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Levamisole as an Immunomodulatory Agent for PPR Vaccinated Goats

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Abstract

In the present study, levamisole was used as an immunomodulator with PPR cell culture vaccine (live attenuated type). Elevated antibody titer was obtained when levamisole was used prior to the PPR vaccination in treatment groups. Due to vaccination there was also increase in the immunity which can be denoted from 14 day to 30 day. There was gradually an increase in the immune response because after vaccination the antibody producing cells activity increased. So, in the serum the vaccine titer was elevated, but after some time when the activity of antibody producing cells decreased, there was also a decline in immune status. Levamisole is a non-specific immunostimulator which can increase both the humoral and cellular immunity. 12 goats aged 6 months-1 year old were considered in the present investigation to assess the effect of levamisole as an immunomodulator on PPR vaccinated animals. Four animals were considered in each group with one group as control. In the present study, when the vaccine was administered with levamisole then the activity of immune effector cells also increased.

Keywords: Goats, Immunomodulator, PPR, Vaccine

Contents

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1. Introduction

Peste des petits ruminant's virus (PPRV) belongs to the family *Paramyxoviridae* and genus Morbillivirus**.** It is roughly spherical and enveloped RNA virus. Gibbs et al.[1] classified the PPRV as fourth member of genus Morbillivirus. It is closely related to the rinderpest virus of cattle and buffalo, the measles virus of human, the distemper virus of dogs and wild carnivores and the morbilliviruses of aquatic mammals**.**

Levamisole is a broad spectrum anthelmintic which functions in a manner similar to thymopoietin, a thymic hormone**.** It stimulates T-cell differentiation and T-cell response to antigens. It also elicits cell-mediated cytotoxicity, lymphokine production, phagocytosis by macrophages and neutrophils. Levamisole may therefore be of assistance in the treatment of chronic infections and neoplastic diseases, but may exacerbate disease caused by excessive T-cell function[2,3]. Cattle, sheep, goat, pig, chicken, mice, fish treated with levamisole show enhanced cellular and humoral immune response providing protection against different types of bacterial and viral infections**.** The present study was undertaken with the objective of assessing immunomodulatory effect of levamisole in goats thereby determining the efficacy of levamisole on PPR vaccine in goats.

2. Materials and Methods

Experimental treatment groups:

The goats were divided into three groups' of 4 goats each. *viz.*, A, B and C. In Group A, 4 goats were treated with levamisole HCl by S/C route @ 2.5 mg/kg body weight every other day for a week. In Group B, 4 No. goats were treated with levamisole HCl by S/C route @ 5.0 mg/kg body weight every other day for a week, and in Group C, which acted as Control group, 4 goats were treated only with equivalent saline subcutaneously.

Experimental design:

After 7 days interval of treatment with levamisole, all the animals were vaccinated with standardized freeze dried PPR cell culture vaccine (live attenuated type). Total 12 goats were vaccinated with 1.0 ml of reconstituted vaccine obtained from commercial source by S/C route at neck region. All the animals were aged between 6 months to 1 year.

Collection of serum:

Blood (5 ml) was collected with vacutainer fitted with appropriate needle, from jugular vein of the animals and allowed to clot for 45 min. After clotting of blood, the obtained serum was used for Indirect ELISA (i-ELISA). This type of ELISA was conducted as per the procedure described by Engvall & Perlman[4] by using controls in each plate. The serum was collected at 0 day, 7 day, 14 day, 21 day and 30 day. After collection of test serum from Groups A, B and C. Pooled serum was made from each individual group for each day. Detection the antibody titer of test and pooled serum was carried out by i-ELISA and readings were finally read ELISA plate reader at 492 nm[4]. The average of the OD value of negative controls was calculated and compared with the test OD values. The OD value of tests higher than the average OD value of the negative controls was considered as the positive samples. The measured ODs were correlated with the antibody concentration.

Quantitative assay of antibody titre of pooled serum by i-ELISA:

The pooled serum samples were serially diluted. Serial dilution was done at 1:100, 1:200, 1:400, 1:800 and so on. Ten tubes (capacity 1.5 ml each) were taken for 0 day, 7 day, 14 day, 21 day and 30 day each for Groups A, B and C. 990 µl PBS was added in first tube and 500 µl in other nine tubes. Then 10 µl pooled serum was mixed in first tube. After that 500 µl diluted serum was diluted serially up to the 10^{th} tube and 500 µl was discarded. The data was subjected to analysis of variance applicable to a completely randomised design. Statistical analysis of the data generated from mean ± standard error (S.E.).

3. Results and Discussion

Vaccination response:

Control Group C was treated only with vaccine. Table 2 presents the increase in antibody titer from day 7 to day 30. Group A was treated with both vaccine and levamisole @2.5mg/kg. Antibody titer increased from 7 day to 30 day (Table 1). Group B was treated with vaccine and levamisole @5.0mg/kg. Antibody titer increased from 7 day to 30 day (Table 1). The comparison between Group A and Group C represented OD value was higher in Group A than Group C.

Table 1. OD value of i-ELISA based on test serum samples of different groups

Group	OD value				
	0 Day	**7 Day**	**14 Day**	**21 Day**	**30 Day**
A	$0.161^{c} \pm 0.013$	$0.213^{b} \pm 0.019$	$0.261^{b} \pm 0.020$	$0.337^{ay} \pm 0.036$	$0.339^{ay} \pm 0.018$
B	$0.168^{c} \pm 0.012$	$0.230^{b} \pm 0.015$	$0.282^{b} \pm 0.018$	$0.402^{ax} \pm 0.031$	$0.414^{ax} \pm 0.034$
C	$0.149^{c} \pm 0.007$	$0.207^{b} \pm 0.016$	$0.211^{b} \pm 0.030$	$0.289^{az} \pm 0.012$	$0.313^{az} \pm 0.026$

Data were expressed as mean ± standard error (S.E.). Values bearing different superscripts in a row differed significantly ($P < 0.05$)

Table.2 OD value of i-ELISA based on pooled serum samples of different groups

Group	OD value				
	0 Day	**7 Day**	**14 Day**	**21 Day**	**30 Day**
A	0.152	0.244	0.265	0.345	0.372
B	0.140	0.253	0.290	0.412	0.438
C	0.114	0.222	0.248	0.308	0.363

Assessment the titre of antibody between the groups based on pooled serum samples:
Groups A, B and C showed increased antibody titer from 7 day to 30 day (Table 2). The comparison between Group A and Group C represented the OD value to be higher in Group A than Group C. The comparison between Group C and Group B represented that the OD value of Group B was higher than Group C. The OD value was higher in Group B than Group A. So, accordingly the titre of antibody as denoted by OD value showed to be highest in Group C.

Assessment the titre of antibody between the groups based on pooled serum samples:
The OD value (mean ± S.E.) of test serum in i-ELISA as represented in Table 2 was plotted in line diagram to know the response of levamisole for Groups A, B and C and also to know the antibody titer of PPR vaccine and PPR vaccine with levamisole at 0 day, 7 day, 14 day, 21 day and 30 days. PPR vaccine administered to goat and after 14^{th} day post vaccination, there was a gradual increase in the antibody titer till 56^{th} day[5]. Sheep administered with levamisole @2.5mg/kg body weight at repeated doses prior to blue tongue virus (BTV) vaccination and levamisole showed good anthelmintic and immunomostimulating properties[6]. Non-specific immunostimulant increased the level of immunoglobulin in colostrum and in cell-mediated and humoral immune response[7]. Levamisole was used as a non-specific immunostimulator in a concentration of 2.5 mg/kg[8]. Levamisole had immunostimulating effect in turkey when administered together with the CU strain of *Pasteurella multocida*. Vaccinated turkeys treated with levamisole had persistent higher systemic humoral immunity and cell-mediated immune responses than turkeys administered with the vaccine[9] as similar to the finding in the present study. The present study suggested that pre-treatment with levamisole stimulated both cellular and humoral immune response and by this prevent the immunosuppression in normal cell-mediated immunity[10].

4. Conclusion

Vaccination can enhance the immunity of animal. It increases the activity of B-cell and produce antibody within the body. When we influenced the activity of immune system by inducing some other substances, then this is called immunomodulator. When immunomodulator was administered with vaccine then the immune system activity highly increased. So, the level of immunity also increased.

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