ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 3 (2): 140-143, 2004

Evaluation of Alum Precipitated Formalin Killed Fowl Cholera Vaccines with Their Immunologic Responses in Ducks

M.A. Islam[1]*, M.A. Samad[1] and M.B. Rahman[2]
[1]Department of Medicine, Faculty of Veterinary Science,
[2]Department of Microbiology and Hygiene, Faculty of Veterinary Science,
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
E-mail: maislam77@yahoo.com

Abstract: The alum precipitated formalin killed fowl cholera vaccines (FCV) are locally produced from the Livestock Research Institute (LRI), Mohakhali, Dhaka and Bangladesh Agricultural University (BAU), Mymensingh which are being used to control fowl cholera in chickens and ducks in Bangladesh. Efficacy of these two vaccines has been evaluated mostly in chickens but reports on ducks are very limited. Four weeks old 50 Jinding breed of ducks were used to evaluate the efficacy and immune responses of fowl cholera vaccines during the period from October 2002 to March 2003. These 50 ducks were divided into three groups (A = 20, B = 20 & C = 10 ducks) and each duck of group A was inoculated with FCV (LRI) @ 0.5 ml subcutaneously at the age of 8 weeks, and each duck of group B received FCV (BAU) @ 1.0 ml intramuscularly at the age of 12 weeks as manufacturer instruction, whereas ducks of group C served as unvaccinated control. Each duck of group A and B was also injected booster dose after two weeks of primary vaccination with their respective FCV. Each duck of all the three groups (A, B and C) was challenged after two weeks of post-booster vaccination with 1.0 ml inoculum containing 5.4×10^{6} CFU of virulent *Pasteurella multocida* intramuscularly. The results of challenged experiment revealed that one (5.0%) duck of group A, two (10.0%) ducks of group B died within 2 to 3 days of post-challenged, whereas 10 (100%) unvaccinated control ducks of group C died within 1 to 3 days of post-challenged. Therefore, the FCV® (LRI) conferred protection to 95% and FCV (BAU) conferred protection to 90% of vaccinated birds against challenged infection after two weeks of booster vaccination. The mean values of Total leukocytic count (TLC), Total serum protein (TSP) and Passive haemagglutination assay (PHA) antibody titre of ducks in both the groups A and B were found significantly ($p < 0.01$) increased at two weeks of post-primary and two weeks of post-booster vaccination, and also two weeks of post-challenged infection in comparison to the respective pre-vaccination values. These results indicate that the FCV of LRI induced comparatively higher TLC, TSP and PHA antibody titre than FCV of BAU in ducks. These results showed that the locally prepared fowl cholera vaccines induced sufficient cellular and humoral immune responses which resulted satisfactory level of protection against duck cholera and therefore both the locally prepared FCV could be recommended to control duck cholera under filed conditions in Bangladesh.

Key words: Fowl cholera, immunization; duck, immune response, protection

Introduction

There are about 13 million ducks in Bangladesh (Anonymous, 2000) and most of them are maintained by the scavenging rearing duck systems, which are still considered to be organic farming in nature. However, the major population of ducks is reared by the farmers of costal areas and who live by the side of river, canals, haors, jhils etc. due to the costly feed are not required in these areas. The diseases associated with morbidity and mortality in ducks are very limited in number in comparison to chicken. The causes of mortality of duck, have been investigated from Bangladesh by Baki *et al.* (1993) who reported fowl cholera (duck cholera) (10.91%) and duck plague (54.55%) are the major malady associated with high mortality in ducks. Duck cholera, caused by *Pasteurella multocida* which is mainly prevented by vaccination in Bangladesh. Vaccines against fowl cholera are being prepared from two different institutes (FCV, LRI and FCV, BAU) in Bangladesh and made available in the local market for field use to control fowl cholera in chicken and ducks. Some reports on the immune response and efficacy of locally prepared fowl cholera vaccines in chicken have been made from Bangladesh (Choudhury *et al.*, 1988, 1990; Mondal *et al.*, 1988a,b; Khan *et al.*, 1994) but similar reports are very limited in ducks (Ali and Sorwar, 1975). Therefore, this study was undertaken to determine the immune response with comparative efficacy of locally prepared two fowl cholera vaccines in ducks.

Materials and Methods

Experimental ducks: Four-week-old 50 Jinding breed of healthy ducks of either sex with no previous history either

Table 1: Results of immunization of ducks with commercial fowl cholera vaccines and their state of protection

S/N Parameters	Unit	Fowl cholera vaccine used		Control
		FCV (BAU) (n=20)	FCV (LRI) (n=20)	(n=5+5 =10)
Age of primary vaccination	-	8 weeks	12 weeks	-
Dose and route	-	0.5 ml, SC	1.0 ml, IM	-
Age of booster dose	-	10 weeks	14 weeks	-
Age of challenged	-	12 weeks	16 weeks	12/16 weeks.
No. of birds challenged	-	20	20	5/5
Challenged with * 1 ID	-	1.0 ml, IM	1.0 ml, IM	1.0 ml, IM
Ducks showed clinical signs	No.	1/20	2/20	10/10
	%	5	10	100
Duck survived after challenged	No.	19/20	18/20	0/10
	%	95.0	90.0	0

n = Number of ducks used * 1 ID (Infective dose) = 5.4×10^6 CFU/ml of virulent *Pasteurella multocida*

vaccination or of fowl cholera infection were purchased from the Government Duck Farm, Kishoregonj. These ducks were divided into three main groups (A, B and C) of which groups A and B consisting of 20 ducks in each, whereas group C consisting of 10 ducks. These three groups of ducks were maintained separately with intensive care and adequate commercial feed (Quality Feeds Ltd., Dhaka) and water supply throughout the experiment. In addition to general feed and water supply, vitamin-mineral premix (Megavit®, Novartis, Bangladesh Ltd., Dhaka) was also supplied in the drinking water daily.

Vaccines: Alum precipitated formalin killed fowl cholera vaccine prepared by the Poultry Biologic Unit (BAU), Mymensingh was purchased directly from the manufacturing unit, and fowl cholera vaccine manufactured by the (LRI) Mohakhali was obtained from the district veterinary hospital, Mymensingh were used for this study.

Immunization of ducks: Two doses of fowl cholera vaccines were administered at interval of 14 days as per manufacturer immunization of ducks. Each duck of group A (n = 20) was primarily vaccinated at the age of 8 weeks with fowl cholera vaccine prepared by LRI @ 0.5 ml SC followed by booster vaccination with same vaccine at the age of 10 weeks of age. Primary vaccination with fowl cholera vaccine prepared by BAU was made in each of the duck of group B (n =20) at the age of 12 weeks @ 1.0 ml intramuscularly with same vaccine followed by booster vaccination with at the age of 14 weeks. Ducks of group C (n=10) served as unvaccinated and challenged control.

Challenge of experimental ducks: The locally isolated virulent *Pasteurella multocida* serotype I (Strain X-73) maintained in the laboratory (Choudhury *et al.*, 1986) was used as challenge organisms. Each of the bird of all the three experimental groups (A, B and C) was challenged intramuscularly with 1.0 ml inoculum contained 5.4×10^6 CFU (ID). All the challenged birds were observed daily for two weeks to detect the signs and symptoms, morbidity and mortality by the challenged inoculum.

Collection and examination of blood: Blood was collected from the wing vein of each of the experimentally vaccinated duck of groups A and B in two separate test tubes. One contained double oxalate as anticoagulant which is used for total leukocytic count and other one without adding any anticoagulant which is used for separation of serum to determine the antibody titre and total serum protein. Blood samples were collected from the ducks at pre-vaccination, two weeks of post-primary vaccination, two weeks of post-booster vaccination and two weeks of post-challenge. Sera were separated from the blood collected without adding any anticoagulant and stored at -20 ^{o}C until tested.

Total Leukocytic Count (TLC): Oxalated veinous blood taken up to 0.5 mark of a RBC diluting pipette and then drawn the diluting fluid up to the 101 mark, thus made a 1:200 dilution and mixed well with 8 knot method. First one or two drops discarded and then pour of one drop on counting chamber with cover slip and counted the leukocytes on the 9 large squares having a total area of 9 sq.mm and counted the average leukocyte, multiplied by 2000 to gave the TLC in 1 sq. mm of blood as described by Sastry (1979).

Antibody response: The passive haemagglutination assay (PHA) first was used to determine the antibody titre of ducks immunized with fowl cholera vaccines according to the method described by Tripathy *et al.* (1970) with slight modification. Tripathy *et al.* (1970) described the PHA in fowl pox virus with PBS pH 6.4, 1:25000 tannic acid solution, $Na_2HPO_4 \cdot 12H_2O$ 0.15 M, $KH_2PO_2 \cdot 2H_2O$ 0.15 M and sensitized RBC 0.5% whereas pH 7.2, 1:20000, 0.2M, 0.2M and 0.7% were used in this study respectively.

Table 2: Immune responses in ducks immunized with fowl cholera vaccines (FCV)

S/N Parameters	Vaccines used	Pre-vaccination	Post-vaccination		Post-challenged[3]
			Pre-booster[1]	Post-booster[2]	
PHA titre	FCV, LRI	4.0-8.0	32.0-64.0	64.0-256.0	128.0-512.0
		5.6±1.96	48.0±16.0	124.6±52.83	263.11±99.8
		(n=20)	(n=20)	(n=19)	(n=18)
	FCV, BAU	4.0-8.0	32.0-128.0	64.0-256.0	128.0-512.0
		4.80±1.6	51.2±26.52	128.0±57.69	256.0±118.81
		(n=20)	(n=20)	(n=16)	(n=14)
Total leuko cytic count (TLC,10^3/mm^3)	FCV, LRI	33.1-53.4	61.3-86.3	72.1-96.3	85.8-108.4
		42.1±5.69	73.7±6.26	82.6±6.45	95.7±6.27
		(n=20)	(n=20)	(n=19)	(n=18)
	FCV, BAU	29.5-55.2	58.7-88.2	75.3-89.2	81.3-102.4
		43.4±6.94	71.10±9.62	83.3±4.48	91.6±7.07
		(n=20)	(n=20)	(n=16)	(n=14)
3. Total serum protein (TSP, g/dl)	FCV, LRI	2.6-3.9	3.6-5.0	4.8-5.8	4.8-6.1
		3.18±0.46	4.34±0.39	5.30±0.31	5.58±0.37
		(n=20)	(n=20)	(n=19)	(n=18)
	FCV, BAU	2.8-3.8	3.6-4.8	4.6-6.0	5.0-6.2
		3.22±0.27	4.37±0.36	5.24±0.35	5.56±0.35
		(n=20)	(n=20)	(n=16)	(n=14)

PHA = Passive hemagglutination assay, n = Number of ducks tested, LRI = Livestock Research Institute, BAU = Bangladesh Agricultural University, [1]After two weeks of primary vaccination, [2]After two weeks of booster vaccination, [3]After two weeks of challenged

Total serum protein (TSP): The TSP in vaccinated duck with fowl cholera was estimated using TS meter as described by Samad (2001).

Post-challenge necropsy and bacterial isolation: The ducks which were died at post-challenged were necropsied as soon as they were found dead. In addition three ducks in each the three challenged groups (A, B and C) were randomly selected and sacrificed at 14 days of post challenged. Swabs taken from liver and heart were streaked onto blood agar plates, which were incubated at 37 ^{o}C for 24 hours for the growth of organisms. The positive cases showing growth of *P. multocida* were confirmed by the standard bacteriological procedure (Cowan and Steel, 1985).

Statistical analysis: Results are analyzed statistically with the help of Student's 't' test for significance as described by Gupta (1982).

Results and Discussion

Fowl cholera caused by *P. multocida* is an important and endemic disease causes high morbidity and mortality in chickens and ducks in Bangladesh. The alum precipitated formalin killed fowl cholera vaccines are produced locally to control this disease in chickens and ducks. Although the locally produced fowl cholera vaccines have been evaluated in chickens (Choudhury *et al.*, 1988; Mondal *et al.*, 1988a,b; Khan *et al.*, 1994) but very limited information is available on immune response and protection of fowl cholera vaccine in ducks.

The results of immunization of ducks with commercial fowl cholera vaccines and their stage of protection are presented in Table 1. The ducks immunized with FCV (LRI) and FCV (BAU) are challenged with a virulent isolate of *P. multocid*a which showed 95 and 90% protection rate respectively. This variation of protection role might be due to individual antigenic variation vaccine strains. Although the response on the efficacy of the fowl cholera vaccines in ducks are limited in the available literature (Ali and Sorwar, 1975) but these results support the earlier report of Khan *et al.* (1994) who reported 80% protection of chickens vaccinated with FCV (LRI) and challenged with 1 ID (4.8×10^5 CFU/ml) of virulent *P. multocida*.

Clinical reactions: Of the 20 (group A) and 20 (group B) ducks immunized with FCV (LRI) and FCV (BAU) and challenged with virulent *P. multocida* of which only one (5%) in group A and two (10%) in group B showed clinical signs at 24 hours of post-challenged. The clinically affected duck of group A and one affected duck of group B died at 48 hours, whereas the other affected duck of group B died at 72 hours of post-challenged. All the 10 (100%) non-vaccinated challenged control ducks died with characteristic clinical signs of fowl cholera at different interval of post-challenged. Of the 10 control ducks, 4 (40%) died at 24 hours 3 (30%) at 48 hours and the remaining 3 (30%) at 72 hours of post-challenged. Although the ducks immunized with fowl cholera vaccines showed the similar clinical signs like non-immunized challenged control ducks but the severity was found comparatively less in vaccinated ducks.

Leukocytic response: The cellular response in ducks

immunized with fowl cholera vaccines was assessed by counting the blood total leukocytes at pre-and-post vaccination and post-challenged stages. The TLC was found significantly ($p < 0.01$) increased at post-vaccination and post-challenged phases in comparison to pre-vaccination values (Table 2). This finally supports the report of Choudhury *et al.* (1990) who reported significantly ($p < 0.01$) increased TLC in chickens immunized and challenged against fowl cholera. A similar observation was also reported by Maheswaran and Thies (1979) and Collins (1977) who reported significantly increased of leukocytes in turkeys and chickens vaccination against fowl cholera.

Humoral response: The PHA antibody titre in ducks immunized with both the fowl cholera vaccines (FCV, LRI and FCV, BAU) was found significantly ($p < 0.01$) increased at post-vaccination (124.6 ± 52.83 and 128.0 ± 57.69) and post-challenged (263.11 ± 99.8 and 256.0 ± 118.81) period in comparison to pre-vaccination (5.60 ± 1.96 and 4.80 ± 1.60) values (Table 2). It indicates that active humoral immune response is induced in ducks immunized with both fowl cholera vaccines. There observations are in conformity with the earlier finding of Choudhury *et al.* (1987) and Mondal *et al.* (1988a) who reported significantly ($p < 0.01$) increased IHA antibody titre in chickens immunized with fowl cholera vaccines. It was also observed that the immunized ducks with PHA antibody titre <1:64 died with virulent challenged infections.

Effects on total serum protein: The total serum protein (TSP) values in ducks vaccinated with fowl cholera vaccines (FCV, LRI/BAU) showed significantly ($p < 0.01$) increased at post-vaccination and post-challenged stages in comparison to pre-vaccination values (Table 2). This finding supports the report of Choudhury *et al.* (1990) who reported increased TSP in chickens immunized and challenged against fowl cholera.

The experimental immunization of ducks with fowl cholera vaccines induced significantly increased ($p < 0.01$) PHA antibody titre, TLC and TSP which showed a significant activity in protection against virulent challenge infection. This also indicates the humoral and cell mediated immune responses are induced in duck cholera and both might have activity in protection as had been reported by Mondal *et al.* (1988a and 1988b). Although the FCV, LRI was found better than FCV (BAU) on the basis of results of protection, PHA test, TLC and TSP but both fowl cholera vaccines may be recommended to control duck cholera in Bangladesh.

Acknowledgements

The authors are grateful to the authority of the Small Holder Livestock Development Project-2 (SLDP-2) for financial support to conduct this research. We also wish to thanks Dr. Jonathan Bell Senior Adviser, SLDP-2, (DANIDA) for assistance and cooperation during this study.

References

Ali, M.I. and M.G. Sorwar, 1975. Studies on duck cholera (Pasteurellosis) vaccine. Bangladesh Vet. J., 9: 29-30.

Anonymous, 2000. FAO Production Year Book 54: 211-214.

Baki, M.A., M.L. Dewan and M.M.H. Mondal, 1993. An investigation on the causes of mortality of ducks in Bangladesh. Progressive Agriculture, 4: 27-33.

Choudhury, K.A., M.M. Amin and A.J. Sarker, 1986. Serotyping of Pasteurella multocida from chickens and ducks. Bangladesh Vet. J., 20: 55-56.

Choudhury, K.A., M.M. Amin, A.J. Sarker, M.R. Ali and A.R. Ahmed, 1987. Immunization of chickens against fowl cholera with oil-adjuvanted broth culture vaccine. Bangladesh Vet. J., 21: 63-73.

Choudhury, K.A., M.M. Amin, A.J. Sarker and M.R. Ali, 1988. Studies on the comparative efficacy of fowl cholera vaccine. Bangladesh Vet. J., 22: 101-108.

Choudhury, K.A., S.K. Mondal, M.M. Rayman, M.M. Amin and A.J. Sarker, 1990. Changes in leukocytes, total serum protein and immunoglobulin levels in chickens immunize against fowl cholera. The Bangladesh Veterinarian, 7: 27-30.

Collins, P.M., 1977. Mechanism of acquired resistance to Pasteurella multocida. Cornell Veterinariu 67: 103-137.

Cowan, S.T. and K.S. Steel, 1985. Manual for Identification of Bacteria, 2nd edn.

Gupta, S.P., 1982. Statistical methods. 19th edn. Sultan Chand and Sons, Publishiers, 23. Daiyagorj, New Delhi, India.

Khan, M.A.H.N.A., P.M. Das, K.A. Chowdhury and M.R. Islam, 1994. Efficacy of alum precipitated fowl cholera vaccine in chicken. Bangladesh Vet. J., 28: 25-30.

Maheswaran, S.K. and E.S. Thies, 1979. Pasteurella multocida antigen induced *in vitro* lymphocyte immunostimulation using whole blood from cattle and turkeys. Res. Vet. Sci., 26: 25-31.

Mondal, S.K., K.A. Chowdhury, M.M. Amin, M.M. Rahman and A.J. Sarker, 1988a. Immune response in chickens induced by alum precipitated fowl cholera vaccine: Humoral immune response. Bangladesh Vet. J., 22: 63-69.

Mondal, S.K., K.A. Chowdhury, M.M. Amin, M.M. Rahman and A.J. Sarker, 1988b. Immune response in chickens induced by alum precipitated fowl cholera vaccine: II. Cell mediated immune response. Bangladesh Vet. J., 22: 71-75.

Samad, M.A., 2001. Poultry Palon O Chikitsavidya (Poultry Husbandry and Medicine). 3rd edn.,Published by M. Bulbul, Bangladesh Agricultural University campus, Mymensingh, Bangladesh.

Sastry, G.A., 1979. Veterinary Clinical Pathology. 2nd edn., 465, Bhavaninagar, TIRUPATI (AP), India.

Tripathy, D.N., L.E. Hanson and W.L. Mayrs, 1970. Passive hemagglutination assay with fowl poxvirus. Avian Disease, 14: 29-38.