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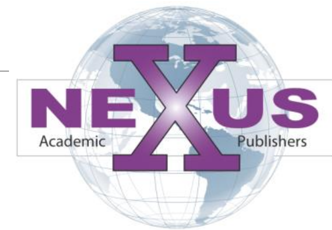

Research Article

Segregation of Plasma Cells in Lymphoid Organs by Various Routes of Vaccination against Newcastle Disease in Broiler Chickens

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ABSTRACT

One hundred ninety five (195) chicks were used to collect three different lymphoid organs i.e Thymus, Bursa of Fabricious and Harderian gland after vaccinating with Newcastle disease virus to observe the histopathological changes. The chicks were divided into three groups and vaccinated via coarse spry, eye drop and drinking water at the age of five days and then boasted at twenty six days of age. The histopathological changes show high number of Plasma cells in these lymphoid organs. The segregation of plasma cells was significantly higher in Harderian gland using NDV vaccine by eye drop method. All of the three routes of immunization provided comparable protection against the challenged infection at 6 weeks of age. However, the coarse spray method of vaccination provided significantly high level of circulating antibodies as compared to ocular and drinking water vaccination.

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INTRODUCTION

Newcastle disease (ND) is an infectious, highly contagious and widespread disease of avian species. It has a considerable economic impact on poultry industry, especially chickens. Since its recognition in 1926 in Indonesia and Newcastle-upon-Tyne, England also occurred in Ranikhet, India in 1927, ND is regarded as being endemic in many countries (Bwala et al., 2009). The morbidity and mortality of susceptible birds is 100% in the severe form of the disease and unvaccinated birds are more prone to the disease (Alexander, 2003; Manchang et al., 2004). The poultry industry loses millions of dollars annually from mortality due to ND. Its importance is mainly due to the resulting economic losses that occur upon infection with virulent strains (Susta et al., 2011; Diel et al., 2012).

Immune responses of lymphoid organs to ND vaccine are influenced by routes (ocular, injection, drinking water, aerosol or nasal) of vaccination. Vaccines given through mucosalsurfaces (oral, eye drop and nasal) generate mucosal immune responses so they are more effective routes of vaccination for viruses entering in the body through mucosal surfaces, such as NDV (Salam, 2003). As the vaccinal strain is deposited directly onto respiratory mucosa, the spray route is suitable for respiratory type vaccines. Coarse spray is preferred vaccination method as it is proposed to deliver large droplets of vaccine to the upper respiratory tract and eye. In addition the moistened feathers of birds stimulating preening which further increase the chance of vaccine uptake (Breytenbach, 2005).Giambrone (1985) reported that birds vaccinated with a live vaccine by coarse spray have highest NDV HI titers; resistance to challenge is also greatest. Eye drop or oral administration of NDV vaccines were also proved to be effective in eliciting mucosal immunity mediated by IgA antibodies as reported by (Jayawardane and Spradbrow, 1995; Parry and Aitken, 1973). Inactivated vaccines do not induce local immunity in the respiratory and digestive tracts; however, immunity is established rather slowly. Also the killed vaccines are expensive and difficult to administer than live vaccines (Van Eck, 1987).

The humoral and cell-mediated immunity acquired by vaccination play vital role for protecting the birds against field infection (Chandraseker et al., 1989). Live vaccines provide better protection as they provide instant protection to field virus challenge on later exposure. A vaccine that induces an immune response by uplifting mucosal immunity is a valuable way of targeting the respiratory pathogens before infection occurs (Brandtzaeg, 2004; Holmgren, 2005). Mucosal vaccination, in contrast to parenteral vaccination can produce both systemic and mucosal immunity by secreting both IgA and IgG immunoglobulins whereas parenteral vaccines only induce serum IgG antibodies (Yuki, 2003). This study was carried out for comparing the immune status of the broiler chickens and the morphological changes in lymphoid organs and its role, if any, to elicit the antibody response and to assess the efficacy of various routes of vaccination.

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Table1. Experimental Design

Groups	Subgroups	Rotes of VACCINATION						ChallengedLD 50		
		Spray		Ocular		D/W		Spray	Ocular	D/W
		5	26	5	26	5	26		26	
A	A1	+	+					+		
	A2	+	+						+	
	A3	+	+							+
B	B1			+	+			+		
	B2			+	+				+	
	B3			+	+					+
C	C1					+	+	+		
	C2					+	+		+	
	C3					+	+			+
D	D1	-	-	-	-	-	-	+	ND	ND
	D2	-	-	-	-	-	-	ND	+	ND
	D3	-	-	-	-	-	-	ND	ND	+
E		-	-	-	-	-	-	-	-	-

Note: ND: Not done

MATERIAL AND METHOD

Experimental Chicks

Day- old broiler chicks (n= 195) were procured from local commercial hatchery. They were brooded for 5 days in a semi specified pathogen free area at the University of Veterinary and Animal Sciences, Lahore.

Vaccination of Experimental Chicks

A commercially manufactured LaSota virus vaccine was purchased from the local market and administered via spray, eye droppings and drinking water route (Table-1).

Challenge Virus

A virulent field isolate of velogenic NDV (vNDV) previously characterized as velogenic by the research workers (unpublished data) in the (Quality Operation Laboratory), University of Veterinary and Animal Sciences, Lahore was selected. The biological titer of the virus was $10^{5.5}$ LD50 and the challenge dose was 0.1 ml (100 × LD50) per bird.

On day 26, all experimental birds were challenged with velogenic NDV at dose rate of 0.1ml/ bird through Eye drop, drinking water and spray as shown in the Table 1.

Experimental Design

The experiment lasted for 42 days. On day 5, birds were individually identified with numbered wing tags after being randomly divided into four treatment groups i.e. A, B, C, D and one control group E containing 45 birds each. Then chicks of former four groups were subdivided into A1, A2,A3, B1, B2, B3,C1, C2,C3,D1,D2,and D3 containing 15 birds each on day 26. The experiment design is given in Table 1.

Collection of Samples

Blood samples were collected from 5 birds of each subgroup selected randomly on days, 5, 12, 19, 26, 33, 40 and serum samples were separated to assess the antibody titers against NDV. Lymphoid organs (Harderian gland, Bursa of Fabricius and thymus) from 5 randomly selected birds of each subgroup were collected on days 26, 33, 40 for histo-pathological studies.

Antibody titration against NDV

After collection, serum samples were stored at -40 C till used. Antibody titer against NDV in serum was determined by haemagglutination inhibition (HI) test as described by (Allan and Gough, 1974).

Histopathology of Lymphoid Organs

Harderian Gland, bursa of Fabricius and thymus were collected and kept in 10% Neutral Buffered Formalin (NBF) in pre labeled containers. They were allowed to fix for 48 hours and were processed for histopathological studies (Nakamura et al., 2008; Bwala et al., 2012; Zakeri and Kashefi, 2012).

Plasma cell counting in Harderian gland

Plasma cells were counted in separate Harderian gland fields (200 x). Plasma cells numbers were expressed as Plasma cells per mm2 (Salam, 2003).

Post-challenge Mortality

Post-challenge mortality was recorded in all experimental birds. The symptoms and lesions were recorded by postmortem examination of the dead birds.

Statistical analysis

The data collected was statistically analyzed by Analysis of Variance and least significant difference (LSD) using SPSS 16.0 software , significance was accepted at $P<0.05$.

RESULTS AND DISCUSSION

Newcastle disease continues to be a major threat to the poultry industry despite the wide spread use of various vaccines and route of vaccination. One way of controlling this problem is to improve the efficacy of vaccines by experimenting different routes of immunization. In the present study, three routes (spray, ocular and drinking

Table 2: Geometric Mean titer (GMT) HI of different groups before and after infection

(GMT) HI	At day	Groups: Spray	Eye Drop	Drinking water	Control	E
Prior To Challenge	5	10.3	9.1	8.4	12	5.7
	12	129.0	82.1	70.5	7.8	4.8
	19	340.3	219.8	158.9	5.0	3.9
	26	482.1	335.0	254.7	2.9	2.5

(GMT) HI	At day	Subgroups: A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E
Post Challenged	33	260.0	230.2	195.7	184.2	64.0	43.9	156.9	56.2	32.0	37.0	23.6	-	1.8
	40	1024.0	895.0	793.9	778.3	622.5	588.1	698.3	545.9	476.7	-	-	-	1.7

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Table 3: Histopathological changes in Bursa of Fabricius, Thymus and Harderian gland on day 26.

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

N= Normal; PD= Plasma cell degeneration; L= Lymphoid follicals; I.S= Interfollicular space; E= edema; LD= Lymphoid depletion; P= Plasma cells; H= Hyperemia; Ne= Necrosis.

Table 4: Histopathological changes Bursa of Fabricius, Thymus and Harderian gland on day 33.

Organs		Subgroups												
		A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E
Bursa of Fabricius	N	-	+	+	±	+	+	-	+	-	-	-	-	+
	E	+	-	+	+	-	-	±	-	+	+	+	+	-
	LD	+	-	-	-	-	-	±	-	+	+	+	+	-
	↑I.S	+	-	-	-	-	-	+	-	+	+	+	+	-
Thymus	N	-	+	+	-	+	+	-	+	-	-	-	-	+
	LD	+	-	-	±	-	-	+	-	+	+	+	+	-
	Ne	-	-	-	-	-	-	-	-	+	+	±	+	-
Harderian gland	N	-	-	+	-	-	+	-	-	+	-	-	-	+
	H	+	+	-	+	+	-	+	+	-	+	+	±	-
	PD	+	+	-	+	+	-	±	+	-	+	+	+	-
	↑P	+	+	+	+	+	+	+	+	+	+	+	+	-
	↑L	+	+	+	+	+	+	+	+	+	+	+	±	-

Table 5: Histopathological changes in Bursa of Fabricius, Thymus and Harderian gland on day 40.

Organs		Subgroups												
		A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E
Bursa of Fabricius	N	+	+	+	+	+	+	+	+	-	-	-	-	+
	E	-	-	-	-	-	-	-	-	±	-	-	-	-
	LD	-	-	-	-	-	-	-	-	±	-	-	-	-
	↑I.S	-	-	-	-	-	-	-	-	±	-	-	-	-
Thymus	N	+	+	+	+	+	+	+	+	-	-	-	-	+
	LD	-	-	-	-	-	-	-	-	±	-	-	-	-
	Ne	-	-	-	-	-	-	-	-	±	-	-	-	-
Harderian gland	N	+	+	+	±	+	+	±	±	+	-	-	+	+
	H	-	-	-	-	-	-	-	-	-	-	-	-	-
	PD	-	±	±	±	±	-	-	±	-	-	-	-	-
	↑P	+	+	+	+	+	±	±	±	±	-	-	-	-
	↑L	+	+	+	±	±	±	±	±	±	-	-	-	-

water) of vaccination against ND were used on 5 and 26 days of age. Geometric mean titers (GMT) of different groups at different days are shown in table 2. GMT of groups A, B and C showed a rise in antibody titer from 12th day of age, which reached the peak level at day 26. There was higher rise in antibody titer in group A and B as compared to group C as 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 C (Tizard, 1996). Decrease in HI antibody titer was observed in subgroups A1, A2, A3, B1, B2, B3, C1, C2 and C3 and sharp

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rise in antibody titer in sub groups D1, D2 and D3 on 33th day of experiment (7 day post challenge), as shown in Table 2. These observations are in coordination with (Tizard, 1996) who described a decrease in antibody titer due to neutralization of virus with circulating antibodies and rise in antibody titer due to activation of immune system against challenge due to which rise in antibody titer noted in serum.

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

Groups	At day	Plasma Cells (cells / mm^2) counted before infection
A	26	8253 ± 7.59
B		7150 ± 6.50
C		4678 ± 46.30
D		2650 ± 80.96
E		2290 ± 120.90
	Plasma Cells (cells / mm^2) counted after infection	
Subgroups	At day 33	At day 40
A1	9886± 86.70	9991± 23.79
A2	9456± 115.77	9956± 26.70
A3	9180± 50.38	9489± 87.71
B1	8956± 76.37	9257± 15.77
B2	8260± 26.10	8956± 36.80
B3	7956± 80.09	8999± 67.47
C1	6956± 2.76	7796± 16.20
C2	5956± 50.67	6986± 39.07
C3	5236± 22.89	6160± 59.19
D1	4563± 16.43	-
D2	3569± 56.75	-
D3	2563± 45.38	-
E	1360± 17.90	8563± 69.08

A sharp rise in antibody titer was also observed in subgroups A1, A2, A3, B1, B2, B3, C1, C2 and C3 on day 40 (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 titer. However, no histopathological changes were shown in parts of Harderian gland, bursa of fabricius and thymus of groups A, B, C, D and E on day 26, Table 3.

Table 7: Mortality percentages of birds of different groups after infection

Subgroups	Live Birds/Total Birds	Mortality (%)
A1	15/15	0
A2	15/15	0
A3	15/15	0
B1	15/15	0
B2	14/15	6.7
B3	14/15	6.7
C1	13/15	13.3
C2	15	13.3
C3	15	20.0
D1	15	93.33
D2	15	86.66
D3	15	73.33
E	15	0

Histopathological changes in Harderian gland, bursa of fabricius and thymus of different groups at day 33 and 40 are shown in Tables 4 and 5. On day 33, no histopathological lesions were noted in Harderian Gland of Subgroups A3, B3, C3 and D3 and E. On day 33, no histopathological lesions were seen in bursa of fabricius and thymus of subgroups B2, B3, C2 and E, while severe lesions were seen in subgroups D1, D2 and D3 (Table 4). Histopathological lesions in Harderian Gland, Interfollicular edema and necrosis of lymphoid follicles were seen in bursa of Fabricius while thymus showed necrotic centers and less dense population of lymphocytes in subgroups A1, A2 and A3. A few birds of subgroup C3 also showed mild degree of same lesions. Our results are similar to previous reports (Salam, 2003; Giambrone, 1985) Who noted necrosis and degeneration in bursa of fabricius and thymus after NDV challenge.

Mean plasma cell counts in sections of Harderian gland of different groups at different days are presented in Table-6. On day 26, significantly ($P<0.05$) higher number of Plasma cells were seen in stroma of Harderian gland in groups A, B and C as compared to D and E. These results are in line with the findings of (Jayawardane and spradbrow, 1995; Giambrone, 1985) who studied significant increase in plasma cells in sections of Harderian gland after vaccinations. Plasma cell count in group A was significantly ($P<0.05$) higher than that of group B and C. Hyperemia and vascularization along with increased number of plasma cells were seen in subgroups A1, A2, B1, B2, C1, C2, D1 and D2. Our findings are supported by those of (Salam, 2003). Increase in plasma cell number was also observed in orally challenged birds (Subgroups A3, B3, C3 and D3) but this increase was not as significant as that of coarse spray challenged birds (Table 6).

No mortality at twenty six (26) days post challenge of different subgroups A1, A2, A3 and B1 was recorded. This shows that the spray method of vaccination not only provides good protection against field challenge but also no pathological changes observed in the lymphoid organs.

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