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COMPARATIVE IMMUNOPATHOLOGICAL AND IMMUNOSUPPRESSIVE EFFECTS OF THREE DIFFERENT GUMBORO VACCINE STRAINS AGAINST NEWCASTLE DISEASE VACCINATION IN BROILERS

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ABSTRACT

This project was carried out to study the comparative immunosuppressive effects of three different Gumboro live vaccine strains on Newcastle disease (ND) vaccination. A total of 100 chicks were divided into four equal groups (A, B, C and D). Birds of all the groups were vaccinated against ND on 5th and 21st day of age. Specific Gumboro vaccine strain was given to specific group at 14th and 28th day of age. Group A, B and C were vaccinated with 228-E, D-78 and Bursine-2, respectively, while group D was kept as control. Immune organs including bursa, thymus and spleen were examined for their gross and histopathological changes, before and after Infectious Bursal disease (IBD) vaccination. For this purpose, these organs were collected at 13th, 17th and 31st days of age. At 13th day (before IBD vaccination) no gross and histopathological lesions were observed in any bird of any group. At 17th and 31st day, severe lesions were noted in group A, moderate lesions in group B, mild lesions in group C and no lesions were observed in immune organs of group D. Haemagglutination inhibition (HI) test showed that 228-E vaccine strain (group A) was highly immunosuppressive, D-78 vaccine strain (group B) was less immunosuppressive while Bursine-2 vaccine strain (group C) was least immunosuppressive. No humoral immunosuppression was observed in unvaccinated control group D. This study suggests the use of Bursine-2 strain of IBD vaccine in a flock having risk of ND infection, as it has least immunosuppressive effect against ND vaccination.

Key words: Gumboro vaccines, immunosuppression, Newcastle disease, broilers.

INTRODUCTION

Newcastle disease (ND) is the most prevalent and devastating disease of birds of all ages occurring throughout the year (Jaffery, 1981). For controlling ND, vaccination programmes are strictly carried out in poultry farms. It is necessary to chalk out different factors which have immunosuppressive effects on ND vaccination. Infectious Bursal agent (IBA) reduces the immunological responses to ND vaccine as well as increases susceptibility of chickens to ND infection, despite prior ND vaccination (Allan *et al*., 1972). IBD appears to be worldwide and is recognized as of considerable economic significance (Winterfield, 1969), because immunodeppressive effects of IBD virus may result in economic losses to the poultry industry. Protection against IBD is an essential component of health-management protocols in commercial chicken flocks (Sharma *et al*., 1989). Live virus IBD vaccines have been used extensively for well over a decade (Snedeker *et al*., 1967; Winterfield, 1969). Several vaccines have been developed, but most of them produce, at least, a mild form of the disease having an effect on the bursa of Fabricius, which can lead to immunosuppression (Cessi and Nardelli, 1974; Box and Farminger, 1975; Baxendale, 1976). The immunity to ND is suppressed by infection with Gumboro disease and to some extent also in chickens vaccinated against Gumboro disease (Mallick, 1978). All the IBD vaccines have an immunosuppressive effect on HI antibody titre against ND vaccination (Khan *et al*., 1998). So this project was planned to study the comparative immunosuppressive effects of three different Gumboro live vaccine strains on ND vaccination in broilers.

MATERIALS AND METHODS

Experimental birds

One hundred day-old broiler chicks were procured from a local hatchery. These birds were reared in the of Pathology Department, University of Veterinary and Animal Sciences Lahore, under standard managemental conditions. They were given feed and water *ad-libitum*.

Experimental design

Experiment was carried out for 35 days.

Vaccines

Experimental birds were divided into four groups, A, B, C and D, each group comprising of 25 birds. Group A was given Gumboro live vaccine intermediate-plus strain 228-E, group B vaccine strain D-78 and group C strain Bursine-2, while group D was kept as control. These Gumboro vaccines were given at day 14 (as priming dose) and at day 28 (as booster dose) in respective groups. All the four groups were given ND vaccine at day 5 (as priming dose) and at day 21 (as booster dose).

Experimental parameters

Haemagglutinition inhibition (HI) antibody titre against ND vaccination was carried out (Allan and Gough, 1974). For this purpose, blood and then serum was collected (from 8 birds from each group) at days 13, 20, 27 and 35.

Histopathological study of immune organs (bursa, thymus and spleen) was also carried out (Drury and Wallington, 1980). For this purpose, these immune organs were collected at days 13, 17 and 31 of age.

Statistical analysis

Data collected from HI antibody titre were subjected to statistical analysis by applying paired t-test, one-way analysis of variance and Least Significant Difference test (Steel and Torrie, 1980).

RESULTS

Haemagglutination inhibition test

The data collected in both HI observations i.e. first and second HI observations (Table 1) showed that there was a statistically significant difference ($P<0.05$) in GMT of anti-ND HI titres of groups A, B and C before and after IBD vaccination. This indicates that all the IBD vaccine strains had immunosuppressive effects as evident from reduced anti-ND titres after vaccination. Similarly, it was found that there was a significant difference among all these groups (A, B and C) as well, which showed that all the IBD vaccine strains varied in their immunosuppressive effects against ND vaccination.

Examination of immune organs

No gross and histopathological lesions were noted on day 1. Plate 1 is from normal bursa showing normal epithelium (group D). However in Plate 2 severely hyperplastic epithelium is visible in bursa of chicks of group A.

Bursa of Fabricius

Gross and histopathological changes of bursa of Fabricius of all the groups are presented in Tables 2 and 3, respectively. Generally, in groups A, B and C, these lesions were of severe (BLS=4), marked (BLS=3) and moderate (BLS=2) levels respectively. While in group D no lesion (BLS=0) was observed. Bursa showed presence of haemorrhages, follicular hypertrophy, swollen bursal folds and presence of fluid. Histopathological lesions included lymphoid necrosis, epithelial hyperplasia, inter-epithelial cyst formation, macrophage infiltration, bursal atrophy, oedematous fluid presence, lymphoid depletion and fibrous tissue presence.

Thymus

Gross and histopathological changes of thymus of all the groups are presented in Table 4. Generally in groups A, B and C histopathological lesions were of marked (TLS=3), moderate (TLS=2) and mild (TLS=1) levels, respectively. Group D was normal (TLS=0). Grossly, thymus glands were examined for hypertrophy and haemorrhage while histopathologically presence

Table 1: Geometric mean titres (GMT) of anti-ND HI antibody titres of various groups (first and second HI observations)

Group	Vaccines	First HI observation		Second HI observation	
		GMT at day 13 (before Ist IBD vaccination)	GMT at day 20 (after Ist IBD vaccination)	GMT at day 27 (before 2nd IBD vaccination)	GMT at day 35 (after 2nd IBD vaccination)
A	228-E	64^{a}	2.6^{b}	20.7^{e}	2.8^{h}
B	D-78	64^{a}	24.8^{c}	165.9^{f}	107.6^{i}
C	Bursine-2	64^{a}	53.8^{d}	331.9^{g}	215.2^{j}
D	Unvaccinated	64^{a}	98.7^{a}	255.9^{g}	331.9^{g}

Values having different superscripts differ significantly (both row wise and column wise) from each others ($P<0.05$).

Plate 1: Normal bursa of Fabricius of chickens of group D showing normal epithelium (H & E staining, 400x)

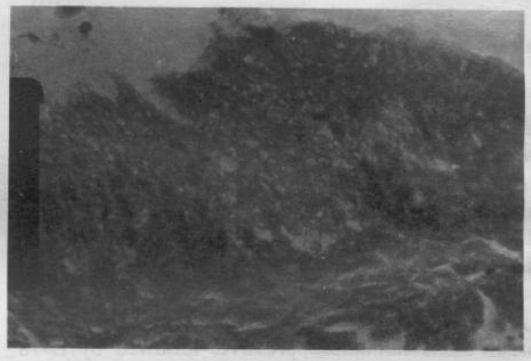

Plate 2: Bursa of Fabricius of chickens of group A showing severely hyperplastic thickened epithelium (H & E staining, 400 x).

Table 2: Gross bursal lesions scores in bursa of Fabricius of various groups after first and second IBD vaccination

Day	Group	Vaccine strain	Haemorrhage	Hypertrophy	Swollen bursal folds	Fluid presence
Day 17	A	228-E	3	1	4	4
	B	D-78	2	0	3	3
	C	Bursine-2	1	0	1	2
	D	Unvaccinated	0	0	0	0
Day 31	A	228-E	3	1	4	4
	B	D-78	2	0	3	3
	C	Bursine-2	1	0	1	2
	D	Unvaccinated	0	0	0	0

0 = No lesion 1 = Mild lesions 2 = Moderate lesions
3 = Marked lesions 4 = Severe lesions

Table 3: Histopathological bursal lesions scores in bursa of Fabricius of various groups post first and second IBD vaccination

Day	Group	Vaccine strain	LN	LD	EH	FT	CF	MP	BA	OF
Day 17	A	228-E	4	3	4	3	4	4	4	3
	B	D-78	3	2	2	2	0	2	2	2
	C	Bursine-2	2	1	0	0	0	2	0	0
	D	Unvaccinated	0	0	0	0	0	0	0	0
Day 31	A	228-E	4	3	4	3	4	4	3	3
	B	D-78	3	2	2	2	0	2	2	2
	C	Bursine-2	2	1	0	0	0	2	0	0
	D	Unvaccinated	0	0	0	0	0	0	0	0

LN = Lymphoid necrosis LD = Lymphoid depletion EH = Epithelial hyperplasia
FT = Fibrous tissue presence CF = Cyst formation MP = Mononuclear cellular presence
BA = Bursal atrophy OF = Oedematous fluid
0 = No lesions 1 = Mild lesions 2 = Moderate lesions
3 = Marked lesions 4 = Severe lesions

of macrophage and oedematous fluid was noted. No hypertrophy was seen in any group. Severe haemorrhage (TLS=4) was noted in groups A and B; while marked haemorrhages (TLS=3) were seen in group C.

Spleen

Gross and histopathological changes of spleen of all the groups are presented in Table 5. Grossly, spleens were examined for haemorrhage and hypertrophy. Gross lesions (SLS=0) were not observed in any group. Histopathological lesions included macrophage and fluid presence. These lesions were of moderate (SLS=2) levels in only group A. While no lesion (SLS=0) was observed in all the other groups (B, C and D).

Overall results showed that vaccine strain 228-E was the most immunosuppressive, followed by D-78 and Bursine-2 strain.

DISCUSSION

In the present study, there was a significant difference ($P<0.05$) in the GMT values of groups A, B and C before and after first IBD vaccination at day 13 and second IBD vaccination at day 28, which showed humoral immunosuppression as evident through decreased H1 antibody titre after IBD vaccination. There was no difference in the GMT values of group D before and after vaccination which showed no humoral immunosuppression. Muskett *et al.* (1979), Lucio and Hitchner (1980), Edwards *et al.* (1982) and Reece *et al.* (1982) reported humoral immunosuppressive effects of Gumboro vaccination against ND vaccination. When the comparison was done on the basis of statistical analysis of data, it was found that two intermediate IBDV vaccines used in the present study, D-78 and Bursine-2 strain, varied significantly in their immunosuppressive effects. The D-78 strain was more

Table 4: Gross and histopathological lesions in thymus of various groups post first and second IBD vaccination

Day	Group	Vaccine strain	Gross lesions		Histopathological lesions	
			HY	HM	MP	FP
Day 17	A	228-E	0	4	3	2
	B	D-78	0	4	2	3
	C	Bursine-2	0	3	1	1
	D	Unvaccinated	0	0	0	0
Day 31	A	228-E	0	4	3	2
	B	D-78	0	4	2	3
	C	Bursine-2	0	3	1	1
	D	Unvaccinated	0	0	0	0

Table 5: Gross and histopathological lesions in spleen of various groups post first and second IBD vaccination

Day	Group	Vaccine strain	Gross lesions		Histopathological lesions	
			HY	HM	MP	FP
DAY 17	A	228-E	0	0	2	2
	B	D-78	0	0	0	0
	C	Bursine-2	0	0	0	0
	D	Unvaccinated	0	0	0	0
DAY 31	A	228-E	0	0	2	2
	B	D-78	0	0	0	0
	C	Bursine-2	0	0	0	0
	D	Unvaccinated	0	0	0	0

Keys for Tables 4 and 5

HY = Hypertrophy
HM = Haemorrhage
MP = Macrophage presence
FP= Fluid presence
0 = No lesions
1 = Mild lesions
2 = Moderate lesions
4 = Severe lesions
3 = Marked lesions

immunosuppressive than Bursine-2 strain. In this regard, Mazariegos *et al.* (1990) reported that intermediate vaccine strains were highly variable in their virulence and immunosuppressive properties. In the present study high immunosuppressive effects of 228-E strain, moderate immunosuppressive effects of D-78 strain and mild immunosuppressive effects of Bursine-2 strain were observed. These variations of different Gumboro vaccine strains are in line with the results found by Thangavelu *et al.* (1998), who reported that some strains of IBD live virus vaccines were highly immunosuppressive, some were moderately and some others were mildly immunosuppressive.

The highly immunosuppressive effects of D-78 strain of IBD vaccine, found in the present study, are similar to those reported by Thangavelu *et al.* (1998), who reported that D-78 vaccine strain greatly depressed HI antibody response to ND virus. Mousa *et al.* (1988) reported that Intervet D-78 produced mild bursal lesions and was not immunosuppressive; but highly immunogenic in both immune and susceptible chicks.

Histopathological study of immune organs showed that all the IBD vaccine strains damaged bursa of Fabricius and other immune organs variably. Mousa *et al.* (1988) reported that different viruses varied widely in pathogenicity in terms of bursal damage. Bursal lesions in various IBD vaccines treated groups included infiltration of deep staining mononuclear cells, severe depletion of cells from medullary areas of the follicles, obvious infiltration of larger mononuclear cells and heavily infiltrated interlobular septae with mononuclear cells. These results are in accordance with those of Ezeokoli *et al.* (1990). Similarly, hyperplasia of the epithelial cells of the bursa and cyst formation was also observed which are in line with Winterfield and Thacker (1978).

The histopathological damage of both thymus and spleen was less extensive than in the bursa of Fabricius. These findings are in agreement with the observations of Ezeokoli *et al.* (1990).

Contrary to the findings of the present study, Zanella *et al.* (1977) have reported that the Gumboro vaccine virus 1-65 pv did not suppress the immune response to ND virus. Rhee *et al.* (1985) indicated that the vaccine did not damage the bursa of Fabricius. This may be related to the specific nature of the strain used as vaccine.

On the basis of observations of the present study, it is suggested that different IBD virus strains may have varying indications for field use, depending upon circumstances and requirements. The need exists for effective IBD vaccine, low in virulence, which can be applied by a mass vaccination procedure. Such a vaccine would minimize immunosuppression (Winterfield *et al.*, 1978) and also immunize young chicks possessing passively conferred IBD immunity (Winterfield and Thacker, 1978).

The present study suggests the careful choice of IBD live vaccine strain particularly in the flock already at the risk of ND. It is concluded that Bursine-2 strain of IBD live virus vaccine can be used in a flock having risk of ND, on account of it's least immunopathological effects on immune organs and least immunosuppressive effects on humoral immune response to HI antibody titre against ND vaccination.

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