EFFECT OF DIFFERENT ROUTES OF VACCINATION AGAINST NEW-CASTLE DISEASE ON LYMPHOID ORGANS OF BROILERS

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ABSTRACT

This project was designed to compare two routes (intraocular and drinking water) of vaccination against Newcastle disease in terms of protection against velogenic field isolate of Newcastle disease virus (NDV). The immune response and morphological changes in lymphoid organs (Harderian gland, bursa of fabricius and thymus) of broilers were noted. The role of Harderian gland to generate local and humoral immunity in response to eye drop and drinking water vaccination against NDV was also evaluated. This experiment showed that ocular vaccination resulted in significantly high level of circulating antibodies as compared to drinking water vaccination. No histopathological changes were observed in lymphoid organs after NDV challenge in ocularly vaccinated birds. There were significantly ($P<0.05$) higher number of plasma cells in sections of Harderian gland after eye drop vaccination. It was concluded that ocular vaccination stimulated Harderian gland to produce strong local protective immunity in ocular as well as in oral mucosa.

Key words: Newcastle disease, ocular route, drinking water, lymphoid organs, plasma cells.

INTRODUCTION

Newcastle disease (ND) is one of the most infectious, highly contagious, fatal viral disease of chickens, characterized by respiratory, digestive and nervous symptoms. ND also produces lesions in lymphoid organs i.e. degeneration, depletion and necrosis (Mishra *et al.*, 2000). The total average losses in poultry due to ND are presently estimated to be 40-60% inspite of heavy vaccination and medication schedule. In this scenario, it is a major challenge for poultry experts to device measures for improving efficacy of vaccination by exploiting new programs of vaccination particularly stressing on the importance of immune response due to different routes of immunization.

Immune responses of lymphoid organs to ND vaccine are influenced by routes (ocular, injection, drinking water, aerosol or nasal) of vaccination. Eye drop vaccination stimulates Harderian gland to produce necessary local antibodies (Dohms *et al.*, 1988). Oral vaccination results in significant increase in plasma cells in sections of Harderian gland (Jayawardane and Spradbrow, 1995). These findings suggest an important role for Harderian gland in protective immunity.

Vaccines given through mucosal surfaces (oral, eye drop and nasal) generate mucosal immune response. Also vaccines given through mucosal routes are more effective against viruses entering in the body through mucosal surfaces, such as NDV (Calnek *et al.*, 1997). Therefore, this project was designed for comparison of two major routes (intraocular and drinking water) of vaccination in terms of protection against velogenic strain (GB Texas) isolate by noting immune response and morphological changes in lymphoid organs of broilers.

MATERIALS AND METHODS

Experimental chicks

A total of 160 day-old broiler chicks were procured from local commercial hatchery. The chicks were reared under standard managemental conditions at University of Veterinary and Animal Sciences, Lahore. The chicks were provided feed and water *ad libitum*.

Experimental design

The experiment lasted for 42 days. On day 7, 160 chicks were randomly divided into four major groups i.e. A, B, C and D, containing 40 birds each. Then chicks of former three groups were subdivided into A1, A2, B1, B2, C1, C2 containing 20 birds each on day 28. The experimental design is given in Table 1.

Table 1. Experimental design

Groups	Sub-groups	Vaccination				Infection	
		Eye dropping		Drinking water		Eye dropping	Drinking water
		Day 7	Day 28	Day 7	Day 28		
A	A1	+	+	-	-	+	-
	A2	+	+	-	-	-	+
B	B1	-	-	+	+	+	-
	B2	-	-	+	+	-	+
C	C1	-	-	-	-	+	-
	C2	-	-	-	-	-	+
D	-	-	-	-	-	-	-

Vaccination of experimental chicks

Ampoules of freeze dried ND vaccine LaSota (Vetycare) were reconstituted and administered according to instructions of manufacturer.

Preparation of inoculum

Newcastle disease virus was procured from Veterinary Research Institute, Lahore. Embryo infective dose (EID_{50}) and 100 EID_{50} were determined (Read and Muench, 1938).

Newcastle disease virus challenge

On day 28 birds of subgroups were challenged against NDV inoculum at dose rate of 0.1ml/bird through intraocular and drinking water, as shown in Table-1.

Collection of samples

Blood samples from 5 randomly selected birds of each subgroup were collected on days, 7, 14, 21, 28, 35, 42 and serum was separated for estimation of antibody titers against ND. Lymphoid organs (Harderian gland, bursa of Fabricius and thymus) from 5 randomly selected birds of each subgroup were collected on days 28, 35 and 42 for histopathological studies.

Experimental parameters

Following parameters were studied:

1) Determination of antibody titers against NDV

After collection, serum samples were stored at –20°C till used in HI testing. Prior to conducting HI, the serum samples were thawed. Phosphate buffer saline (PBS) solution having a pH of 7.2 was used in HA and HI tests. Erythrocytes from 6-8 weeks old SPF chicken blood were washed and their 1 percent solution was used in HA and HI tests. The HA and HI tests were conducted according to the protocol described by Allan and Gaugh (1974).

2) Histopathological study of lymphoid organs

Harderian gland, bursa of Fabricius and thymus were processed for histopathological studies (Drury and Willington, 1980).

3) Plasma cell counting in sections of Harderian gland

Plasma cells were counted from 3 fields per area of Harderian gland. The areas included the stroma near the outside of the gland, areas along collecting ducts near the middle of the gland, and the area adjacent to the central collecting duct. Plasma cells were counted in 9 separate Harderian gland fields (200 x). Plasma cell numbers were expressed as plasma cells per mm^2 (Dohms *et al.*, 1988).

4) Post-challenge mortality

Post challenged mortality in chicks of all groups was recorded.

Statistical analysis

The data collected were subjected to statistical analysis by analysis of variance and least significant difference (LSD). Analysis was done using computer based Statistical Analysis System SPSS 10.0, software (SPSS INC, 1996). Significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Newcastle disease continuous to be a major threat to poultry industry despite of wide spread use of different types of vaccinations. One way of controlling this problem is improving efficacy of vaccination by

introducing different routes of immunization. In the present study, two routes (ocular and drinking water) of vaccination against ND were used on 7 and 21 days of age. Geometric mean titre (GMT) of different groups at different days is shown in Table-2. GMT of groups A and B showed a gradual rise in antibody titre from 14th day of age, which reached the peak level at day 28. There was higher rise in antibody titer in group A as compared to group B. This is in line with Nachimuthu *et al.* (1982), Jayawardane and Spradbrow (1995) and Sun *et al.* (1997). Our results are also in line with Tizzard (1996), who reported that gastric secretions provided a non-specific barrier against invaders and destroyed them. Thus, some of the vaccinal virus given through oral route got denatured resulting in reduced antibody titer in group B. Decrease in HI antibody titer was observed in subgroups A1, A2, and B1 and B2 on 35th day of experiment (7 days post challenge), as shown in Table 2. These observations are in coordination with Tizzard (1996), who reported a decrease in antibody titre due to neutralization of virus with circulating antibodies. A sharp rise in antibody titre in sub groups C1 and C2 was observed on 35th day. Tizzard (1996) also reported a rise in antibody titre due to activation of immune system against challenge due to which rise in antibody titre was noted in serum. A sharp rise in antibody titer was also observed in subgroups A1, A2, and B1 and B2 on day 42 (14 days post challenge). Similarly, Manzoor (1999) observed that in vaccinated birds challenged virus was neutralized by circulating antibodies and the immune system was boosted up resulting in increase in antibody titre.

Histopathological changes in sections of Harderian gland, bursa of fabricius and thymus of different groups at day 28 are shown in Table-3. Results showed no histopathological changes in the sections of Harderian gland, bursa of fabricius and thymus of groups A, B, C and D on day 28. Mean plasma cell counts in sections of Harderian gland of different groups at different days are presented in Table-6. On day 28, significantly ($P<0.05$) higher number of plasma cells were seen in stroma of Harderian gland in groups A and B as compared to groups C and D. These results are in line with Jayawardane and Spradbrow (1995), who studied significant increase in plasma cells in sections of Harderian gland after vaccination. Statistical analysis showed that plasma cell count in group A was significantly ($P<0.05$) higher than that of group B. Our results are similar to Survashe and Aitken (1979) and Russell (1993). Statistical analysis also showed that plasma cell count of groups C and D was significantly ($P<0.05$) lower than the plasma cell count of groups A

Table 2. Geometric mean titer (GMT) HI of different groups before and after infection

Groups	(GMT) HI before infection on day				Subgroups	(GMT)HI after infection on day	
	7	14	21	28		35	42
A	10.6	128.0	338.0	477.0	A1	256.0	1024.0
					A2	229.0	891.0
B	9.2	84.4	223.0	337.0	B1	194.0	776.0
					B2	64.0	588.0
C	12.1	8.0	4.6	3.06	C1	36.0	-
					C2	27.0	-
D	6.0	5.27	4.0	2.6	D	2.0	2.0

Table 3. Histopathological changes in Harderian gland, bursa of fabricius and thymus on day 28

Groups	Organs											
	Harderian gland					Bursa of fabricius				Thymus		
	N	↑P	↑L	H	PD	N	↑I.S	E	LD	N	Ne	LD
A	+	+	+	-	-	+	-	-	-	+	-	-
B	+	+	+	-	-	+	-	-	-	+	-	-
C	+	-	-	-	-	+	-	-	-	+	-	-
D	+	-	-	-	-	+	-	-	-	+	-	-

N=Normal, P=Plasma cells, L=Lymphoid follicles, H=Hypraemia P D=Plasma cell degeneration, I.S=Inter follicular space, E=Edema, LD=Lymphoid depletion, Ne=Necrosis, ↑=Increased.

and B. Avram and Bucur (1982) also found low plasma cell count in non-vaccinated group than vaccinated group with lentogenic NDV.

Histopathological changes in Harderian gland, bursa of fabricius and thymus of different groups at days 35 and 42 are shown in Tables 4 and 5. On day 35, no histopathological lesions were noted in Harderian gland of subgroups A2, B2, C2 and D. Hyperemia and vascularization along with increased number of plasma cells were seen in subgroups A1, B1 and C1. Our findings are supported by those of Davelaar and Kouwenhoven (1976) and Dohms *et al.* (1988). Increase in plasma cell number was also observed in orally challenged birds (subgroups A2, B2 and C2) but this increase was not as significant as that of ocularly challenged birds (Table 6). These results are in line with Survashe and Aitken (1979). This was because ocular challenge directly stimulated Harderian gland and led to development of active immunity in this gland. On day 35, no histopathological lesions were seen in

Table 4. Histopathological changes in Harderian gland, bursa of fabricius and thymus on day 35

Subgroups	Organs											
	Harderian gland					Bursa of fabricius				Thymus		
	N	↑P	↑L	H	PD	N	↑IS	E	LD	N	Ne	LD
A1	-	+	+	+	+	+	-	-	-	+	-	-
A2	+	+	+	-	-	+	-	-	-	+	-	-
B1	-	+	+	+	+	+	-	-		+	-	-
B2	+	+	+	-	-	-	+	+	+	-	+	+
C1	-	+	+	+	+	-	+	+	+	-	+	+
C2	-	+	±	±	+	-	+	+	+	-	+	+
D	+	-	-	-	-	+	-	-	-	+	-	-

Table 5. Histopathological changes in Harderian gland, bursa of fabricius and thymus on day 42

Subgroups	Organs											
	Harderian gland					Bursa of fabricius				Thymus		
	N	↑P	↑L	H	PD	N	↑IS	E	LD	N	Ne	LD
A1	±	+	±	-	±	+	-	-	-	+	-	-
A2	+	±	±	-	-	+	-	-	-	+	-	-
B1	±	±	±	-	±	+	-	-	-	+	-	-
B2	+	±	±	-	-	-	±	±	±	-	±	±
C1	-	-	-	-	-	-	-	-	-	-	-	-
C2	+	-	-	-	-	-	-	-	-	-	-	-
D	+	-	-	-	-	+	-	-	-	+	-	-

Table 6. Mean (±S.E.) values of plasma cells in Harderian gland (cells/mm^2)

Groups	Plasma cells counted before infection	Subgroups	Plasma cells counted after infection	
	Day 28		Day 35	Day 42
A	7104 ± 6.33	A1	8948 ± 73.37	9904 ± 17.20
		A2	8280 ± 116.79	9440 ± 26.83
B	4656 ± 34.29	B1	7216 ± 50.35	8004 ± 192.91
		B2	6608 ± 2.61	7242 ± 118.76
C	2500 ± 90.55	C1	5108 ± 59.86	-
		C2	4352 ± 62.80	-
D	2424 ± 117.20	D	1840 ± 34.05	1448 ± 76.56

Table 7. Mortality percentage of birds of different groups after infection

Subgroups	Total birds	Live birds	Dead birds	Mortality (%)
A1	20	20	0	0
A2	20	20	0	0
B1	20	20	0	0
B2	20	18	2	10
C1	20	5	15	75
C2	20	1	19	95
D	20	20	0	0

bursa of fabricius and thymus of subgroups A1, A2, B1 and D, while severe lesions were seen in subgroups C1 and C2 (Table 4). Interfollicular edema and necrosis of lymphoid follicles were seen in bursa of Fabricius while thymus showed necrotic centers and less dense population of lymphocytes. A few birds of subgroup B2 also showed mild degree of same lesions. Our results are similar to Mishra *et al.* (2000), who noted necrosis and degeneration in bursa of fabricius and thymus after NDV challenge.

Post challenge mortality percentage of different groups is shown in Table-7. After day 28, in subgroups A1 and A2 no mortality was noted. It indicated that ocular vaccination had given 100% protection. Our results are similar to Winterfield *et al.* (1980) and Musa (1984). No mortality was noted in subgroup B1. In subgroups B2, C1 and C2 10, 75 and 95% post challenge mortality was noted, respectively. Our results are in line with Ibrahim *et al.* (1980).

This study leads to the conclusion that ocular vaccination results in good local protective immune response and does not result in any pathological change in lymphoid organs.

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