum-precipitated vaccine, immune response, PHA test, quail

Introduction

Fowl cholera (FC) is one of the most important contagious bacterial diseases of poultry caused by the bacterium *Pasteurella multocida*. It occurs sporadically or enzootically in most countries of the world including Bangladesh (Heddleston & Rhoades, 1978; Choudhury *et al.*, 1985). Most reported outbreaks of FC are in chickens, turkeys, ducks, geese and also in quails (Heddleston and Watko, 1965; Choudhury *et al.*, 1985). FC typically occurs as a fulminating disease with massive bacteraemia and high morbidity and mortality. Vaccination is practiced as preventive measures in Bangladesh like in other countries of the world to reduce the incidence of the disease (Choudhury *et al.*, 1988). Both humoral and cell mediated immunity are considered to be of primary importance in the protection of animals and birds against infectious diseases (Collins, 1977; Mondal *et al.*, 1988). The immune responses (Mondal *et al.*, 1988; Choudhury *et al.*, 1990), efficacy of oil adjuvanted broth culture (Choudhury *et al.* 1987), alum-precipitated

vaccines (Khan *et al.*, 1994; Islam *et al.*, 2004) and comparative efficacy of different FCV (Choudhury *et al.*, 1988) have been evaluated under local conditions. But vaccination has not yet been practiced in quail against FC. Quail is reported to be more resistant to many infections as reported earlier (Heddleston & Rhoades, 1978) but presently there are many unpublished reports from farmers of the incidence of FC like infection in quail. Although various studies have been conducted on the FC in experiment models (Islam *et al.*, 2004), but there is no available information regarding the immune responses against FC vaccine in this bird. The present research therefore was conducted to determine the efficacy and safety level of alum-precipitated FCV with their immunogenic response in quail.

Materials and Methods

A total of 30 eight-week-old quails *Coturnix japonica* (Galliformes:Mimidae) used for this study were purchased from the Bangladesh Agricultural University (BAU) poultry farm, Mymensingh.

These quails were reared in the poultry shed of the Department of Microbiology and Hygiene, BAU, Mymensingh supplying food and water *ad libitum* with maintaining proper biosecurity. *Pasteurella multocida* (PM-38) serotype 1(X-73) was collected from the same Department and used for the preparation of formalin killed alum-precipitated FCV as well as challenge virulent organisms. The quails were equally divided into three experimental groups namely A, B, and C and were maintained in separate cages. Each of the quails of A and B groups was immunized with 0.5 ml of 5×10^7 CFU of the FCV through subcutaneous (SC) and intramuscular (IM) route, respectively. Booster immunization was administered in A and B groups after 15 days of primary immunization. Quails of the group C served as unimmunized control throughout the experimental period.

The immune response was studied by using passive haemagglutination assay (PHA) and protection tests to determine the presence of antibody against *P. multocida* in the serum of quails immunized with the prepared FCV. PHA test was conducted according to the procedure described by Tripathy *et al.* (1970) and Islam *et al.* (2004). The protection test was conducted on both immunized and control groups of quails with the same dose rate mentioned above through IM route as described by Choudhury *et al.* (1985) and Islam *et al.* (2004). The challenged quails were observed up to 10 days for the development of any clinical signs and symptoms of FC. *P. multocida* was reisolated from challenged quails according to the method of Choudhury *et al.* (1987). Statistical significance was evaluated using student t test. All the analyses were performed using SPSS (10.0 version). A p-value of <0.05 being considered as statistically significant.

Results and Discussion

PHA test. The test was conducted to determine the immune response induced in quails having been inoculated to 8-week-old quails with the antigen containing *P. multocida*. In this respect, Choudhury *et al.* (1985); Chang (1987); Mondal *et al.* (1988) and Sarker *et al.* (1992) stated that PHA test was useful for assay the serum antibody titres following administration of FC vaccine. The mean antibody titres of quails of group A and B were 51.2 ± 16.52 and 57.6 ± 29.4 , respectively after primary immunization which is similar to the findings of Coates *et al.* (1977) and Mondal *et al.*

(1988) whereas the mean antibody titres were 102.4 ± 33.05 in both groups of quails after booster immunization which corresponds with the findings of Choudhury *et al.* (1987) and Mondal *et al.* (1988). The mean pre-vaccination PHA titre of sera samples of all immunized and control quails were found to be 4 ± 0 , which correlated with finding of Mondal *et al.* (1988) (Fig. 1).

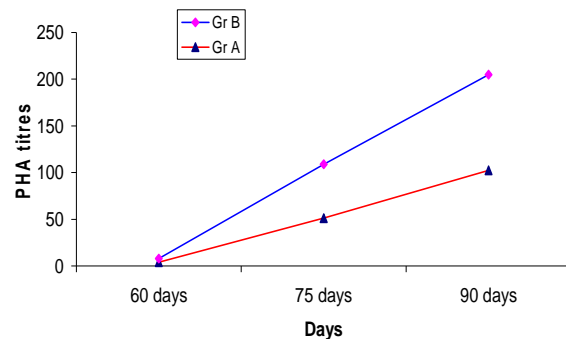


Fig. 1 Comparison of serum PHA titres of quails vaccinated with fowl cholera vaccine through SC in Gr A and IM in Gr B

Protection test. Quails of groups A and B showed 100% protection against challenge infection whereas quails of the group C showed 0% protection (Fig. 2). Immunized quails protected challenge infection showed no clinical signs except for dullness, depression and drowsiness. In controls, the clinical signs first observed at 12 hours post inoculation (PI) were characterized by dullness and depression. There were dullness, depression, slight rise of body temperature (42.5°C) and increased respiratory rates (30-45/min) at 24 hours PI. At 48 hours PI, severe weakness, drowsiness, anorexia, rise of body temperature (43.6°C), increased respiratory rates (45-55/min), lameness and whitish (chalky) diarrhoea with mucous were the important clinical manifestations which was previously observed by many authors' (Sharma *et al.* 1974; Gordon and Jordan, 1985; Rhoades and Rimler, 1990). The clinical signs at 96 hours PI were almost similar to that of 48 hours. Death of one quail was observed first at 24 hours PI, while two died at 48 hours and others died at 96 hours PI.

Post challenge isolation of bacteria: *P. multocida* were reisolated from heart, blood, liver and brain of quails of all groups.

Independent samples t-tests revealed that immune response was non significant due to route variation. Paired samples t-tests showed that

immune response of secondary immunization was significantly higher (102.4) than the primary immunization. The mean PHA titre after primary and secondary immunization induce better immune response in quail but no remarkable variation in immune response were recorded between two routes of vaccination. Thus it may be concluded that the route did not cause any variation in production of immune response. The present finding did not correlate with many of the investigators. The experimental FCV conferred 100% protection ($P < 0.01$) against challenge infections.

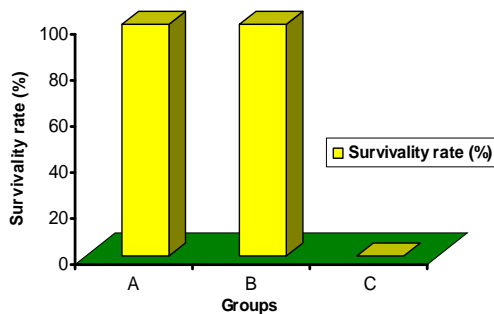


Fig. 2. Survivability of quails immunized with formalin killed allum precipitated FCV.

Therefore, it may be suggested the formalin killed alum-precipitated FCV prepared with highly antigenic strain of *P. multocida* should be used to provide better protection against the epidemic FC in quail. However, further study with large number of quails to determine the efficacy of routes of immunization and establishment of Enzyme linked immunosorbent assay test instead of PHA for determination of immune response is necessary to conclude about the present study.

References

- Chang, C.F. 1987. In vitro assay for humoral and cell mediated immunity to pasteurellosis in ducks. *J. Chi. Soc. Vet. Sci.* **13**(1):67-74.
- Choudhury, K.A., Amin, M.M., Rahman, A. & Ali, M.R. 1985. Investigation of natural outbreak of fowl cholera. *Bang. Vet. J.* **19**(1-4):49-56.
- Choudhury, K.A., Amin, M.M., Sarker A.J. & Ahamed, A.R. 1987. Immunization of chickens against fowl cholera with oil adjuvanted broth culture vaccine. *Bang. Vet. J.* **21**(1-2):63-73.
- Choudhury, K.A., Amin, M.M., Sarker, A.J. & Ali, M.I.L. 1988. Studies on the comparative efficacy of fowl cholera vaccines. *Bang. Vet. J.* **22**(1-2):101-108.
- Choudhury, K.A., Mondal, S.K., Rahman, M.M., Amin, M.M. & Sarker, A.J. 1990. Changes in leukocytes, total serum protein and immunoglobulin levels in chickens immunized against fowl cholera. *The Bang. Vet.* **7**(1):27-30.
- Coates, R.S., Jensen, J.M. & Brown, E.D. 1977. The response of turkey to varying doses of live oral *Pasteurella multocida* vaccine. *Inter. J. Poult. Sci.* **56**:273-276.
- Collins, F.M. 1977. Mechanisms of acquired resistance of *Pasteurella*. *Cornell Vet.* **67**:103-167.
- Gordon, R.F. & Jordan, F.T. (eds.). 1985. *Poultry Diseases*. (2nd edn.), ELBS, Baillier Tindall, London, pp. 44-46.
- Heddleston, K.L. & Rhoades, K.R. 1978. *Avian pasteurellosis in diseases of poultry*. (7th edn.), Iowa State University Press, Ames, Iowa, USA, pp. 181-199.
- Heddleston, K.L. & Watko, L.P. 1965. Fowl cholera: comparison of serologic and immunogenic responses of chicken and turkeys. *Avian Dis.* **9**:367-376.
- Islam, M.A., Samad, M.A. & Rahman, M.B. 2004. Evaluation of alum precipitated formalin killed fowl cholera vaccines with their immunologic responses in ducks. *Inter. J. Poult. Sci.* **3**:140-143.
- Khan, M.A.H.N.A., Das, P.M., Choudhury, K.A. & Islam, M.R. 1994. Pathology of experimentally induced fowl cholera in chickens. *Bang. Vet. J.* **31**(1-2):28-34.
- Mondal, S.K., Choudhury, K.A., Amin, M.M., Rahman, M.M. & Sarker, A.J. 1988. Immune response in chickens induced by Alum precipitated fowl cholera vaccine. I Humoral immune response. *Bang. Vet. J.* **22**:7163-69.
- Rhoades, K.R. & Rimler, R.B. 1990. *Pasteurella multocida* colonization and invasion in experimentally exposed turkey poults. *Avian Dis.* **34**(2):381-383.
- Sarker, A.J., Amin, M.M. & Hossain, W.M.A. 1992. Testing and quality control of poultry vaccines and its monitoring in the field. *Bang. Agri. Uni. Res. Pro.* **6**:249-257.
- Sharma, R.N., Bhalla, N.P. & Lall, J.M. 1974. Observation of experimental infection of *Pasteurella septica* (avian) in domestic fowl. *Indi. J. Ani. Heal.* **13**(2):105-109.
- Tripathy, D.N., Hanson, L.E. & Myers, W.L. 1970. Passive haemagglutination test with fowl pox virus. *Avian Dis.* **14**:29-38.