EFFECTS OF IMMUNOSTIMULANTS ON BROILERS SUFFERING FROM INFECTIOUS BURSAL DISEASE

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ABSTRACT

This project was aimed to evaluate immunostimulatory effects of three therapeutic substances in broilers suffering from infectious bursal disease (IBD). For this purpose, 150 chicks were divided into five equal groups i.e. A, B, C, D and E having 30 birds each. Group A, B, C and D were challenged with infectious bursal disease virus. There were three immunostimulatory treatments i.e. levamisole (group A), vitamin E (group B), and bursinex (group C). Groups D and E were untreated control. Bursa body weight index, histopathology of bursa of Fabricius, plasma cell counting in Harderian gland and estimation of antibody response against infectious bursal disease virus was recorded. Vitamin E played a major role in improving the condition of birds suffering from infectious bursal disease, as it showed increased bursa body weight index (BBIx), less histopathological lesions in bursa of Fabricius, increased number of plasma cells in Harderian gland and high antibody response in infectious bursal disease infected broilers as compared to levamisole and bursinex. Levamisole played a minor role in improving condition of birds, while bursinex did not seem to be much effective against infectious bursal disease virus in this study.

Key words: Infectious bursal disease, immunostimulants, broilers.

INTRODUCTION

Infectious bursal disease (IBD) is a commonly encountered lymphocytolytic disease that adversely affects the defensive mechanism of birds and results in immunosuppression and failure to develop satisfactory immunity (Amin *et al.*, 1991). The target organ of infectious bursal disease virus (IBDV) is the bursa of Fabricius, which is specific reservoir for B lymphocytes. IBDV causes destruction and depletion of B cells in bursa of Fabricius (Mazariegos *et al.*, 1990). Immunosuppression induced by IBDV is caused by apoptosis (Vasconcelos and Lam, 1994). Immunostimulation of a bird may lead to increased phagocytosis by macrophages and increase in antibody production (Spallholz *et al.*, 1973). The immunostimulatory effect of levamisole in chicks immunocompromised by IBD is the modulation of a number of lymphocytes and macrophage function (Hadden *et al.*, 1975). Vitamin E plays an important regulatory role in many biological processes in enhancing immune response (Balker, 1993). There are certain herbal medicines, which delay the occurrence and reduce the severity of symptoms, incidence of IBD lesions and rates of mortality among infected chickens (Jian and Zhu, 1997). The ultimate success of any therapeutic substance used in commercial poultry flocks, is based not only on the direct effect on a pathogen but also substantiation by an effective immune response (Qureshi, 1999). This project is designed to evaluate the immunostimulatory effects of three therapeutic substances in broilers suffering from experimental IBD. The results of this study may hel in improving immune status of birds suffering from IBD.

MATERIALS AND METHODS

Experimental chicks

A total of 150 day-old broiler chicks wer purchased from local commercial hatchery. The study wa conducted in the Veterinary Research Institute, Lahor The chicks were provided feed and water *ad libitu* Experimental period was of 42 days.

Experimental design

The chicks were randomly divided into five equ groups A, B, C, D and E on day 21. The experiment design is shown in Table 1.

Table 1: Experimental design

Treatments	Routes	Days	Groups				
			A	B	C	D	E
IBD virus challenge	S/C	21st	+	+	+	+	
Levamisole	D/W	23rd–25th	+	-	-	-	
Vitamin E	D/W	23rd–25th	-	+	-	-	
Bursinex	D/W	23rd–25th	-	-	+	-	

Preparation of inoculum and challenge

IBDV inoculum was prepared in Veterina Research Institute, Lahore. On day 21 birds of groups

B, C and D were challenged against IBDV inoculum at a dose rate of 0.5ml/bird through subcutaneous route.

Collection of samples

On days 28, 35 and 42 five birds from each group were randomly selected for collection of lymphoid organs (Harderian gland and bursa of Fabricius) for histopathology and serum for estimation of antibody titre against IBDV.

Experimental parameters

1) Determination of bursa body weight index (Dohms *et al.*, 1988).
2) Histopathological examination of bursa of Fabricius (Dohms *et al.*, 1988).
3) Plasma cell counting in histopathological sections of Harderian gland. Cells with eccentrically located nuclei with coarse chromatin and abundant basophilic cytoplasm were considered to be plasma cells (Dohms *et al.*, 1981).
4) Determination of antibody titre against IBDV through ELISA (Patnayak *et al.*, 1997). For this purpose, 20X wash solution and 4X sample diluent were diluted with deionized water. Then 1:104 of samples dilutions, calibrators and control sera were prepared. Then 100 µl of diluted samples and control sera were dispensed into appropriate wells and incubated for 30 minutes at room temperature. Plates were washed three times with 1X solution, 100 µl of conjugate was dispensed into each well and incubated for 30 minutes at room temperature. Then plates were washed with 1X solution, 100 µl of substrate was dispensed into each well and incubated at room temperature. Then 100 µl of stop solution was dispensed into each well and the absorbance was recorded at 410 nm wavelenhgth.

Statistical Analysis

The data thus collected were statistically analyzed using one way analysis of variance technique (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

In the past a number of immunomodulators have been evaluated for their ability to overcome immunosuppressive conditions. However, sufficient information regarding immunostimulation in IBD affected birds with different therapeutic substances have not yet been much investigated. In the present study immunostimulatory effects of levamisole, vitamin E and bursinex were seen in broilers suffering from experimental IBD.

IBDV primarily induces lesions in bursa of Fabricius, which results in corresponding decrease in bursal weight and size. Bursae with index lower than 0.70 were considered as atrophied (Lucio and Hitchner, 1980). On day 28, 35 and 42 BBIx of groups A, B , C and E were higher than 0.70 whereas BBIx of group D was found to be lower than 0.70 (Table-2). Group B showed highest BBIx as compared to other treated groups (Table-2). Statistical analysis showed that on day 28, 35 and 42 BBIx of group B was significantly higher ($p<0.05$) from groups A, C and D, while on day 35 and 42 there was no significant differences in group B and E.

Table 2: Mean (±S.E.) bursa body weight index of five groups on different post challenge days.

Groups	Day 28	Day 35	Day 42
A	$1.13^{a} \pm 0.15$	$1.59^{b} \pm 0.12$	$1.90^{b} \pm 0.04$
B	$1.53^{b} \pm 0.19$	$1.96^{a} \pm 0.10$	$2.07^{a} \pm 0.01$
C	$0.95^{a} \pm 0.12$	$1.04^{c} \pm 0.14$	$1.58^{c} \pm 0.01$
D	$0.52^{c} \pm 0.01$	$0.60^{d} \pm 0.02$	$0.67^{d} \pm 0.02$
E	$1.95^{d} \pm 0.07$	$1.95^{a} \pm 0.07$	$2.17^{a} \pm 0.06$

Values with different superscripts within a column differ significantly ($P<0.05$).

Our findings are similar to those of Shadaksharappa *et al.* (1997), who reported that mean BBIx of chickens treated with vitamin E was significantly higher than those treated with levamisole and bursinex. He also reported hyperplastic changes of lymphoid cells of bursa of Fabricius in levamisole treated birds. As far as group C is concerned statistical analysis showed that on day 28, 35 and 42 BBIx of group C was significantly different ($p<0.05$) from groups B, D and E while on day 28 there was no significant difference of group C with group A (Table-2). Our results are in line with Xin and Zhu (1996), who reported that herbal treatment did not provide complete protection against challenge with virulent IBD.

Group D showed most predominant histopathological lesions in bursa of Fabricius like depletion of lymphoid cells, increased interfollicular spaces, presence of macrophages and thickening of bursal epithelium (Tables-3, 4 and 5) These results are in line with findings of Okoye and Uzoukwu (1984) and Suveges (1998) who reported same lesions. Group C also showed marked histopathological lesions (Tables- 3, 4 and 5). These results are also supported by Jian and Zhu (1997), who reported that herbal treatment could reduce the severity of symptoms and number of IBD lesions but did not provide complete protection against challenge with virulent IBDV. Group A showed moderate histopathological lesions (Tables-3, 4 and 5), which are in accordance with Panda and Rao (1994a), who reported that histopathological features in levamisole treated and untreated birds, infected with IBDV, were same but not all the follicles were affected in treated birds, also in treated birds the cortical lymphocytes were densely populated indicating some stimulation of immune system by the

drug. Group B showed mild histopathological lesions (Tables-3, 4 and 5). These findings corroborate with the findings of Lawrence (1985), who reported that vitamin E decreased PGF2 alpha values in bursa of Fabricius, as higher values of PGF2 alpha caused greater immunodepression.

Table 3: Histopathological lesions scores in bursa of Fabricius of different groups on day 28.

Groups	BA	LD	OIFS	MP	ET	FT	LN
A	2	2	2	2	2	2	1
B	1	1	1	1	0	0	0
C	3	3	3	4	3	3	3
D	4	4	4	4	4	3	4
E	0	0	0	0	0	0	0

BA = Bursal atrophy, LD = Lymphoid depletion,
OIFS = Oedematous interfollicular space,
MP = Macrophage presence, ET = Epithelial thickness,
FT = Fibrous tissue, LN = Lymphoid necrosis.
Bursal lesion score:
0 = No lesion, 1 = Mild lesion, 2 = Moderate lesion,
3 = Marked lesion, 4 = Severe lesion

Table 4: Histopathological lesions scores in bursa of Fabricius of different groups on day 35.

Groups	BA	LD	OIFS	MP	ET	FT	LN
A	2	2	2	2	2	1	1
B	1	1	1	1	0	0	0
C	3	3	3	4	3	3	3
D	4	4	4	4	4	3	4
E	0	0	0	0	0	0	0

Table-5: Histopathological lesions scores in bursa of Fabricius of different groups on day 42.

Groups	BA	LD	OIFS	MP	ET	FT	LN
A	2	2	2	2	1	1	1
B	1	1	1	1	0	0	0
C	3	3	2	3	3	3	3
D	4	4	4	3	3	3	3
E	0	0	0	0	0	0	0

Harderian gland of group D showed severe depletion of plasma cells on day 28 (Fig. 1), however restoration of plasma cell number started gradually on day 35 and 42 (Table-6). Statistical analysis showed that on day 28, 35 and 42. Plasma cell count of group D was significantly lower ($p<0.05$) from groups A, B and E, while on day 35 and 42 there was no significant difference in plasma cell count of group D and C. These results are in line with Dohms *et al.* (1988), who reported that in broilers inoculated with IBDV at 3 weeks of age plasma cells were reduced by 51% on day 28. However numbers were normal on day 42. In group C on day 28 the number of plasma cells markedly decreased (Fig. 2), however restoration of numbers started gradually on day 35 and 42 (Table-6). Statistical analysis showed that on day 28, 35 and 42 group C was significantly different ($p<0.05$) from groups A, B and E while on day 35 and 42 there was no significant difference in group C and D. These results are in agreement with Jian and Zhu (1997), who reported that herbal treatment did not provide complete protection against IBD. In group A on day 28 the number of plasma cells were moderately decreased (Fig. 3), however the numbers started to restore and on day 35 and 42 the numbers increased to a greater extent (Table-6). Statistical

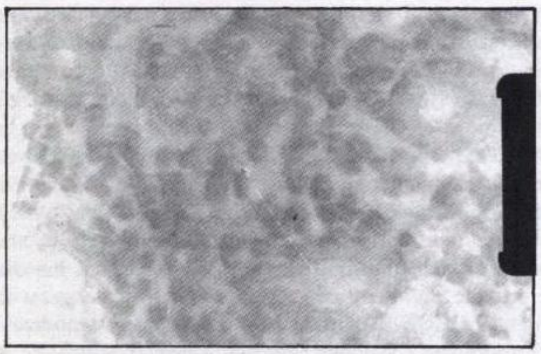

Fig. 1 Histopathological section of Harderian gland of group D birds showing severe depletion of plasma cells (H and E. 400X).

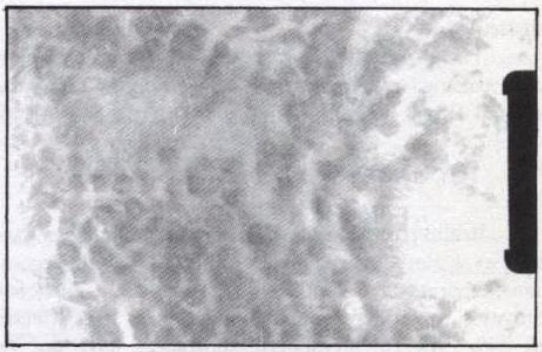

Fig. 2 Histopathological section of Harderian gland of birds of group C showing marked depletion of plasma cells (H and E. 400X).

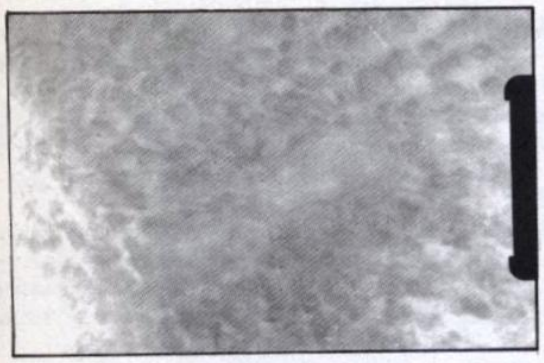

Fig. 3 Histopathological section of Harderian gland of birds of group A showing moderate depletion of plasma cells (H and E. 400X).

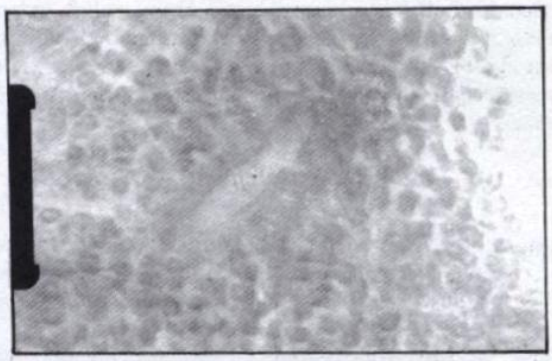

Fig. 4 Histopathological section of Harderian gland of birds of group B showing mild depletion of plasma cells (H and E. 400X).

analysis showed that on day 28, 35, and 42 group A was significantly different from groups B, C, D and E. These results are supported by Hadden *et al.* (1975), who reported that levamisole enhanced lymphocyte proliferation and modulation of numbers of lymphocytes. In group B on day 28 plasma cell numbers were moderately decreased (Fig. 4), however on day 35 and 42 number increased and were near to normal. Statistical analysis showed that on day 28, 35 and 42 group B was significantly different ($p<0.05$) from groups A, C, D and E (Table-6). Our results are supported by Confantoris (1988), who reported that vitamin E supplementation protected bursa and other lymphoid organs i.e Harderian gland from damage in infections such as IBD.

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

Groups	Day 28	Day 35	Day 42
A	4559^{c}±140.4	5501^{c}±226.3	5915^{d}±38.4
B	5400^{b} ± 88.1	6132^{b}±49.4	6306^{c}±31.0
C	3381^{d}±135.5	4746^{a}±243.1	5804^{a}±110.3
D	3035^{e}±34.6	4496^{a}±119.3	5641^{a}±73.5
E	6951^{a}±53.2	7119^{a}±56.0	7271^{b}±33.9

Values with different superscripts within a column differ significantly (P<0.05).

Table 7: Mean (±S.E.) ELISA titres observed in different groups on different post challenge days.

Groups	Day 28	Day 35	Day 42
A	60^{b} ± 1.80	90^{c} ±4.83	73^{b}±6.71
B	80^{a} ± 1.82	120^{b} ± 9.91	101^{a} ± 3.80
C	51^{c} ± 3.39	66^{a} ± 2.59	59^{c} ± 3.67
D	44^{d} ± 1.87	55^{a} ± 4.30	37^{d} ± 3.61
E	02^{e} ± 1.02	01^{d} ± 0.63	00^{e} ± 0.00

Values with different superscripts within a column differ significantly (P<0.05).

ELISA is one of the most sensitive technique commercially employed to detect the antibody titre against IBDV. In the present study on days 28, 35 and 42 group A showed moderate protective serum antibody level (Table-7). Statistical analysis showed that group A was significantly different ($p<0.05$) from groups B, C D and E on days 28, 35 and 42. Our results are congruent with Shadaksharappa *et al.* (1998), who reported that in IBD vaccination levamisole treatment developed a better immune response assessed by measurement of antibody titre. Group B showed strongly protective serum antibody level on day 28, 35 and 42 (Table-7). Statistical analysis showed that group B was significantly different ($p<0.05$) from groups A, C, D and E on days 28 35 and 42. Results of our study are in conformity with a number of workers. Panda and Rao (1994b) reported that vitamin E selenium treatment significantly boosted the GMT in IBD infected birds in comparison with untreated uninfected birds. Shadaksharappa *et al.* (1997), reported that serum antibody titre against IBDV at 42 day of age were higher in vitamin E treated groups compared to untreated birds. Group C showed relatively low mean antibody titer as compared to group A and B (Table-7). Statistical analysis showed that on day 28 and 42 group C was significantly different ($p<0.05$) from groups A, B, D and E , whereas on day 35 there was no significant difference in group C and D. Our results are in agreement with Jian and Zhu (1997), who reported that herbal treatment in IBDV infected birds did not provide complete protection.

CONCLUSION

It was concluded that vitamin E treatment provided better protection as compared to levamisole and

bursinex in broilers suffering from IBD. Vitamin E treatment resulted in higher bursa body weigh index, increased number of plasma cells in Harderian gland, less histopathological lesions in bursa of Fabricious and increased antibody titre as compared to levamisole and bursinex.

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