

EFFECT OF IBD VACCINE AND LEVAMISOLE ON BURSAL INDEX IN HPS INFECTED CHICKS

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ABSTRACT

Hydropericardium syndrome (HPS) is a viral infectious disease of broiler chicks causing significant economic losses to the poultry industry. The present study was conducted to observe the effect of infectious bursal disease (IBD) vaccine and levamisole on bursal index in hydropericardium syndrome affected broiler chicken. There was a significant reduction in the bursal index due to IBD vaccination indicating immunosuppression. Levamisole was able to significantly improve the bursal index in uninfected and IBD vaccinated birds. The HPS infection alone also reduced significantly the bursal index at 3 days post infection, indicating that HPS alone is also an immunosuppressant but of lower degree. Levamisole was able to slightly improve the bursal index in HPS alone and IBD vaccinated plus HPS infected birds

Key words: IBD vaccine, levamisole, hydropericardium syndrome, chickens

Hydropericardium syndrome (HPS) is primarily a disease of broilers and was reported for the first time in Angara Goath area near Karachi in Pakistan during 1987 (Jaffery 1988, Khawaja *et al.*, 1988). It spreaded to most of the regions of India within very short time (Gowda and Satyanarayan, 1994). The disease is caused by Fowl Adenovirus type-4 (Jadhao *et al.*, 1997, Ganesh *et al.*, 2002, Dahiya *et al.*, 2002). The disease is characterized by severe hydropericardium resembling peeled off litchi fruit along with its sudden occurrence with high rate of mortality even up to 80% in broiler chicks (Akhtar, 1994, Asrani *et al.* 1997) and low mortality of less than 10% in layers (Cheema *et al.*, 1989).

Due to the stability of infectious bursal disease (IBD) virus in the environment, the principal method of its control is vaccination. Intermediate and hot vaccines are more frequently used now a day by the farmers, as fully attenuated IBD vaccines do not induce immunity in chickens in the presence of maternal antibodies (Wood *et al.*, 1981). Although these vaccines are useful in inducing antibody levels against IBD virus but may also lead to simultaneous immunosuppression against many antigens and increased susceptibility to

various infections (Onaga *et al.*, 1989, Nakamura *et al.*, 1990, Saif, 1991, Khan *et al.*, 1998). Kumar and Kharole (1999) observed high incidence of HPS at poultry farms where IBD occurred or IBD vaccine had earlier been used. Role of immunosuppression in precipitation and aggravation of HPS under field conditions has also been suggested by Toro *et al.* (2000) but little work has been done on the effect of immunosuppressive agents on the pathology of HPS in chicken.

Mohanty *et al.* (2000) evaluated that the administration of levamisole before IBD vaccination boosted humoral immune response against ND virus and significantly increased bursal body weight index indicating its immunopotentiating activity. Keeping in view the above facts, the present work was undertaken to study the effect of levamisole along with IBD vaccine on bursal index in hydropericardium syndrome infected chickens.

MATERIALS AND METHODS

Two hundred and seventy five, commercial broiler chicks were procured from Department of Animal Breeding, CCS HAU, Hisar. The chicks were reared under strict hygienic conditions in the departmental

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animal house. All the birds were given standard chick feed ad-libitum and provided clean drinking water throughout the experiment. An intermediate plus IBD vaccine was procured from a commercial source and given to the birds at 14th day of age as per the manufacturer's recommendations. Levamisole hydrochloride [L, 2, 3, 5, 6 Tetra-hydro, 6-phenylmidiazol 2(1-6) thiazol] was obtained as pure powder. It was freshly dissolved in distilled water and was given at the dose rate of 15mg/kg body weight orally for 3 days (14-16th days of age).

Hydropericardium syndrome seed virus was procured and infective dose was calculated as per the method of Reed and Muench (1938). A dilution of 10⁻³ of 20% HPS inoculum was found to be optimum for producing the protracted disease.

The chicks were reared upto 14th day of age. On 14th day, 3 birds were sacrificed before vaccination for bursal index and the rest were divided randomly into four groups: A, B, C and D with 68 chicks in each. Each bird in group C and D was given IBD vaccine at 14th day. Whereas the birds of groups B and D were given levamisole orally @ 15 mg/kg body weight from 14th to 16th day of age. Three birds each from groups A, B, C, D were sacrificed on 4 and 6 day post vaccination (DPV) and at 20th day of age prior to HPS challenge. Rest of the birds in each group were further subdivided into two sub groups i.e. group A into A1 and A2, group B into B1 and B2, group C into C1 and C2 and group D into D1 and D2 with 19 birds in groups A1, B1, C1, D1 while 40 birds each in groups A2, B2, C2, D2. Each bird of groups A2, B2, C2, and D2 was injected with 0.5 ml of the diluted [10⁻³] HPS inoculum (ID50) subcutaneously, on 21st day of age whereas the birds of groups A1, B1, C1 and D1 were injected with 0.5 ml of PBS. Bursal index was studied at 0, 4, 6, 8/1, 10/3, 12/5, 14/7 and 16/9 (DPV/DPI). Three birds each from all the groups were weighed along with the weighing of bursa at the intervals mentioned above throughout the experiment. Bursal index (Dohms et al., 1988) and mean of each group was calculated at each sampling.

RESULTS AND DISCUSSION

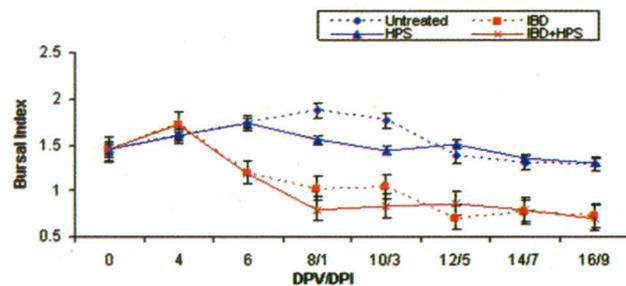
Bursal index reflects the relative changes in

weight of bursa of Fabricius with respect to body weight. The mean bursal index in different groups of broiler chicks at various intervals has been presented in Table and Fig 1. The bursal index was lower in IBD vaccinated and levamisole untreated birds (group C1) from 6 DPV onwards with a significant difference up to 16 DPV as compared to uninfected levamisole untreated control (group A1). Decreased bursal body weight index on IBD virus inoculation has also been reported by Lucio and Hitchner (1979), when virus was inoculated at 7 and 14 days of age. Similarly, a decrease in weight of bursa of Fabricius without any clinical signs due to IBD vaccine was observed by Reece *et al.* (1982). Thangavelu *et al.* (1998) also reported a significant reduction in bursal and body weight indices due to various IBD vaccines used in India. The reduction in index was more by intermediate vaccines as compared to mild vaccines. Gupta and Singh (2001) reported a significant decrease in bursal index from 11 to 28th day post IBD virus inoculation. Luengo *et al.* (2001) and Kumar (2002) also reported decreased bursal and body weight ratio in IBD vaccinated birds.

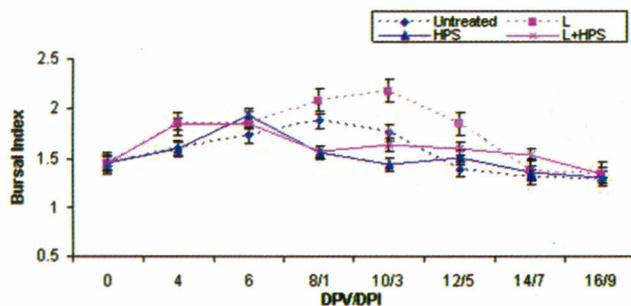
Levamisole treatment in IBD vaccinated birds (group D1) significantly increased the bursal index from 8 to 16 DPV as compared to IBD vaccinated levamisole untreated birds (group C1). Levamisole treatment also increased significantly the bursal index in uninfected control birds (group B1) on 8, 10 and 12th DPV with higher values at all other intervals too. This indicated that levamisole helped in reducing immunosuppression caused by IBD vaccine to some extent although it could not be brought back to normal level.

Challenge with HPS (group A2) decreased bursal index on 8 and 10 DPV (1 and 3 DPI) with a significant decrease at 10 DPV (3 DPI) as compared to uninfected untreated control birds (group A1) indicating mild immunosuppressive effect of HPS itself. Levamisole treatment in HPS challenged birds (group B2) slightly increased the bursal index at most of the intervals as compared to group A2 (HPS) birds, but the increase was not significant statistically at any interval.

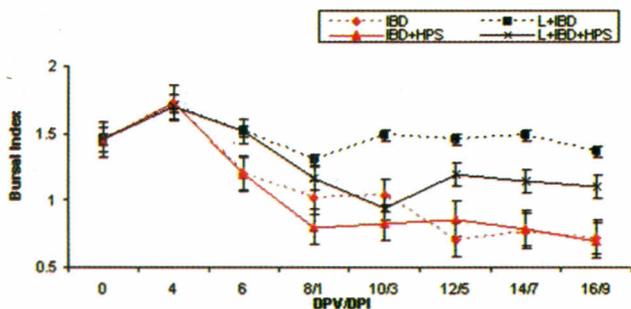
The higher values of bursal index in group D2 (L+IBD+HPS) as compared to group C2 (IBD+HPS)



Effect of IBD vaccine



Effect of levamisole



Effect of levamisole along with IBD vaccine

Fig 1. Effect of IBD vaccine and levamisole on bursal index in HPS infected chicks of different experimental groups.

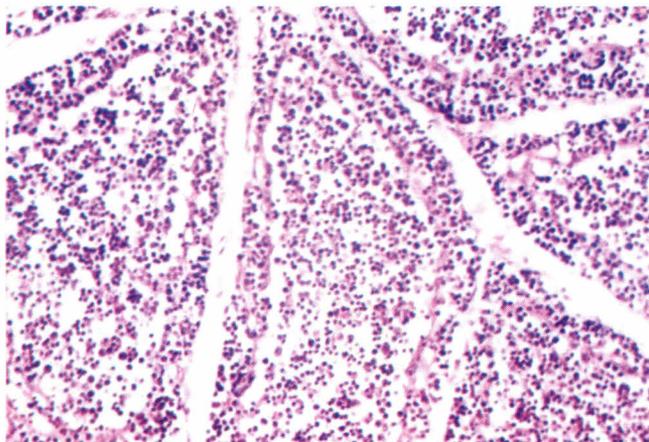


Fig2. Section of bursa with mild degree of depletion and degeneration of lymphocytes in IBD vaccinated chicks (4 DPV). (H. & E. x 66)

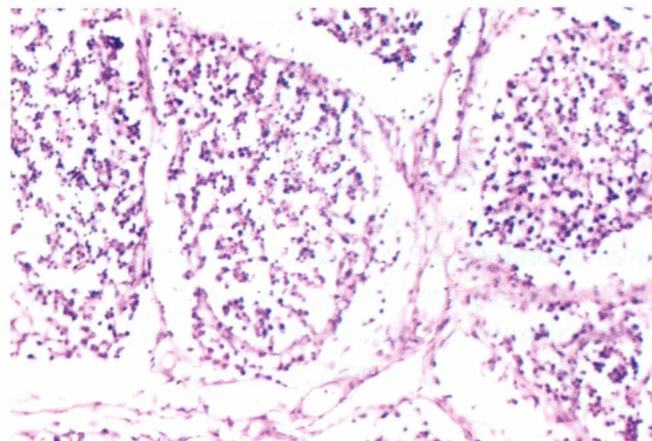


Fig3. Section of bursa with marked depletion, degeneration along with mild degree of R. E. cell proliferation in IBD vaccinated chicks (12 DPV). (H. & E. x 66)

with statistical significant difference only at 5 DPI/12 DPV also indicated that levamisole neutralised the IBD immunosuppression to some extent in HPS affected chicks. Kumar (2002 a) reported that the immune response of birds given levamisole was enhanced in IBD vaccinated birds as assessed by serum globulins and HI titres against ND vaccine.

Bursal index was significantly lower in IBD vaccinated plus HPS challenged birds (group C2) as compared to unvaccinated HPS challenged birds (group A2) from 6 DPV onwards till the end of experiment. Levamisole treatment in IBD vaccinated plus HPS challenged birds (group D2) slightly increased the bursal index at most of the intervals with significant increase from 12 to 16 DPV when compared to group C2 (IBD+HPS) birds. Bursal index in chicks of groups C1 (IBD vaccine alone) and C2 were comparable at all the intervals post vaccination and post infection.

The decrease in bursal index in IBD vaccinated birds correlated with the sequential microscopic changes observed in bursa. Mild depletion was observed on 4 DPV (Fig 2) followed by moderate to severe depletion and formation of some cystic cavities on 10 DPV. On 12 to 14 DPV mild reticular cell hyperplasia was also seen (Fig 3). Edwards *et al.* (1982) reported a severe damage with destruction of follicular architecture, necrosis, depletion of lymphocytes and connective tissue proliferation after IBD vaccination. Mazariëgos *et al.* (1990) demonstrated that some intermediate vaccines were almost as pathogenic as a known virulent virus with respect to bursal atrophy and

Table
Effect of IBD vaccine and levamisole on bursal index in HPS infected chicks

Days	Non-infected						IBD vaccinated						HPS challenged						IBD vaccinated plus HPS challenged						
	AI		BI		CI		DI		A2		B2		C2		D2		LU		LU		LU		LU		
	LU	DPV	LU	DPV	LU	DPV	LU	DPV																	
14	1.453 ^{a1} ±0.023	0	1.453 ^{a1} ±0.023	0	1.453 ^{a1} ±0.023	0	1.453 ^{a1} ±0.023	0	1.453 ^{a1} ±0.023																
18	1.596 ^{a1} ±0.083	4	1.853 ^{a1} ±0.244	4	1.726 ^{a1} ±0.018	4	1.696 ^{a1} ±0.008	4	1.596 ^{a1} ±0.083	4	1.853 ^{a1} ±0.244	4	1.726 ^{a1} ±0.018	4	1.696 ^{a1} ±0.008	4	1.596 ^{a1} ±0.083	4	1.853 ^{a1} ±0.244	4	1.726 ^{a1} ±0.018	4	1.696 ^{a1} ±0.008	4	1.596 ^{a1} ±0.083
20	1.737 ^{a1} ±0.280	6	1.860 ^{a1} ±0.169	6	1.200 ^{b1} ±0.120	6	1.516 ^{a1} ±0.068	6	1.737 ^{a1} ±0.280	6	1.860 ^{a1} ±0.169	6	1.200 ^{b1} ±0.120	6	1.516 ^{a1} ±0.068	6	1.737 ^{a1} ±0.280	6	1.860 ^{a1} ±0.169	6	1.200 ^{b1} ±0.120	6	1.516 ^{a1} ±0.068	6	1.737 ^{a1} ±0.280
22	1.876 ^{a1} ±0.101	8	2.090 ^{a2} ±0.078	8	1.020 ^{b1} ±0.011	8	1.310 ^{a2} ±0.017	8	1.876 ^{a1} ±0.101	8	2.090 ^{a2} ±0.078	8	1.020 ^{b1} ±0.011	8	1.310 ^{a2} ±0.017	8	1.876 ^{a1} ±0.101	8	2.090 ^{a2} ±0.078	8	1.020 ^{b1} ±0.011	8	1.310 ^{a2} ±0.017	8	1.876 ^{a1} ±0.101
24	1.763 ^{a1} ±0.063	10	2.183 ^{a2} ±0.057	10	1.040 ^{b1} ±0.015	10	1.483 ^{a2} ±0.063	10	1.763 ^{a1} ±0.063	10	2.183 ^{a2} ±0.057	10	1.040 ^{b1} ±0.015	10	1.483 ^{a2} ±0.063	10	1.763 ^{a1} ±0.063	10	2.183 ^{a2} ±0.057	10	1.040 ^{b1} ±0.015	10	1.483 ^{a2} ±0.063	10	1.763 ^{a1} ±0.063
26	1.383 ^{a1} ±0.080	12	1.853 ^{a2} ±0.080	12	0.710 ^{b1} ±0.085	12	1.460 ^{a2} ±0.236	12	1.383 ^{a1} ±0.080	12	1.853 ^{a2} ±0.080	12	0.710 ^{b1} ±0.085	12	1.460 ^{a2} ±0.236	12	1.383 ^{a1} ±0.080	12	1.853 ^{a2} ±0.080	12	0.710 ^{b1} ±0.085	12	1.460 ^{a2} ±0.236	12	1.383 ^{a1} ±0.080
28	1.303 ^{a1} ±0.269	14	1.373 ^{a1} ±0.138	14	0.770 ^{b1} ±0.115	14	1.490 ^{a2} ±0.047	14	1.303 ^{a1} ±0.269	14	1.373 ^{a1} ±0.138	14	0.770 ^{b1} ±0.115	14	1.490 ^{a2} ±0.047	14	1.303 ^{a1} ±0.269	14	1.373 ^{a1} ±0.138	14	0.770 ^{b1} ±0.115	14	1.490 ^{a2} ±0.047	14	1.303 ^{a1} ±0.269
30	1.293 ^{a1} ±0.169	16	1.352 ^{a1} ±0.132	16	0.725 ^{b1} ±0.116	16	1.365 ^{a2} ±0.272	16	1.293 ^{a1} ±0.169	16	1.352 ^{a1} ±0.132	16	0.725 ^{b1} ±0.116	16	1.365 ^{a2} ±0.272	16	1.293 ^{a1} ±0.169	16	1.352 ^{a1} ±0.132	16	0.725 ^{b1} ±0.116	16	1.365 ^{a2} ±0.272	16	1.293 ^{a1} ±0.169

LU- Levamisole untreated, L- Levamisole treated, DPV- Days post vaccination, DPI- Days post infection,

abc- small alphabets in superscripts used to denote statistical difference within a row between "LU" subgroups at 5% level of significance (To see effect of IBD vaccine).

123- numerical number in superscripts used to denote statistical difference within a row between "LU" and "L" subgroups at * 5% and ** 1 % level of significance

(To see effect of levamisole)

Weight of bursa

Bursal index = $\frac{\text{Weight of bursa}}{\text{Body weight}} \times 1000$

Body weight

histopathological lesions. Thangavelu *et al.* (1998) also reported IBD vaccine strain dependent bursal damage. Histopathologically, bursal damage was more severe due to intermediate vaccine as compared to mild Lukert strain vaccine. Lymphoid depletion along with other bursal damages had also been reported by Gupta and Singh (2001) on IBD virus inoculation and by Kumar, (2002) on vaccination with intermediate plus IBD vaccine.

Reduction in the number of lymphocytes particularly B cells and their actively dividing precursors in bursa of Fabricius was probably the most important cause of immunosuppression (Saif, 1991) due to IBD virus. Stimulation of suppressor cells could be the other possible mechanism (Sharma *et al.*, 1989).

On the basis of the findings of the present study, it may be concluded that IBD vaccination enhanced the severity of HPS infection as evident by bursal index and pathological changes in bursa suggesting the role of B-cells in limiting the pathology and pathogenesis of hydropericardium syndrome. Use of levamisole as an immunomodulator along with IBD vaccine may reduce the severity of HPS to some extent.

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