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Assessment of Immunologic Responses in Khaki Cambell Ducks Vaccinated Against Duck Plague

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Abstract: The duck plague vaccine (DPV) is locally produced from the Livestock Research Institute (LRI), Mohakhali, Dhaka which is used to control the duck plague viral disease in ducks in Bangladesh. Efficacy of this vaccine reports has no, so to say on ducks in Bangladesh. Three weeks old 50 Khaki Cambell Ducks were used to evaluate the induced of immune responses of duck plague vaccine during the period from February to April 2003. These 50 ducks were divided into three groups (A = 15, B = 15 and C = 5 ducks) and each duck of group A and group B were inoculated primarily with duck plague vaccine @ 1.0 ml. intramuscularly at the age of 4 weeks and whereas ducks of group C served as unvaccinated control. Then each duck of group A was also injected booster dose after 2 weeks of primary vaccination with same vaccine, dose and route. The mean value of TLC, TSP and PHA antibody titre of ducks of group A was found significantly ($p < 0.1$) increased at two weeks and four weeks of post-primary vaccination and two weeks and four weeks of post-booster vaccination in comparison to the pre-vaccination values. The mean value of TLC, TSP and PHA titre of ducks of group B was also found significantly increased ($p < 0.1$) at two, four, six and eight weeks of post-primary vaccination in comparison to the pre-vaccination values. These results indicate that booster vaccination of duck plague vaccine induced comparatively higher TLC, TSP and PHA antibody titre than single primary vaccination in ducks. The mean value of TLC, TSP and PHA in unvaccinated control group C was more or less nearer at the age of four weeks and 12 weeks in ducks. These results showed that the locally prepared duck plague vaccine induced sufficient both cellular and humoral immune responses with booster-vaccination than primary vaccination in ducks. Therefore, it could be recommended to booster dose of vaccination of duck plague vaccine to control duck plague under field condition in Bangladesh.

Key words: Khaki cambell ducks, duck plague, immune responses

Introduction

Duck Plague (Duck virus enteritis) is an acute contagious herpes virus infection of wild and domestic ducks, geese and swans. Outbreaks of duck virus enteritis (DVE) are sporadic and the morbidity and mortality in a flock may range from 5 to 100%, depending on the virulence of the virus and the immunologic status of the birds (Campagnolo *et al.*, 2001). Research works on various aspects of DVE, especially outbreaks, isolation of causative agent, antigenic relationship with imported vaccine strains, pathogenicity, pathology, and evaluation of immunodiagnostic tests, immunogenicity, and protective potential cell - mediated immune response have been reported from Bangladesh (Samad, 2001). Although ducks are routinely vaccinated against duck plague, the dearth of information on the immune status of the DVE vaccinates prompted the present study.

Materials and Methods

Experimental ducks: Three-week-old 50 Khaki Cambell breed of healthy ducks of either sex with no previous history either vaccination or of duck plague infection

were purchased from the Government Duck Breeding Farm, Narayanganj on 27 February, 2003. These ducks were maintained in a newly constructed experimental duck house of the Department of Veterinary Medicine, Bangladesh Agricultural University, Mymensingh with intensive care, adequate commercial feed (Quality Feed Ltd., Dhaka) and water supply throughout the experimental period from 27 February to 25 April, 2003. After acclimatization of these 50 experimental ducks, of which only 35 ducks were selected randomly for this immunization study. These selected 35 ducks were divided into three groups (A, B and C), of which group A and B consisting of 15 ducks in each, whereas group C consisting of 5 ducks. These three groups of ducks were maintained separately with intensive care and management system. In addition to general feed and water supply, vitamin-mineral premix (Megavit[®], Novartis Bangladesh Ltd., Dhaka) was also supplied in the drinking water daily.

Immunization of ducks: Duck plague vaccine (LRI, Mohakhali, Dhaka) was obtained from the district of Veterinary hospital, Mymensingh, used for immunization

Table 1: Humoral and cellular responses in ducks immunized with the locally produced commercial Duck plague vaccine

Parameters	Groups	No. of Ducks used	Age of ducks with responses				
			Pre-vaccination (PV) 4 weeks ¹	Post-primary vaccination (PPV)		Post-booster vaccination (PBV)	
				6 weeks ²	8 weeks ³	9 weeks ⁴	10 weeks ⁵
PHA	A	15	04 – 04	16 – 32	32 – 128	64 – 128	128 – 256
			4.00 ± 0.0	27.73 ± 7.32	70.4 ± 24.79	128.0 ± 41.88	196.2 ± 69.86
	B	15	04 – 04	16 – 32	64 – 128	64 – 128	64 – 128
			4.00 ± 0.0	29.87 ± 05.62	76.80 ± 26.50	102.40 ± 32.45	115.2 ± 49.57
	C	05	04 – 04	–	–	–	04 – 08
			4.0 ± 0.0	–	–	–	06.40 ± 1.96
TLC (10 ³ /mm)	A	15	38.6 – 58.8	52.5 – 69.6	60.7 – 78.1	77.4 – 92.1	84.3 – 97.4
			47.37 ± 5.30	61.79 ± 4.99	69.25 ± 4.71	84.28 ± 5.75	93.5 ± 4.64
	B	15	39.5 – 54.3	54.2 – 68.6	58.7 – 74.1	68.1 – 86.7	84.3 – 97.4
			46.77 ± 4.69	61.17 ± 4.62	68.65 ± 4.39	78.47 ± 5.31	83.55 ± 4.09
	C	05	42.4 – 52.4	–	–	–	43.5 – 58.6
			46.76 ± 3.39	–	–	–	51.30 ± 5.38
TSP (g/dl)	A	15	2.40 – 3.20	2.80 – 3.60	3.40 – 4.40	3.80 – 5.00	4.6 – 5.8
			2.83 ± 0.33	3.19 ± 0.27	3.87 ± 0.31	4.33 ± 0.42	5.29 ± 0.31
	B	15	2.4 – 3.4	2.8 – 3.4	3.2 – 4.4	3.6 – 5.0	3.6 – 5.0
			2.82 ± 0.31	3.13 ± 0.18	3.93 ± 0.33	4.31 ± 0.33	4.31 ± 0.33
	C	05	2.4 – 3.2	–	–	–	2.8 – 3.4
			2.84 ± 0.27	–	–	–	3.12 ± 0.20

¹PV at 4 weeks, ²two weeks of PPV, ³four weeks of PPV and before PBV, ⁴two weeks of PBV, ⁵four weeks of PBV, PHA = Passive haemagglutination assay, TLC = Total leukocyte count, TSP = Total serum protein, A = Primary (at 4 weeks) and booster (at 8 weeks) vaccinated, B = Only primary (at 4 weeks) vaccinated, C = Unvaccinated control

of ducks. Each duck of group A (n=15) was primarily vaccinated at the age of 4 weeks with DPV @ 1.0 ml IM, followed by booster vaccination with same vaccine at the age of 8 weeks of age. Each duck of group B (n=15) was vaccinated with DPV at the age of 4 weeks @ 1.0 ml IM as a single vaccine. Duck of group C (n=5) served as unvaccinated control.

Examination of blood: Blood was collected from the wing vein of each of the experimentally vaccinated duck of groups A, B and C in duplicate test tubes. One tube (1.0 ml/bird) contained double oxalate as anticoagulant which was used for total leukocyte count (TLC) and other one (2.0ml/bird) 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 and two weeks interval of post-primary and booster vaccination. Sera were separated from the blood collected without adding any anticoagulant and stored at -20°C until tested.

Total leukocyte count (TLC): The total leukocyte count (TLC) was determined with the oxalate venous blood by the method described by Sastry (1979) and Islam *et al.*, (2004).

Antibody response: The passive haemagglutination assay (PHA) was used to determine the antibody titre of ducks immunized with Duck plague vaccine as

described by Tripathy *et al.* (1970) with slight modified by Islam *et al.* (2004).

Total serum protein (TSP): The total serum protein (TSP) in pre-and post-vaccinated ducks with DPV was estimated by using TS meter as described by Samad (2001).

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

Results and discussion

The result of vaccination in ducks with the locally produced commercial Duck plague vaccine (LRI, Mohakhali, Dhaka) with their humoral and cellular response are presented in Table 1. The ducks, one group (A) was immunized primarily as well as secondarily, whereas birds of group B immunized with only primary vaccination (Table 1). The PHA antibody titre, TLC and TSP responses were found significantly ($p < 0.01$) increased in both the vaccinated groups A and B in comparison to control group C. However, comparatively more responses were recorded in birds of group A which was boosted with the same vaccine after 4 weeks of primary vaccination.

Humoral response: The PHA antibody titre in ducks immunized with the duck plague vaccine was found significantly ($p < 0.01$) increased at two weeks and four

weeks of post-primary vaccination (27.73 ± 7.32 and 70.40 ± 24.79) and two weeks and four weeks of post-booster vaccination (128.0 ± 41.88 and 196.2 ± 69.86) in comparison to pre-vaccination antibody titre (4.0 ± 0.0) in ducks of group A (Table 1). On the other hand the PHA antibody titre in ducks of group B with single vaccination showed significantly increased at two, four, six and eight weeks of post-primary vaccination (29.87 ± 5.62 , 76.80 ± 26.50 , 102.40 ± 32.45 and 115.2 ± 49.57) in comparison to pre-vaccination value (4.0 ± 0.0). The PHA titre in control ducks of group C was little increasing along with advance age (Table 1). It indicates that active humoral immune response is induced in ducks immunized with duck plague vaccine and more immune response is produced due to booster vaccination than single vaccination. These observations are in conformity with the earlier findings of Butterfield and Dardiri (1969a and 1969b) who reported attenuated duck plague vaccine could produce satisfactory levels of humoral immune response either post-primary vaccination and followed by challenge with virulent virus and could protect the virulent challenge.

Leukocytic response: The cellular response in ducks immunized with duck plague vaccine was assessed by counting the blood total leukocytes at pre-and-post vaccination stages. The total Leukocytic count (TLC) was found significantly ($p < 0.1$) increased in ducks of group A at post-primary vaccination (61.79 ± 4.99 and 69.25 ± 4.71) and post-booster vaccination (84.28 ± 5.75 and 93.5 ± 4.64) in comparison to pre-vaccination values (Table 1). In ducks of group B the TLC was also found significantly ($p < 0.01$) increased at post-vaccination (61.17 ± 4.62 , 68.65 ± 4.39 , 78.47 ± 5.31 and 83.55 ± 4.09) in comparison to pre-vaccination values (46.77 ± 4.69). This finally supported the report of Choudhury *et al.* (1990) and Islam *et al.* (2004), who reported significantly ($p < 0.01$) increased TLC in chicken immunized against fowl cholera.

Effects of total serum protein (TSP): The total serum protein (TSP) values in ducks of group A, vaccinated with booster dose of duck plague vaccine showed significantly ($p < 0.1$) increased at post-primary vaccination (3.19 ± 0.27 and 3.87 ± 0.31) and post-booster vaccination (4.33 ± 0.42 and 5.29 ± 0.31) in comparison to pre-vaccination values (2.83 ± 0.33) (Table 1). The TSP values in ducks of group B, which primarily vaccinated with same vaccine, were found significantly ($p < 0.01$)

increased at post-vaccination (3.13 ± 0.18 , 3.93 ± 0.33 , 4.30 ± 0.33 and 4.31 ± 0.33) in comparison to pre-vaccination value (2.82 ± 0.31).

The experimental immunization of ducks with duck plague vaccine induced significantly increased ($p < 0.01$) PHA antibody titre, TLC and TSP either in booster immunization of ducks and primary immunization of ducks. But, after booster vaccination the PHA antibody titre, TLC and TSP values are more induced than single primary vaccination. This indicated that the humoral and cellular mediated immune responses are induced in ducks due to locally prepared duck plague vaccine and would give protection against duck plague infection.

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