PATHOGENIC PROPERTIES OF INFECTIOUS BURSAL DISEASE VACCINES

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ABSTRACT

The study was conducted to test the pathogenic effect of six commercially available infectious bursal disease (IBD) vaccines claimed to be intermediate in their pathogenicity. Three week old chickens were inoculated with these vaccines. The pathogenic effects of the IBD vaccines were evaluated by hemorrhages on the thigh and breast muscles, bursa weight to body weight ratio and virulence; two of the strain were found to be highly virulent; two others were moderate and two could be classified as mild.

Key words: Gumboro, Vaccine, Pathogenicity

INTRODUCTION

Infectious bursal disease (IBD) is an acute and highly contagious viral disease of young chickens having immunosuppressive effect. The virus has lymphoid tissue as its primary target with a special predilection of bursa of Fibricious (Tsukomoto *et al.*, 1995; Reddy *et al.*, 1997). Other lesions inlcude hemorrhages on thigh and breast muscles (Singh and Dhawedkar, 1997). Chicken of 3 to 6 week of age are most susceptible to clinical infection (Ley *et al.*, 1983). The birds infected with IBD at an early age become more prone to other infections (Parveen *et al.*, 1995). The effective mean of controlling IBD is vaccination. At present, 14 live IBD vaccines are imported by Pakistan. Of which six intermediate vaccine strains are most commonly used in Pakistan (Ahmad, 1999). The intermediate vaccine strains have become popular as they can be used in the presence of maternal antibodies. But experimental results reflected that vaccination of susceptible chickens with such vaccines caused outbreaks of IBD (Winterfeild and Thacker, 1978). Similar results have also been reported by Naqi *et al.* (1980) with three commercially available IBD vaccines in USA. Post vaccination outbreaks of IBD on the commercial poultry farms were recorded and diagnosed on the basis of postmartm observations, increased bursal weight to body weight ratio and agar gel precipitation test (Muhammad *et al.*, 1996). The present studies have been designed to test the pathogenic properties of the most commonly used six intermediate IBD vaccines (after a field survey) in three-week-old chickens.

MATERIALS AND METHODS

Vaccine strains

For this study, six commercial available IBD vaccines 228-E, D-78, D.S. Gumbovac, Bursine plus, IBD vac and Bio Gumboro were obtained from the market and were tested for their virulence capability. These were designated as A, B, C, D, E and F., respectively.

Birds and Housing

A total of two hundred and ten, three-week-old chicks free from precipitating antibodies against IBDV, were kept at the experiment unit of Department of Microbiology, University of Agriculture, Faisalabad.

Experimental Design

A total of 210, three-week-old chicken, free from precipitating antibodies against IBDV were equally divided into seven groups A, B, C, D, E, F and G and kept separately. The six group of chickens were inoculated subcutaneously with the intermediate strains namely A, B, C, D, E, and F, respectively, whereas G was kept as unvaccinated control. For the comparative pathogenesis study samples of bursa and muscles from 5 birds each at 24 hours interval upto 96 hours, 7th and 10th day post inoculation (PI) of IBD vaccines were observed for the development of clinical sign and collected for histopathological studies (Sing and Dhawedhar, 1997). Bursa to body weight index (B:BW) was determined for each group of 5 birds and their means were determined as described by Lucio and Hitchner (1980).

RESULTS

Gross Pathological Changes

No significant changes in A,B,C,D,E, and F at 24 hours post inoculation were seen. The changes were seen at 48 hours at 10th day PI in group A to E as compared to control. These changes included

enlargement, hemorrhages and edematous fluid. The birds inoculated with these intermediate vaccines showed significant changes in group B,C,A, F, D and E as compared with the control group as shown in Table 1. Vaccine B and C had significantly higher B: BW index than others.

Muscles

The hemorrhages in thigh and breast mscles started at 24 hours PI to the 10th day in groups (A to E) inoculated with A, B, C, D, E and F, respectively. The intesntiy of changes were relatively lower in birds of groups A and F whereas in group B and C, the intensity was high and still persistent at day 10th, while the lesions in the birds of group D and E were mild and no lesions were in control group (G).

Muscles

The changes started in the muscles of all groups at 24 hours post inoculation as compared to contol group. The changes were congestion, hemorrheges, hyalimization, dispersed muscles fibers and blood cells in between muscle fibers.

Bursa body weight ratio

Five birds from each group (A to E and G) were slaughtered at 24 to 96 hours and at 7th and 10th days of post inoculation for bursa body weight index. Actual weight (g) of the organs were recorded in half an hours of slaughtering. The results of B:BW index are shown in Table 2 . In group B and C, B:BW index increased significantly after PI whereas the index in group A and F was less than those of group B and C, while in group D and E the index was nearly equal to that of control group.

Table 1: Bursal gross lesions of chickens receiving different treatment with IBDV vaccines

Time (PI)	A	B	C	D	E	F	G
24 hrs	SE	SE	SE	N	SE	SE	N
48 hrs	M	SE	SE	SE	SE, ED	SE	N
72 hrs	MH, M	MH, E, ED	ED, MH	MH	SE, ED, MH	MH, E	N
96 hrs	MH, ME	E, ED, MH	E, MH, ED	MH	ED, E, KH	MH, E, ED	N
7th day	MH, E	KE, E	SH, E, ED	MH, E	ED, E, SH	KH, ED, E	SE
10th day	KH, ED	SH	SH, E, ED	KH, E	SH, E	SH, ED	SE

Slightly enlarged = SR; Mild enlarged = M; Moderately enlarge =ME
Enlarged = E; Mild hemorrhanged = MH; Marked hemorrhage = KH
Severe hemorrhage = SH; Edematous = ED; Normal = N

Histophathology

The tissue samples of bursa, and muscles were processed in acetone, benzene and embedded in parffin wax. 4-5 µm thick sections were cut and stained by hematoxylin and eosin method. The results of histopathology are described as under

Bursa

No remarkable changes were observed in vaccine-inoculated groups (A to F) at 24 hours post inoculation as compared with control with control group (G). The significant changes started at 48 hours and remained upto 7th day PI in group A to F inoculated with A, B, C, D, E, and F vaccines, respectively. The changes were necrosis, congestion and hyperplasia of epithelial reticular cells. Lumen contained exudate fluid contained degraded cells or cell debris. Affected parenchymal cell (shrinkage) were nearly similar in all groups A to E with slight variation. The intensity of changes was significantly higher in groups B and C (Fig. 2 & 3), the intensity was moderate in groups A and F (Fig 1 & 6), whereas birds in group D and E showed the least intense lesion compared with unvaccinated group G (Fig. 4 & 5).

DISCUSSION

Many workers have reported on the effect of infectious bursal disease virus vaccines in chickens. Muskett *et al.* (1979) compared two infectious bursal disease vaccine in susceptible chickens and found that one of these caused severe damage to the bursal tissues. Reece *et al.* (1982) and Lucio and Hitchner (1980) observed that "Bursa-Vac" a vaccinal IBD virus in the susceptible chicken from 3 to 42 days of age. Both the virus caused lesion in the bursae and cause immunosuppression. Passaging six times of IBDV in specific pathogens free chicken incresed the virulence of the virus (Muskett *et al.*, 1985). The live intermediate IBD vaccines used in the present study varied greatly in their pathogenic effect. Two of the vaccines tested (B and C) were highly pathogenic as determined by B:BW index as well as bursal and muscles lesions. Moderate pathogenic effect was observed with vaccines A and F. Vaccines D and E induced mild lesions in the bursae and the muscles, yet all are marketed as vaccines with intermediate

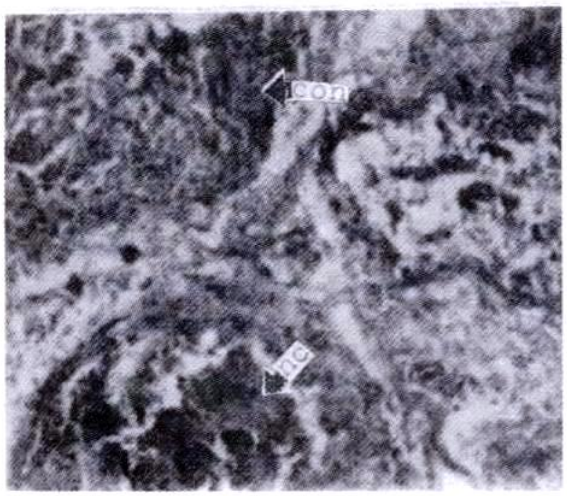

Fig 1: Histopathological changes in bursa of chickens inoculated with D-78 at 96 hours post inoculation (H&E, 400 X) Ne=Necrosis; Con=congestion

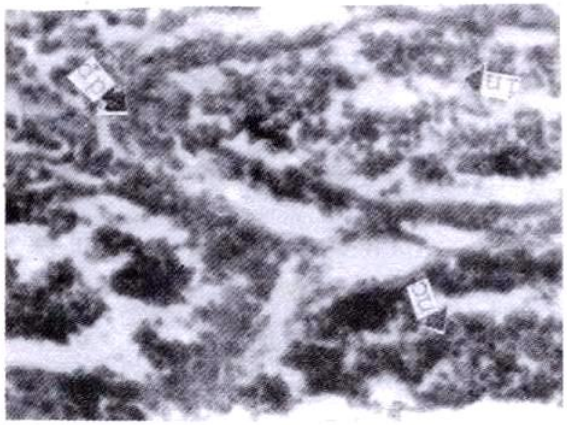

Fig 2: Histopathological changes in bursa of chickens inoculated with 228-E at 96 hours post inoculation (H&E, 400 X), Ne=Necrosis; In=infiltration of lymphocytes; Dp=Degeneration of paranchymal cells

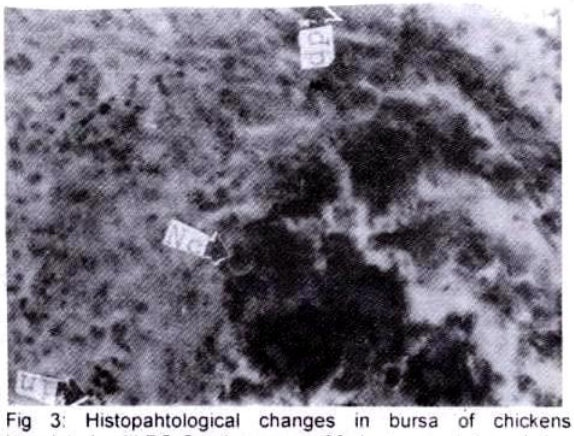

Fig 3: Histopahtological changes in bursa of chickens inoculated withDS-Gumbovac at 96 hours post incoulation (H&E, 400X), NC=Necrosis; In=infiltration of lymphocytes; Dp=Degeneration of paranchymal cells

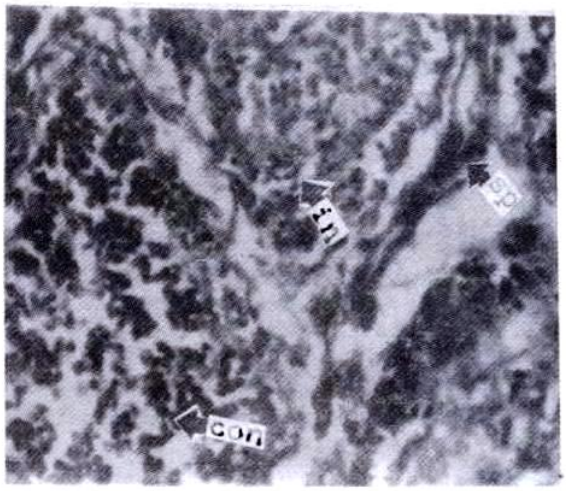

Fig 4: Histopathological changes in bursa of chickens inoculated with Bursine Plus at 96 hours post inoculation (H&E, 400 X), In= infiltration of lymphocytes; Sp= Shrinkage of paranchymal cells; Con= congestion

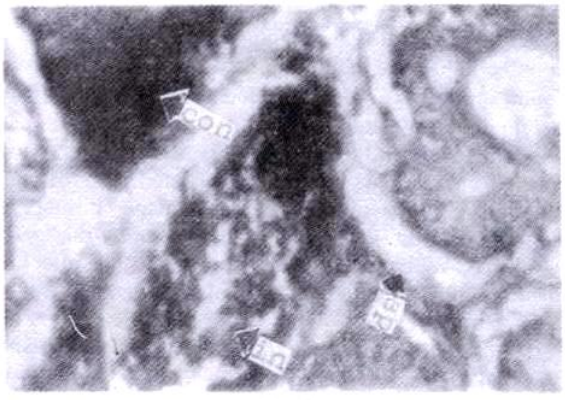

Fig 5: Histopathological changes in bursa of chickens inoculated with IBD Vac at 96 hours post inoculation (H&E, 400X). In=infiltration of lymphocytes; Sp=Shrinkage of paranchymal cells; Con=congestion

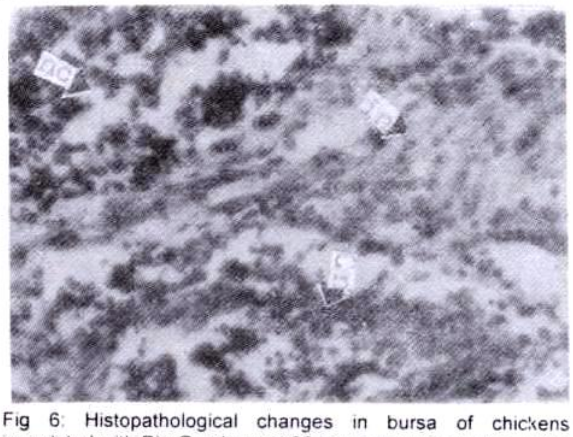

Fig 6: Histopathological changes in bursa of chickens inoculated with Bio-Gumboro at 96 hours post inoculation H&E, 400X0. Nc=Necrosis; In=infiltration; Con=congestion.

Table 2: Comparison of the mean of bursa body weight index (B:BW index) of chicken at different poat inoculation interval receiving different treatments with infectious bursal disease virus vaccine.

No	Days	Total	Mean
1	1	4.750	0.679^{d}
2	2	5.120	0.731^{cd}
3	3	5.570	0.796^{bc}
4	4	6.230	0.890^{b}
5	7	7.650	1.093^{a}
6	10	7.720	1.103^{a}
No	Group	Total	Mean
1	A	5.700	0.950^{ab}
2	B	6.110	1.018a
3	C	5.820	0.970^{ab}
4	D	5.170	0.861^{b}
5	E	5.620	0.936^{ab}
6	F	5.260	0.876^{b}
7	G	3.360	0.560^{c}

Any two mean carrying the same letter are statistically non-significant ($P \leq 0.05$) usind DMR test.

virulence. The demonstration of severe bursal damage by some of the vaccines suggests that they are indistinguishable from the challenge field virus and may cause disease themselves. However, Anjum *et al.* (1996) reported that the imported vacines are different rom the field virus, laking some of the polypeptide band found through sodium dodecyl sulphate poly acrylamide gel electrophoresis.

The results of the present study also reveal that two vacinal virus strains (B and C) persisted upto 10^{th} day PI and caused severe bursal damage throughout experimental period. It may be possible that such virulent vaccines could establish a persistent infection in the poultry population and cause a break down in vaccination program.

Different factors should be considered when using live IBD vaccines. These factors include presence or absence of maternal antibodies, the level of maternal antibodies, the severity of IBDV challenge in the field and management or husbandry factors. It has also been suggested the need for vaccines with various pathogenic characteristics and the use of pathogenic strain as vaccines where IBD is a serious problem (Winterfield *et al.*, 1981). However, results of another study by Mazariegos *et al.* (1990) do not support the need for pathogenic vaccines, since such vaccines may cause severe immunosuppression and the birds may succumb to other infection such as Newcastle disease. Studies on the immunosuppressive effect of these vaccines are under way. Muskett *et al.* (1985) recommended the use of live virus vaccine.

In conclusion, some of the imported commercially used as intermediate IBD vaccines were found to be highly virulent in susceptible chickens i.e. 3-week-old having no IBD antibodies. These vaccines are claimed to be intermediate and are meant to be used in the presence of maternal antibodies which may modify the effect of infection. Such studies of these imported vaccines in the chickens with various level of maternal antibodies are warranted to determine the proper age of vaccination to avoid failure in vaccination programm.

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