

## EFFECT OF LEVAMISOLE ALONG WITH HVT VACCINE ON CELL MEDIATED IMMUNITY AGAINST MAREK'S DISEASE

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### ABSTRACT

Effect of levamisole on Herpes virus of turkey vaccinal immunity in chickens against Marek's disease was studied. Cell mediated immune response was studied using leucocyte adherence inhibition test. Levamisole improved cell mediated immune response significantly during early stages of Marek's disease infection. Leucocyte adherence inhibition was significantly better at 7 days post infection.

**Key words:** Levamisole, HVT vaccine, immunity, Marek's disease

Herpes virus of turkey (HVT) has been used as a vaccinal agent against Marek's disease (MD) in various parts of the world (Okazaki *et al.*, 1971). But there have been increasing reports of MD outbreaks even in the vaccinated flocks (Witter, 1982, Powell and Lombardini, 1986, Otaki *et al.*, 1989, McKimm-Breschkin *et al.*, 1990, Narang *et al.*, 2003). Increased incidence of vaccine failure has posed a serious problem to the prophylactic programmes against MD. Live fowl cholera vaccine (Maheswaran *et al.*, 1980), New Castle disease vaccine (Chenchev *et al.*, 1981, Mohan *et al.*, 1987, Vyas *et al.*, 1987, Hassan *et al.*, 1989) and coccidiosis vaccine (Giambrone and Klesius, 1985) offered better protection when used along with levamisole, a non-specific immunomodulator (Symoens *et al.*, 1980, Confer *et al.*, 1985). In the present study, the effect of levamisole on the development of HVT vaccinal immunity against MD in chickens is described.

### MATERIALS AND METHODS

**Experimental design:** One hundred and twenty unsexed, unvaccinated day-old White Leghorn (WLH) chicks were procured from Government Hatchery, Hisar. All the chicks were wing banded

and divided at random into eight groups of fifteen each on day 1. The birds of groups 1 to 4 and that of groups 5 to 8 were housed in separate rooms. The details of the treatments given to different groups were : group 1 (unvaccinated, uninfected and levamisole untreated), group 2 (unvaccinated, uninfected and levamisole treated), group 3 (HVT vaccinated, uninfected and levamisole untreated), group 4 (HVT vaccinated, uninfected and levamisole treated), group 5 (MDV infected, unvaccinated and levamisole untreated), group 6 (MDV infected, unvaccinated and levamisole treated), group 7 (MDV infected, HVT vaccinated and levamisole untreated), and group 8 (MDV infected, HVT vaccinated and levamisole treated).

A freeze dried cell free live HVT vaccine (Sri Biobiological Laboratories Pvt. Ltd., Pune) was obtained and stored at -20°C till further use. It was reconstituted just before use in a diluent supplied by the manufacturers as per their instructions, containing 1000 plaque forming unit (PFU) dose. The reconstituted vaccine was inoculated at 3<sup>rd</sup> day of age by sub-cutaneous route in groups 3, 4, 7, and 8. The pathogenic MDV field strain used in this study was obtained from Division of Pathology, Indian Veterinary Research Institute (IVRI), Izatnagar. The infected pooled blood from these birds was transferred to day-old WLH chicks at the dose rate of 0.5 ml i/p per bird. In the experiment,

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the freshly drawn blood from these chicks was used at the dose rate of 0.5 ml i/p per bird as a source of MDV for infecting the experimental birds of groups 5 to 8 on 17<sup>th</sup> day of age. Levamisole hydrochloride was obtained as pure powder. It was freshly dissolved in distilled water and was given at the dose rate of 15 mg/kg body weight orally for 5 days starting from 3 days of age in chicks of groups 2, 4, 6 and 8.

#### **Leucocyte adherence inhibition (LAI) test:**

This test was performed at the age of 24, 38, 52, 66, 80 and 94 days following the method of Halliday and Miller (1972) with slight modifications. Blood was collected from three birds at random from each group. Six ml of blood was collected from these three birds and leucocytes were separated using lymphoprep (Nyegaard and Co., Oslo, P = 1.077). Finally, the cells were suspended in culture medium RPMI -1640 (Hi-Media, Mumbai) so as to achieve a concentration of  $2 \times 10^7$  cells/ml of the medium.

Feather follicle antigen was prepared following the method of Byerly and Dawe (1972). The protein content of the fraction as estimated by the method of Lowry *et al.* (1951) was 1.23 gm/100 ml. The preparation gave positive result by agar gel precipitation test (AGPT) with a known MD positive serum.

To determine the optimum concentration of the antigen which can be employed for leucocyte adherence inhibition studies, serial two fold dilutions (1:2 to 1:64) of the antigen were prepared in 0.1 M PBS (pH 7.2). Per cent adherence inhibitions by these dilutions of antigens were determined. The effect was assessed on leucocyte of normal and MD affected birds/chickens. It was observed that an antigen dilution of 1:32 was optimum as it produced minimum inhibition of adherence on the normal leucocytes and a good effect on leucocytes from birds having MD. Therefore, this dilution of MD antigen having a final protein concentration of  $10.21 \mu\text{g}/0.05 \text{ ml}$  of antigen was used in subsequent studies. A set of three tubes was prepared for each group. The first tube contained equal volumes (0.05 ml) of leucocyte cell suspension and MD antigen and 0.1 ml of foetal calf serum (FCS). In the second tube, equal volumes of leucocyte cell suspension and tissue culture medium were mixed with 0.1 ml of FCS.

The third tube contained equal volumes of cell suspension and MD antigen (0.05 ml each) along with 0.1 ml of MD positive serum. All the three tubes were incubated at 37°C for 30 minutes with gentle shaking every five minutes. After incubation, the mixture from each tube was charged onto a separate haemocytometer chamber with the help of pasteur pipette. The charged chambers were then incubated at 37°C for 60 minutes in a humidified chamber.

After incubation, the nucleated cells present in five squares (used for erythrocytes counting) were counted on both sides of the chamber. Following cell counts, the chambers were immersed gently in a beaker containing Hank's balanced salt solution (HBSS) to remove the coverslips. The chambers were then washed by giving three to four gentle dips in HBSS. Finally, a drop of HBSS was put on the chamber and a fresh coverslip was put in place. The cells present in all the 10 squares (five on each side) were again counted. The percentage of adherence inhibition was calculated as under:

$$\% \text{ adherence} = \frac{\text{Number of cells after wash}}{\text{Number of cells before wash}} \times 100$$

$$\% \text{ adherence inhibition} = 1 - \left( \frac{\% \text{ adherence with antigen}}{\% \text{ adherence without antigen}} \right) \times 100$$

## **RESULTS AND DISCUSSION**

Per cent LAI values in uninfected (groups 1 and 2) and HVT vaccinated groups (groups 3 and 4) remained less than 27 per cent at most of the stages of the experiment (Table 1). Studies of Tong *et al.* (1979) and Thompson and Grosser (1979) showed that LAI values of 20 to 30 per cent were non-specific and might be considered as negative.

Mean per cent LAI values in birds which were unvaccinated, MDV infected (groups 5 and 6) were significantly higher than the values in their respective uninfected controls (groups 1 and 2) indicating depressed CMI. These observations are in agreement with those of Batra (1988) and Gupta *et al.* (1990). The values in the present study were higher immediately after the infection with virulent MDV and dropped suddenly at 63 days PI as also reported by Witter *et al.* (1976) and Batra (1988). The decrease in viraemia in later stages might be causing a decrease in number of leucocytes in



**Table 1**  
**Per cent leucocyte adherence inhibition (LAI) in different groups of chickens at various intervals**

Observation at days			Per cent LAI in different groups							
Age	PV	PI	Uninfected		HVT vaccinated		MDV infected		HVT vaccinated + MDV	
			1	2	3	4	5	6	7	8
			N <sup>a</sup>	L <sup>a</sup>	N <sup>ab</sup>	L <sup>a</sup>	N <sup>c</sup>	L <sup>c</sup>	N <sup>b</sup>	L <sup>ab</sup>
24	21	7	24.05 (5,7)**	21.98 (6,8)**	27.47 (5,7)**	26.00 (6)**(8)*	69.67 (1,3,7)**	66.54 (2,4,8)**	47.92 (1,3,5,8)**	33.99 (2,6,7)**(4)*
38	35	21	22.66 (5,7)**	20.55 (6,8)**	24.23 (5,7)**	25.60 (6)**	63.92 (1,3,7)**	60.59 (2,4,8)**	39.12 (1,3,5)**	31.80 (2,6)**
52	49	35	19.79 (5)**	23.58 (6)**	22.08 (5)**	20.88 (6)**	54.00 (1,3,7)**	58.27 (2,4,8)**	27.20 (5)**	26.70 (6)*
66	63	49	25.92 (5)**	23.16 (6)**	21.34 (5)**	18.49 (6)**	49.49 (1,3,7)**	47.92 (2,4,8)**	23.27 (5)**	21.61 (6)*
80	77	63	19.13 (5)**	17.34 (6)**(8)*	22.54 (5)**	24.96 (6)**	30.52 (1)**	31.00 (2)**	23.62 (5)**	26.07 (2)*
94	91	77	18.24 (5)**	17.48 (6)**	21.65 (6)**	20.25 (6)**	29.23 (1)**(7)*(2)**(4,8)*	28.40 (2)**	20.65 (5)*	18.87 (6)*
Mean % LAI			21.63 (5)**(7)*	20.68 (6)**	23.21 (6)**	22.69 (6)**	49.47 (1,3,7)**	48.78 (2,4,8)**	30.29 (5)**(1)*	26.50 (6)**

Within rows, numbers in parenthesis indicate group numbers that show significant difference \* ( $P \leq 0.05$ ) from the value of that group at that particular time, \*\* ( $P \leq 0.01$ ).

a,b,c - Common superscripts indicate no significant difference, while different superscripts indicate significant difference.

L - Chickens treated with levamisole, N - Untreated chickens, PV - Post-vaccination, PI - Post-infection.

circulation sensitised to MDV as the virus is lymphocytotropic and cell associated which might have led to a decrease in LAI value. It was observed that HVT vaccinated and MDV infected birds (groups 7 and 8) whether treated or not with levamisole showed positive values of LAI on 7<sup>th</sup> and 21<sup>st</sup> day post-infection (PI). This could possibly be attributed to MD viraemia (Witter *et al.*, 1976, Batra, 1988).

Per cent LAI values (Table 1) of levamisole treated, HVT vaccinated and MDV infected birds (group 8) were lower than that of levamisole untreated, HVT vaccinated, MDV infected (group 7) birds at most of stages of experiment and was significantly low at 7 days PI ( $P \leq 0.01$ ). Mean per cent LAI value of group 8 did not differ significantly from its uninfected control (group 2) whereas mean per cent LAI value of group 7 differed significantly ( $P \leq 0.05$ ) from its uninfected control (group 1). This could possibly be due to the levamisole treatment. Soppi *et al.* (1979) also reported an increased cellular immune response in normal chickens treated with levamisole (0.25 mg/kg). The results of the present study indicated that levamisole @ 15 mg/kg body weight when given along with HVT vaccine marginally improved the CMI response, particularly during the early stage of MD infection.

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