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Research Article

EFFECT OF IBD VACCINE AND LEVAMISOLE ON ENZYME PROFILE IN CHICKS INFECTED WITH HYDROPERICARDIUM SYNDROME

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ABSTRACT

The present study was conducted to observe the effect of infectious bursal disease (IBD) vaccine and levamisole on serum enzymes in broiler chicks infected with hydropericardium syndrome (HPS). There was a significant increase in the activities of serum aspartate transaminase, alanine transaminase and creatine phosphokinase.Increased activities of these enzymes were more pronounced in the IBD vaccinated and HPS infected group. Levamisole treatment was able to slightly reduce the activity of these enzymes in HPS and IBD vaccinated plus HPS infected groups but without statistical difference.

Key words: IBD vaccine, levamisole, enzymes, HPS infected chicks

Hydropericardium syndrome (HPS) is primarily a disease of broiler chickens and was reported for the first time in Angara Goath area near Karachi in Pakistan during 1987 (Jaffery, 1988; Khwaja *et al.,* 1988). It spread to most of the regions of India within a short time (Gowda and Satyanarayana, 1994). The disease is caused by fowl adenovirus type-4 (FAV-4) (Jadhao *et al.,* 1997; Dahiya *et al.,* 2002; Ganesh *et al.,* 2002). The disease is characterized by severe hydropericardium resembling peeled off litchi fruit along with high mortality (upto 80%) in broiler chicks (Akhtar, 1994; Asrani *et al.,* 1997) and low mortality (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 frequently used now a days, 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 these also lead to immunosuppression thereby increasing susceptibility to various infections (Faragher, 1972; Onaga *et al.,* 1989; Nakamura *et al.,* 1990; Saif, 1991; Khan *et al.,* 1998). Kumar and Kharole (1999) observed high incidence of HPS in poultry farms where IBD occurred or IBD vaccine had earlier been used. Little work has been done to observe the effect of immuno-potentiating agents on the pathology of HPS in chicken.

Mohanty *et al.* (2000) reported that the administration of levamisole before IBD vaccination boosted humoral immune response against Newcastle disease (ND) virus and significantly increased bursal body weight index indicating its immunopotentiating effect. Keeping in view the above observations, the present study was planned to observe the effect of levamisole along with IBD vaccine on some biochemical parameters in HPS infected chickens.

MATERIALS AND METHODS

Source: Day old commercial broiler chicks were procured from the Department of Animal Breeding, Hisar. The chicks were reared under strict hygienic conditions in the animal house of the department. Birds were given standard chick feed and provided clean drinking water *ad-libitum* throughout the experiment. An intermediate plus IBD vaccine was procured from a commercial source and given to the birds at 14th day of age orally. 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 b. wt. orally for 3 days (14-16th days of age).

The HPS seed virus was procured from Indovax Pvt. Ltd., Hisar and its infective dose was calculated as per the method of Reed and Muench (1938). A

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dilution of 10^{-3} of 20% HPS inoculum was found to be optimum for producing the protracted disease.

Experimental Design: A total of 236, day-old chicks were reared upto 14 days of age. On 14th day, birds were divided randomly into four groups: A, B, C and D with 59 chicks each. Each bird of groups C and D was given IBD vaccine at 14th day whereas each bird of groups B and D was given levamisole orally @ 15 mg/kg b. wt. from 14-16 days. At 21st day of age, 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 each in groups A1, B1, C1 and D1 while 40 birds each in groups A2, B2, C2 and D2. Each bird of groups A2, B2, C2 and D2 was administered 0.5 ml of the diluted (10^{-3}) HPS inoculum (ID50) subcutaneously, whereas, the birds of groups A1, B1, C1 and D1 were administered 0.5 ml of phosphate buffered saline.

Enzymes Studied: Serum was collected at 0, 1, 2, 3, 5, 7 and 9 days post-infection (DPI) for aspartate transaminase (AST), alanine transaminase (ALT) and creatine phosphokinase (CPK). The AST and CPK activities in the serum samples were estimated by the method of Tietz (1986) while ALT activity was estimated by the method of Wroblewski and La Due (1956).

RESULTS AND DISCUSSION

Aspartate Transaminase: Mean serum AST activities in different experimental groups are given in Table 1. The IBD vaccination alone in group C1 birds increased AST activity with a significant increase on 10 days post vaccination (DPV) only as compared to uninfected untreated control birds (group A1). There was an increase in AST activity in all the HPS infected groups as compared to their respective uninfected controls and theincrease was significant in group A2 from 2 to 5 DPI as compared to group A1. Whereas, HPS infection in IBD vaccinated birds (group C2) caused a significant increase in AST activity from 1 DPI in comparison to chicks of control group (group C1). Moreover, AST values were higher in birds of group C2 (IBD+HPS) as compared to that of group A2 (HPS alone) at all the intervals with statistically higher values at 1, 2 and 5 DPI. Also the AST values in group A2 (HPS alone) were comparable with those of group A1 from 7 DPI onwards. The AST values remained significantly higher in group C2 (IBD+HPS) on 7 DPI when compared to uninfected controls (group A1). Levamisole treatment in HPS infected chicks (groups B2 and D2) reduced the AST values, however, the difference was not significant statistically at any interval when compared to their respective levamisole untreated birds (groups A2 and C2).

Serum AST concentration was higher in all the HPS infected birds during the early acute phase of the infection. The increase due to HPS was more severe in IBD vaccinated birds (groups C2 and D2). Moreover AST values in HPS infected birds (group A2) were comparable with uninfected untreated controls (group A1) on and after 7 DPI but not so in IBD vaccinated

Table 1

Effect of IBD vaccine and levamisole on the activity of serum aspartate transaminase in HPS infected chicks

Day			Uninfected		IBD Vaccinated		HPS Challenged		IBD Vaccinated + HPS Challenged	
Age	DPV	DPI	A1	B1	C1	D1	A2	B2	C2	D2
			LU	L	LU	L	LU	L	LU	L
21	7	0	$328.10^{a1}\pm1.45$	$326.33^{1}\pm0.82$	$320.03^{a1}\pm4.73$	$292.13^{1}\pm9.41$	$328.10^{a1}\pm1.45$	$326.33^{1}\pm0.82$	$320.03^{a1}\pm4.73$	$292.03^{1}\pm9.49$
22	8	1	$326.47^{ab1}\pm3.40$	$310.66^{1}\pm7.10$	$301.03^{b1}\pm6.35$	$306.00^{1}\pm3.04$	$299.33^{b1}\pm9.35$	$326.13^{1}\pm6.00$	$365.17^{a1}\pm24.28$	$376.86^{1}\pm50.12$
23	9	2	$280.20^{c1}\pm3.11$	$315.20^{1}\pm6.33$	$328.70^{bc1}\pm24.19$	$292.40^{1}\pm1.34$	$363.37^{b1}\pm33.80$	$343.40^{1}\pm22.93$	$524.23^{a1}\pm19.78$	$472.93^{1}\pm17.80$
24	10	3	$297.93^{c1}\pm2.62$	$304.33^{1}\pm6.33$	$309.86^{b1}\pm13.39$	$309.40^{1}\pm5.17$	$471.53^{a1}\pm28.09$	$436.20^{1}\pm17.91$	$511.03^{a1}\pm10.98$	$442.56^{1}\pm23.86$
26	12	5	$284.23^{c1}\pm5.71$	$310.20^{1}\pm18.36$	$317.86^{c1}\pm13.02$	$316.40^{1}\pm2.45$	$395.20^{b1}\pm17.48$	$334.63^{1}\pm36.96$	$484.33^{a1}\pm35.22$	$415.63^{1}\pm20.06$
28	14	7	$286.16^{b1}\pm19.51$	$288.23^{1}\pm16.22$	$316.30^{ab1}\pm18.40$	$324.86^{1}\pm15.08$	$334.96^{ab1}\pm47.54$	$349.33^{1}\pm31.08$	$398.40^{a1}\pm8.23$	$394.56^{1}\pm68.58$
30	16	9	$280.40^{a1}\pm13.85$	$275.43^{1}\pm26.04$	$323.66^{a1}\pm6.55$	$294.76^{1}\pm1.99$	$277.03^{a1}\pm28.64$	$279.76^{1}\pm10.82$	$332.46^{a1}\pm17.09$	$323.83^{1}\pm18.68$

LU=Levamisole untreated; L=Levamisole treated; DPV=Days post vaccination; DPI=Days post infection; abc=small alphabets in superscripts denote statistical difference within a row between LU subgroups at 5% level of significance (to see the effect of IBD vaccine); 123=numerical number in superscripts denote statistical differencewithin a row between LU and L subgroups at *5% and **1 % level of significance (to see the effect of levamisole)

Table 2

Effect of IBD vaccine and levamisole on the activity of serum alanine transaminase in HPS infected chicks

Day			Uninfected		IBD Vaccinated		HPS Challenged		IBD Vaccinated + HPS Challenged	
Age	DPV	DPI	A1 LU	B1 L	C1 LU	D1 L	A2 LU	B2 L	C2 LU	D2 L
21	7	0	$10.66^{a1}\pm0.63$	$11.93^{1}\pm0.52$	$11.06^{a1}\pm0.52$	$10.70^{1}\pm0.41$	$10.66^{a1}\pm0.63$	$11.93^{1}\pm0.52$	$11.06^{a1}\pm0.52$	$10.70^{1}\pm0.41$
22	8	1	$12.36^{a1}\pm1.12$	$9.55^{1}\pm0.10$	$12.49^{a1}\pm0.11$	$12.86^{1}\pm0.17$	$14.03^{a1}\pm1.90$	$12.70^{1}\pm1.78$	$13.23^{a1}\pm1.41$	$11.76^{1}\pm1.23$
23	9	2	$12.06^{b1}\pm0.12$	$10.63^{1}\pm0.77$	$11.26^{b1}\pm0.34$	$10.66^{1}\pm0.34$	$13.36^{ab1}\pm1.02$	$12.76^{1}\pm1.22$	$15.23^{a1}\pm0.93$	$15.16^{1}\pm1.29$
24	10	3	$10.60^{d1}\pm0.23$	$10.36^{1}\pm0.43$	$12.86^{c1}\pm0.37$	$11.76^{1}\pm0.86$	$18.56^{b1}\pm0.51$	$17.76^{1}\pm1.44$	$23.50^{a1}\pm0.92$	$18.30^{1}\pm1.60$
26	12	5	$12.16^{a1}\pm0.98$	$10.46^{1}\pm0.72$	$11.70^{a1}\pm0.15$	$10.10^{1}\pm0.20$	$15.10^{a1}\pm1.07$	$14.60^{1}\pm0.41$	$15.30^{a1}\pm1.66$	$15.86^{1}\pm1.39$
28	14	7	$12.16^{a1}\pm0.43$	$11.88^{1}\pm0.11$	$10.53^{a1}\pm0.63$	$12.36^{1}\pm0.80$	$13.10^{a1}\pm1.07$	$13.03^{1}\pm1.41$	$12.56^{a1}\pm0.92$	$13.06^{1}\pm1.73$
30	16	9	$9.86^{a1}\pm0.72$	$9.93^{1}\pm0.76$	$12.53^{a1}\pm0.75$	$12.36^{1}\pm0.57$	$9.66^{a1}\pm0.96$	$12.10^{1}\pm0.78$	$10.06^{a1}\pm1.24$	$10.00^{1}\pm0.70$

LU=Levamisole untreated; L=Levamisole treated; DPV=Days post vaccination; DPI=Days post infection; abc=small alphabets in superscripts denote statistical difference within a row between LU subgroups at 5% level of significance (to see the effect of IBD vaccine); 123=numerical number in superscripts denote statistical difference within a row between LU and L subgroups at *5% and **1 % level of significance (to see the effect of levamisole).

plus HPS infected birds (group C2). Increased concentration of AST in HPS infected birds have earlier been reported (Iqbal *et al.,* 1994; Kumar, 1997; Deepak *et al.,* 2001). Similarly, it has been reported that AST levels due to HPS were higher in cyclosporin-A treated (Kumar, 2002) or cyclophosphamide treated broiler chicks (Narender, 2004). Since this enzyme is present in large quantities in the cardiac muscles (Benjamin, 1978), the increased activity of this enzyme in serum might be due to myocarditis induced by HPS infection as evident from the histopathological examination. Levamisole treatment alone in HPS infection slightly reduced the AST values without statistical difference indicating little or no immunomodulatory effect alone in reducing the severity of the disease.

Alanine Transaminase: Mean serum ALT activities in different experimental groups are given in Table 2. An increase in ALT activity was observed in all the HPS infected groups as compared to their respective uninfected controls. The ALT activity was higher at most of the intervals in birds of group A2 (HPS infected) with statistically higher value only at 3 DPI when compared to uninfected untreated controls (group A1). However, significantly higher levels of ALT were observed at 2 and 3 DPI in IBD vaccinated and HPS infected birds (group C2). The HPS infection in IBD vaccinated birds (group C2) significantly increased serum ALT activity as compared to birds given HPS infection alone (group A2) at 3 DPI.

Levamisole treatment in HPS infected birds (groups B2 and D2) slightly reduced the ALT values as compared to their respective levamisole untreated birds (groups A2 and C2), however, the difference was not statistically significant.

Mean serum ALT activity was significantly higher in all the HPS infected chickens. However, the increase was more pronounced in group C2 (IBD+HPS) birds as compared group A2 (HPS infection alone). Levamisole treatment in HPS infected birds (groups B2 and D2) slightly reduced the ALT activity as compared to levamisole untreated HPS infected birds (groups A2 and C2). Increased ALT activity in HPS infected birds have also been reported in broiler chicks by various workers (Kumar, 1997; Soni, 1999; Deepak *et al.*, 2001). Similarly, significantly higher activity of ALT due to HPS had been observed in cyclosporin-A (Kumar, 2002) and cyclophosphamide treated birds (Narender, 2004). The increased activity of serum ALT observed in the HPS infected groups might be due to excessive release of this enzyme from the liver as a result of hepatic necrosis and hepatitis. Increase in the activity of serum ALT is considered to be sensitive indicator of hepatic cell damage and alteration in the permeability of hepatic cell membrane (Christen and Metzler, 1985).

Creatine Phosphokinase: Mean CPK activity in different experimental groups is given in Table 3. There was an increase in CPK activity in all the HPS infected groups. The increase was more pronounced up to 9 DPI in IBD vaccinated and HPS infected birds (group C2) as compared to birds infected with HPS alone (group A2). Significant increase in CPK activity was noticed from 1 DPI in group C2 (IBD+HPS) while it increased from 2 DPI in group A2 (HPS alone). The values of CPK were statistically higher on 1 and 2 DPI in birds of group C2 (IBD+HPS) as compared to birds of group A2 (HPS alone). The value of CPK in chicks of group A2 (HPS alone) was comparable to value of uninfected levamisole untreated control group (group

Table 3

Effect of IBD vaccine and levamisole on the activity of serum creatine phosphokinase in HPS infected chicks

Day			Uninfected		IBD Vaccinated		HPS Challenged		IBD Vaccinated + HPS Challenged	
Age	DPV	DPI	A1 LU	B1 L	C1 LU	D1 L	A2 LU	B2 L	C2 LU	D2 L
21	7	0	148.22^{a1}±10.64	161.77^{1}±9.17	140.96^{a1}±5.02	145.15^{1}±17.69	148.22^{a1}±10.64	161.77^{1}±9.17	140.96^{a1}±5.02	145.15^{1}±17.66
22	8	1	157.06^{b1}±24.72	149.89^{1}±5.15	178.92^{ab1}±6.95	160.55^{1}±6.73	132.32^{b1}±17.28	150.34^{1}±16.02	222.15^{a1}±18.07	185.60^{1}±15.20
23	9	2	161.09^{b1}±6.46	159.96^{1}±15.23	185.70^{b1}±13.37	159.39^{1}±3.87	164.27^{b1}±18.38	187.49^{1}±8.63	284.74^{a1}±17.28	246.02^{1}±13.75
24	10	3	155.70^{b1}±18.80	165.67^{1}±14.40	191.62^{ab1}±2.51	144.39^{1}±9.16	223.05^{a1}±30.83	212.64^{1}±4.02	279.90^{a1}±8.93	236.35^{1}±5.48
26	12	5	183.33^{b1}±13.37	173.81^{1}±8.89	204.44^{b1}±6.479	199.75^{1}±7.29	317.77^{a1}±23.12	287.93^{1}±5.97	358.25^{a1}±1.72	284.07^{1}±35.28
28	14	7	139.27^{c1}±2.26	128.81^{1}±17.83	146.13^{bc1}18.14	167.91^{1}±2.72	180.09^{ab1}±23.23	202.21^{1}±36.32	241.98^{a1}±26.51	174.51^{1}±6.61
30	16	9	151.19^{b1}±23.44	148.12^{1}±22.89	173.89^{ab1}±21.29	116.24^{1}±2.13	171.00^{ab1}±18.51	158.37^{1}±15.14	263.05^{a1}±52.35	167.62^{1}±9.66

LU=Levamisole untreated; L=Levamisole treated; DPV=Days post vaccination; DPI=Days post infection; abc=small alphabets in superscripts denote statistical difference within a row between LU subgroups at 5% level of significance (to see the effect of IBD vaccine); 123=numerical number in superscripts denote statistical difference within a row between LU and L subgroups at *5% and **1 % level of significance (to see the effect of levamisole).

A1) on 9 DPI while it was more in group C2 (IBD+HPS).

Levamisole treatment in HPS infected birds (groups B2 and D2) slightly reduced the CPK values at some intervals but without statistical difference as compared to their respective levamisole untreated (groups A2 and C2) birds.

Challenge of birds with HPS increased mean CPK activity. The CPK activity in IBD vaccinated plus HPS infected chickens (group C2) increased significantly from 1 DPI and remained significantly higher upto 9 DPI as compared to birds in group A2 (HPS) infection alone) where CPK activity increased significantly only from 3 to 7 DPI indicating increased severity of HPS in IBD vaccinated birds. Increased serum CPK activity has also been reported in natural and experimental cases of HPS affected chicks (Zaman and Khan, 1991; Akhtar, 1994; Soni, 1999; Deepak *et al.,* 2001). Levamisole treatment in HPS infected birds of groups B2 and D2 was able to slightly decrease the activity of serum CPK indicating only a little decrease in the severity of disease in levamisole treated groups. The elevated CPK activity in HPS infected chicks might be attributed to cardiac lesions (Benjamin, 1978; Cotran *et al.,* 1994). Furthermore, the difference in the results of serum CPK activity due to infection in IBD vaccinated and unvaccinated groups might be due to differences in the severity of cardiac lesions. Thus, this enzyme may have a diagnostic value to assess the magnitude of cardiac damage due to HPS infection. Moreover, the increase in serum CPK activity in human beings has been reported to be a reliable indicator of myocardial infarction (Cotran *et al.,* 1994).

An overview of different serum enzymes reveals that the activities of serum AST, ALT and CPK increased significantly in all the HPS infected groups, though the increase in activity of these enzymes due to HPS was more pronounced in IBD vaccinated birds. Oral administration of levamisole slightly reduced the activity of these enzymes but without statistical difference. On the basis of the findings of the present study, it may be concluded that IBD vaccination may enhance the severity of HPS infection as evident by the activities of serum enzymes. Use of levamisole as an immunomodulator alongwith IBD vaccine may reduce the severity of HPS.

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