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Immune Response of Yak (*Poephagus grunniens*) Following Trivalent Oil Adjuvant FMD Vaccination Along With Immunomodulator

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ABSTRACT

The present investigation was carried out to determine the immunomodulatory effcet of Levamisole on antibody response in yaks using liquid phase blocking enzyme-linked immunosorbent assay (LPBE) following trivalent oil adjuvant FMD vaccine (O, A, Asia-1). Twenty numbers of apparently healthy and young yaks were divided into two groups viz. groups I and II comprising of 10 animals in each group. All the animals of groups I and II were treated with a single dose of broad spectrum anthelmintic, fenbendazole orally prior to vaccination. The animals of group II were injected with Levamisol, six days prior and after FMD vaccination. On 30 days of post vaccination (dpv), there was a sharp rise to the antibody titres against all the 3 serotypes in animals of both the groups and the protective antibody level (log 10 ≥ 1.8) was maintained up to 90 dpv. A drastic fall of antibody titres against all the 3 serotypes was observed at 120 dpv in animals of both the groups. However, the protective antibody titre against the three serotypes at 180 dpv was maintained in few of the animals of the experimental group II but in Group I the protective titre was found up to 150 dpv only.

Keywords: FMD vaccine, immune response, immunomodulator, LPBE, Yak

The yak *(Poephagus grunniens L.)* is a multipurpose animal, which provides meat, milk, hair, wool, fuel and hide.In addition, it is considered to be an excellent pack animal for transportation of goods in difficult terrain (Lensch, 1996). In India, yaks are mostly found in Ladakh and Kargil of Jammu and Kashmir followed by Arunachal Pradesh, Sikkim and Himachal Pradesh (ICAR, 2006). Yak is treated as a wealth and a sacred animal, and worshiped as God by the people in the high hills of *Monpa*s in Arunachal Pradesh. In spite of this cultural, social and economic importance, the yak population has decreased over the past two decades. The hardy and multipurpose animal of this Himalayan region is not reared scientifically and most of the herdsmen are poor, tribal, small and marginal farmers in the remote, in accessible snow bound hilly terrains (3000 to 6000 m above mean sea level) and this animal in particular is the lonely source of livelihood for this deprived section (Bandyopadhyay and Bhattacharya, 2007). During harsh winter the animals migrate to the comparatively lower altitudes where they come in close contact with the other domestic animals like hill cattle, sheep, goats and mithun (Bandyopadhyay *et al.* 2007). Thus yak usually contract the disease from these animals and share the common grazing ground and stream for drinking (Bandyopadhyay *et al.* 2009). At the same time being an insidious reservoir of pathogen, yaks continue to be a real threat to other domestic animals also. Foot and Mouth disease (FMD) is probably the most important livestock disease in the world in terms of economic impact, which is primarily limited to cloven-footed domesticated animals, especially cattle, sheep, goat, pigs and buffalo (Hedger, 1981; Kumar *et al.* 1994). The disease was reported in Yak from various parts of the world including China, Nepal and India (Sarma *et al.* 1985; Weiner *et al.* 2003). Periodic vaccination of susceptible animals has been the only successful method to control foot and mouth disease in developing countries like India. In order to make vaccines more effective and also to combat its adverse effects in terms of residual pathogenicity and stress on animals, it has been emphasized on use of immunomodulatory agents

along with vaccination. Levamisole, the levo isomer of tetramisole, was introduced as an anthelmintic (Thienpont *et al.* 1966) for use in animals and humans since 1960s, and is now widely recognized and employed for its immunomodulatory activity (Symoens and Rosenthal, 1977; Baibiuk and Misra, 1982, Cuesta *et al.* 2004; Shah *et al.* 2011) It has been recommended for simultaneous use alongwith various vaccines (Hogarth- Scott *et al.* 1980). The immunological response against natural infection or vaccination of FMD virus was found to vary widely depending upon the hosts. However, no systematic study has so far been made on the FMD vaccine efficacy and their role in development of immunity in yak, particularly in the North Eastern Region of India. Thus the present study was undertaken to study the immune response of yak after vaccination with FMD polyvalent vaccine along with immuno modulator.

MATERIALS AND METHODS

Animals and experimental design

In the present study, a total of 20 apparently healthy and young yaks belonging to the National Research Centre on Yak, Dirang, Arunachal Pradesh, maintained uner semi-intensive system of management were selected. During night time animals were kept under shade and during day time they were let free to graze in the pasture. There was no history of foot and mouth disease outbreaks in the selected yak herds and no vaccination against FMD wascarried out for a period of one year prior to the present study. The animals were divided into two groups – groups I and II, comprising of 10 animals in each group. Animals of group I and II were treated with a single dose of broad spectrum anthelmintic, Fenbendazole @ 5 mg/kg b.wt.orally, prior to vaccination. After deworming, the animals of group II were injected Levamisol @ 2.5 mg/ Kg body weight 6 days prior and after FMD vaccination by subcutaneous route. The binary ethylene amine (BEI) inactivated oil adjuvanted trivalent (O, A, and Asia-1) FMD vaccine (Clovax, Intervet India Pvt. Ltd., Pune) was used for vaccination against FMD in the present experiment.

Immune response

To study the immune response, serum samples were collected from each yak following 0, 30, 60, 90, 120, 150 and 180 days post vaccination. The serum samples collected were screened for specific antibodies against FMD virus serotypes 'O', 'A' and 'Asia-1' by liquid phase blocking ELISA(LPBE) according to the method described by Central FMD virus Typing Laboratory, India, which was a modification of the method of Hamblin *et al.* (1986). Flat-bottom 96-well ELISA plates (Maxisorp, Nunc) were used to conduct the LPBE test and an ELISA titre less than 1.5 log was considered as negative and serum showing titre of 1.8 log or above was considered as 100% protective.

Statistical analysis

The data obtained from the present study were subjected to statistical analysis in Statistical Package for the Social Sciences 14 (SPSS14) Software (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

In the present study, on the day of vaccination of the entire animals group I and II were found to possess antibody levels below 1.5 $\log_{10}$ titre in LPBE, which was considered sero-negative. At 30 dpv there was a significant rise to the antibody titres (O: 1.95±0.11, A: 1.92±0.10, Asia-1: 1.89±0.10 in group I and O: 2.07 ±0.09, A: 2.04±0.09, Asia-1: 2.01±0.10 in group II) against all the serotypes (Table 1). This result corroborated with the findings of Bandyopadhyay *et al.* (2009), who also recorded the onset of antibody response to FMD vaccination by 30 dpv. However, in cattle rise of antibody titre was comparatively slow and reached to the highest level only at 120 dpv (Monika *et al*. 2006).

In the present study, serum samples from seven animals out of 10 (70%) in group I and nine animals out of 10 (90%) in group II showed protective antibody titre at 30 dpv against FMDV type 'O', 'A', and 'Asia-1'.This indicated that at 30 dpv, protection could be achieved with the oiladjuvanted FMD vaccine in yaks. In the present study, the protective antibody titre was also recorded at 60 dpv for all the three serotypes of FMD virus in most of the animals of both the groups (Group-I: 70, 60, and 60% and Group-II: 90, 80 and 70% against O, A and Asia-1 respectively). This indicated that a high protection rate persisted up to 60 dpv with oil-adjuvanted FMD vaccine. At 90 dpv, the number

Table 1: Serum antibody response of yaks to trivalent oil adjuvant FMD vaccine (O,A, Asia-1) estimated by liquid phase blocking ELISA

Group	LPBE titre (log_{10} values) at different days post vaccination against FMD virus types																							
	0 dpv			30 dpv			60 dpv			90 dpv			120 dpv			150 dpv			180 dpv			210 dpv		
	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1
I (n=10)	<1.5	<1.5	<1.5	1.95 ± 0.11	1.92 ± 0.10	1.89 ± 0.10	1.86 ± 0.10	1.86 ± 0.10	1.83 ± 0.09	1.68 ± 0.21	1.62 ± 0.20	1.50 ± 0.17	1.14 ± 0.25	1.11 ± 0.24	1.08 ± 0.23	0.93 ± 0.25	0.93 ± 0.25	0.90 ± 0.24	0.75 ± 0.25	0.75 ± 0.25	0.60 ± 0.24	0.45 ± 0.22	0.45 ± 0.22	0.30 ± 0.2
II (n=10)	<1.5	<1.5	<1.5	2.07 ± 0.09	2.04 ± 0.09	2.01 ± 0.10	1.98 ± 0.09	1.98 ± 0.10	1.92 ± 0.11	1.86 ± 0.09	1.86 ± 0.11	1.74 ± 0.09	1.32 ± 0.22	1.29 ± 0.22	1.23 ± 0.20	1.29 ± 0.22	1.26 ± 0.21	1.08 ± 0.24	1.23 ± 0.20	1.11 ± 0.22	1.05 ± 0.23	0.75 ± 0.25	0.75 ± 0.25	0.60 ± 0.24

GroupI-Levamisole untreated, Group II-Levamisole treated group

dpv, days post vaccination

Mean±SE antibody titre (Log10 values) against FMD virus types at different days of post vaccination

Table 2: Number of animals showing protective serum antibody titre against different FMD virus types by liquid phase blocking in group-I

Log_{10} values	No. of serum samples positive for FMD virus types																							
	0 dpv			30 dpv			60 dpv			90 dpv			120 dpv			150 dpv			180 dpv			210 dpv		
	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1
<1.5	10	10	10	0	0	0	0	0	0	1	1	1	3	3	3	4	4	4	5	5	6	7	7	8
1.5-1.79				3	3	3	3	4	4	3	4	5	5	5	6	5	5	6	5	5	4	3	3	2
≥ 1.8				7	7	7	7	6	6	6	5	4	2	2	1	1	1	0	0	0	0	0	0	0

Table 3: Number of animals showing protective serum antibody titre against different FMD virus types by liquid phase blocking in group-II

Log_{10} values	No. of serum samples positive for FMD virus types																							
	0 dpv			30 dpv			60 dpv			90 dpv			120 dpv			150 dpv			180 dpv			210 dpv		
	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1	O	A	As-1
<1.5	10	10	10	0	0	0	0	0	0	0	0	0	2	2	2	2	2	3	2	3	3	5	5	6
1.5-1.79				1	1	1	1	2	3	3	4	5	5	6	7	6	6	6	7	5	7	5	5	4
≥ 1.8				9	9	9	9	8	7	7	6	5	3	2	1	2	2	1	1	2	0	0	0	0

Table 4: Analysis of variance of number of animals with protective antibody titre by liquid phase blocking ELISA against different FMD virus types between groups and days post vaccination

Source of Variation	d.f.	Mean Standard		
		Type O	Type A	Asia-1
Group	1	1.89225*	1.701562*	1.463062*
Days	7	9.57825**	9.428705**	9.474991**
Interaction (Group X Days)	7	0.117964NS	0.07642NS	0.096348NS
Error	144	0.343	0.346438	0.319563

* P < 0.05, ** P < 0.01, ns Non-Significant

of animals showing protective level of antibody titer against all the three FMDV types was fewer than that of 30 and 60 dpv in both groups I and II. At 90 dpv, in group I, 60, 50 and 40% of animals and in group II, 70, 60 and 50% of animals possessed protective antibody titre against FMDV types O, A and Asia-1 respectively.

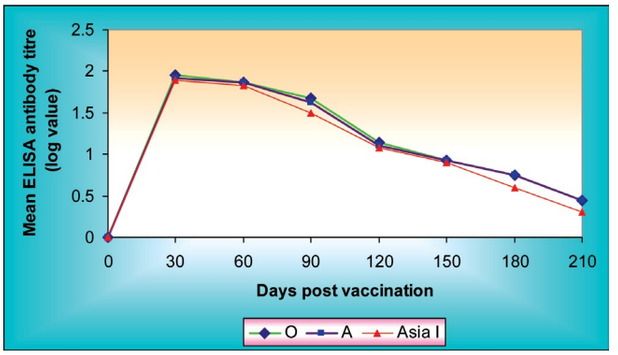

Fig. 1: Changes in LPBE antibody titre (log value) against various types of FMD virus in yaks of group I (Levamisole untreated) at different days post vaccination

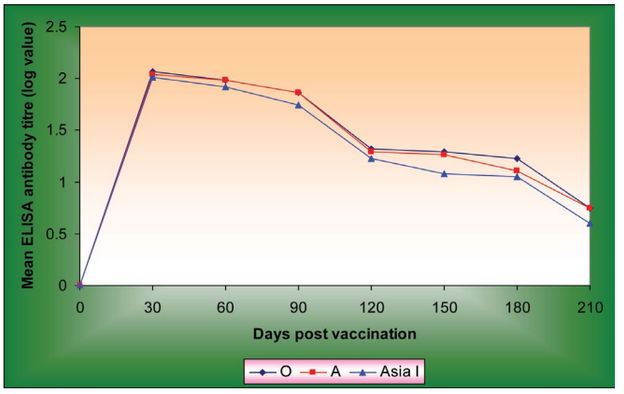

Fig. 2: Changes in LPBE antibody titre (log value) against various types of FMD virus in yaks of group II (Levamisole treated) at different days post vaccination

A declining trend of antibody titres (O: 1.14 ± 0.25, A: 1.11 ± 0.24, Asia-1: 1.08 ± 0.23 in group I and O: 1.32 ± 0.22, A: 1.29 ± 0.22, Asia-1: 1.23 ± 0.20 in group II) against all the three serotype was observed at 120 dpv in both the groups (Figure 1, 2).

The percentages of animals showing protective antibody titre at 120 dpv were 20 and 30% against virus type 'O' ingroups I and II respectively, 20% of animals against type A and 10% against Asia-1 in both the groups. This result corroborated with the findings of Bandyopadhyay *et al.* (2009), who also recorded the decline of antibody response to FMD vaccination by 120 dpv. The protective antibody titre against the three serotypes at 180 dpv was maintained in few of the animals of the levamisole treated group II but in Group I the protective titre was found in few of the animals up to 150 dpv only (Figure 3).

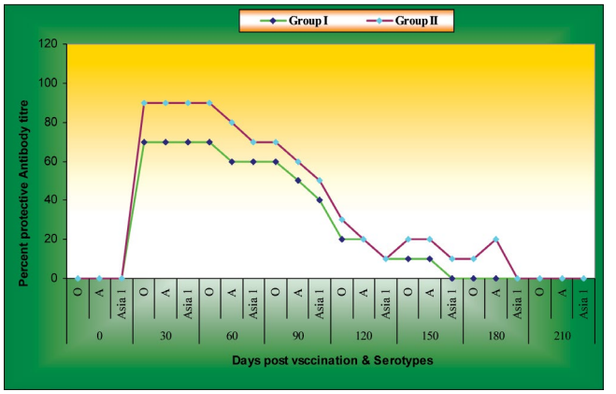

Fig. 3: Percentage of animal showing protective antibody titre after FMD vaccination in Yaks of group I (Levamisole untreated) and group II (Levamisole treated)

In the present study, a wide variation of serum neutralising antibody levels between the different FMD virus types was recorded. The variation in immunogenicity among the strains might be due to variation in antigenicity of the different types and subtypes of FMDV and their concentration in the vaccine. The results of LPBE assay revealed a variation in the number of animals showing protective antibody titre against each type of FMDV at different dpv. Statistical analysis indicated that the variations were significant ($P < 0.05$) between the two vaccinated groups, i.e. groups I and II (Table 4). It was in agreement with the views expressed by Vanselow (1987) that Levamisole enhanced the immune response to vaccination.

In the present study, the variable antibody response found in yaks when compared with pigs or cattle may be due to the inherent genetic differences of the yak. Different studies using molecular genetic tools suggest that yak is quite different from other bovines and subgenus *Poephagus* is adopted instead of *Bos* (Weiner *et al.* 2003).

CONCLUSION

The present study revealed that the Levamisole treated group of animals showed a better antibody response as compared to the untreated trivalent oil adjuvant FMD vaccinated group. Further, the protective antibody titre (≥ log 1.8) could be detected and reached a peak at 30 dpv persisting up to 90 dpv in polyvalent FMD vaccinated yaks.

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