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Evaluation of immune response after foot-and-mouth disease vaccination in yak by liquid phase blocking ELISA

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The yak *(Poephagus grunniens L*), a precious multipurpose animal, is able to withstand extreme harsh weather conditions prevailing at an altitude above 3000 metre mean sea level (msl). Yak is of vital importance and contributes significantly to the human economy as meat, milk, wool, leather, dung and as pack animal for its owners in remote mountainous regions of India adjacent to the Himalayas (Arora 1998). In North East India around 9 000 yaks are present in the West Kameng and Tawang district of Arunachal Pradesh. FMD is a very common and serious disease of yak recorded mostly in the February and March in this region (Bandyopadhyay *et al*. 2007). Commercially available oil adjuvanted inactivated vaccine containing the viral types and subtypes, is widely used for regular immunization in domestic cattle to prevent FMD. However, studies on immune response of this type of vaccine in yaks is scanty. Considering the above fact present study highlighted the immune response after vaccination with inactivated oil adjuvanted trivalent (O, A and Asia-1) FMD vaccine at different time interval and also to establish a vaccination schedule against FMD in yak.

Apparently healthy young yaks (18) of 6 month to 2 years of age of either sex were divided into groups 1, 2 and 3 comprising 6 animals in each group. There is no history of foot-and-mouth disease (FMD) outbreaks in the selected yak herds for last 9 years and no vaccination against FMD was carried out in 1 year prior to the present study.

The binary ethylene amine (BEI) inactivated oil adjuvanted trivalent (O, A and Asia-1) FMD vaccine was used for vaccination against FMD in the present experiment. Vaccine was administered @ 2 ml/animal as per the manufacturer's recommendation. All the animals of groups 1, 2 and 3 received the first dose of FMD trivalent vaccine on '0' day. The animals of group 1 were given booster dose after 1 month of primary vaccination and animals of group 2, were boosted after 2 months of primary vaccination. The animals of group 3 were revaccinated after 6 months of primary vaccination as it is a generally practised schedule in cattle, and animals of groups 1 and 2 were revaccinated after 6 months of booster vaccination.

There was no reference regarding vaccination schedule against FMD in yak hence the above schedule of vaccination was devised.The serum samples were collected on the day of vaccination ('0' day, prior to vaccination) and thereafter at 30^{th}, 60^{th}, 90^{th}, 120^{th},150^{th},180^{th}, 210^{th}, 240^{th} and 270^{th} days post primary vaccination (dppv) from all the groups.

Liquid phase blocking ELISA (LPBE) is the choice of serological test to determine antibody titre against FMD as reported by various workers (Pattnaik *et al.* 1991, Psikal *et al.* 1995, Dus Santos *et al.* 2000, Niedbalski 2004). Serum samples collected from vaccinated yaks were assayed for type-specific antibodies against the FMD virus (FMDV) types O, A and Asia-1 by LPBE test and was performed according to the method described by Project Directorate on Foot-and-mouth Disease, IVRI Campus, Mukteswar, Nainital, India, which was a modification of the method of Hamblin *et al.* (1987). Reference FMDV antigens (O, A and Asia-1) and type specific antisera (rabbit and guinea pig) were also obtained from the PD FMD. Adequate positive controls were kept during the test following standard technique. The optical density of each well of ELISA plate was measured at the wave length of 492 nm. End point titre was expressed as the negative logarithm of the highest dilution of serum-virus mixtures that gave 50% inhibition of colour.

For serological evaluation serum antibody titre of ≥ 1.8 $\log_{10}$, which is equivalent to a serum dilution of 1:64, was taken as the protective antibody titre against FMD virus specific antibody as per protocol of PD FMD. Statistical

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analysis of the experimental data was done as per Snedecor and Cochran (1994).

In the present study, serum samples of all the 3 experimental groups of yaks were tested by LPBE for detecting antibodies against the three FMD virus types, i.e. O, A and Asia-1. Serological evaluation of serum antibody titre of $\geq 1.8 \log_{10}$, which is equivalent to a serum dilution of 1:64, was taken as the protective antibody titre against FMD virus specific antibody. The results of antibody titres against FMD virus types with percentage of animals showing protective antibody titre at different days post vaccination are shown in Table 1.

On the day of vaccination, all the animals of the group 1, 2 and 3 possessed antibody levels below $1.5 \log_{10}$ titre against all 3 FMDV types by LPBE which was considered seronegative. At 30 dppv a sharp rise of antibody titre was detected in all the 3 experimental groups against all the virus types. All the animals (100%) of group 1, 2 and 3 exhibited protective antibody titer against all the serotypes at 30 days of post primary vaccination (Table 1).

Analysis of variance showed that the antibody titre at 30 dppv differed significantly ($P<0.01$) with mean antibody titre on the day of vaccination against FMDV type O, A and Asia-1 (Table 2).

Borua (2006), Mukherjee *et al.* (2006) Venkataraman *et al.* (1994) and Bandyopadhyay *et al.* (2009) also observed the peak antibody titre at 30 dpv following FMD vaccination in cattle and yak. Chitravel *et al.* (1997) reported protective antibody levels in 84, 88, 92 and 92% of cattle against FMD virus types O, A, C and Asia-1, respectively, after 21 days post primary vaccination on the basis of SNT.

The animals of group 1, which received booster dose at 30 dppv showed a peak antibody titre at 60 dppv (Table 1). This result corroborated with the observation of Tekerlekov *et al.* (1983), who reported that booster vaccination at 30 dppv boost up the immune response. Protective antibody titre in this group persisted up to 150 dppv with mean antibody titre 1.90±0.10, 1.85±0.09 and 1.80±0.07 against type O, A and Asia-1 respectively. The percentage of animals that showed protective antibody titre in this group at 150 dppv was 83.33% against all the 3 serotypes of FMDV (Table 1). Tekerlekov *et al.* (1983) observed that revaccination at 1 month could maintain protective immunity up to 5 months in 90% animals. A drastic fall of antibody titre against all the 3 serotypes were recorded at 180 dppv with mean antibody titre 1.45±0.29, 1.45±0.29 and 1.40±0.28 against type O, A and Asia-1 respectively. Percentage of animal showed protective antibody titre was 66.66% against both type O and A and 50% against type Asia-1. Statistically, it was observed that there was significant difference between antibody titre of 150 dppv and 180 dppv against all the 3 serotypes of FMDV (Table 2).

The antibody titre remained below protective level up to 210 dppv and percentage of animals that showed protective

Table 1. Mean (±SE) LPBE FMD virus specific antibody titre (log values) with percentage (%) of animals showing protective antibody titre in different groups of yaks at different days post vaccination

Days	Group 1			Group 2			Group 3		
	Type O <1.50	Type A <1.50	Type Asia1 <1.50	Type O <1.50	Type A <1.50	Type Asia1 <1.50	Type O <1.50	Type A <1.50	Type Asia1 <1.50
0 day	2.05 ± 0.09100*	2.05±0.09100	1.95±0.06100	2.05±0.09100	2.05±0.09100	2.00±0.10100	2.10±0.07100	2.10±0.07100	2.05±0.09100
30 dppv	2.20±0.10100	2.15±0.12100	2.10±0.10100	2.00±0.1283.33	2.00±0.1283.33	1.90±0.1083.33	2.05±0.1283.33	2.00±0.1283.33	1.95±0.1083.33
60 dppv	2.15±0.09100	2.10±0.10100	2.00±0.10100	2.35±0.05100	2.30±0.06100	2.25±0.06100	1.85±0.1266.66	1.80±0.1066.66	1.75±0.0966.66
90 dppv	2.05±0.09100	2.00±0.10100	1.90±0.06100	2.30±0.06100	2.25±0.06100	2.15±0.05100	1.40±0.2850	1.35±0.2733.33	1.30±0.2616.66
120 dppv	1.90±0.1083.33	1.85±0.0983.33	1.80±0.0783.33	2.15±0.09100	2.10±0.07100	2.00±0.06100	1.35±0.2733.33	1.30±0.2616.66	1.25±0.250
150 dppv	1.45±0.2966.66	1.45±0.2966.66	1.40±0.2850	2.00±0.06100	1.90±0.1083.33	1.85±0.0983.33	1.25±0.250	1.25±0.250	1.00±0.320
180 dppv	1.40±0.2850	1.35±0.2733.33	1.35±0.2733.33	1.90±0.1083.33	1.85±0.0983.33	1.80±0.0783.33	2.15±0.09100	2.15±0.09100	2.10±0.07100
210 dppv	2.20±0.10100	2.15±0.09100	2.15±0.09100	1.85±0.0983.33	1.80±0.0783.33	1.75±0.0583.33	2.10±0.07100	2.05±0.09100	1.95±0.1083.33
240 dppv	2.05±0.09100	2.00±0.06100	1.95±0.1083.33	2.30±0.06100	2.25±0.10100	2.20±0.10100	1.95±0.1083.33	1.90±0.1083.33	1.80±0.0783.33
270 dppv									

Group 1, FMD vaccination on '0' day, 30 dppv and 210 dppv; group 2, FMD vaccination on '0' day, 60 dppv and 240 dppv; group 3, FMD vaccination on '0' day and 180 dppv.
* Percentage of animals showing protective antibody titre.

Table 2. Group and day wise variation of average antibody titre (log value) against different types of FMD virus

Groups	Variation of average antibody titre (log value) against TYPE									
	0 day	30 dppv	60 dppv	90 dppv	120 dppv	150 dppv	180 dppv	210 dppv	240 dppv	270 dppv
						'O' FMD virus				
1	$0^{a}{}_{A}$	$2.05^{a}{}_{B}$	$2.2^{a}{}_{BC}$	$2.15^{a}{}_{BCD}$	$2.05^{a}{}_{BCDE}$	$1.9^{a}{}_{BEF}$	$1.45^{a}{}_{G}$	$1.4^{a}{}_{GH}$	$2.2^{a}{}_{BCDE}$	$2.05^{a}{}_{BCDEF}$
2	$0^{a}{}_{A}$	$2.05^{a}{}_{B}$	$2^{b}{}_{BC}$	$2.35^{b}{}_{D}$	$2.30^{b}{}_{DE}$	$2.15^{b}{}_{BCDEF}$	$2^{b}{}_{BCFG}$	$1.9^{b}{}_{BCGH}$	$1.85^{b}{}_{BCGH}$	$2.3^{b}{}_{DEF}$
3	$0^{a}{}_{A}$	$2.1^{a}{}_{B}$	$2^{b}{}_{BC}$	$1.85^{c}{}_{CD}$	$1.4^{c}{}_{E}$	$1.35^{c}{}_{EF}$	$1.25^{c}{}_{EF}$	$2.15^{c}{}_{BG}$	$2.1^{a}{}_{BG}$	$1.95^{a}{}_{BCDG}$
1	$0^{a}{}_{A}$	$2.05^{a}{}_{B}$	$2.15^{a}{}_{BC}$	$2.10^{a}{}_{BCD}$	$2^{a}{}_{BCDE}$	$1.85^{a}{}_{BEF}$	$1.45^{a}{}_{G}$	$1.35^{a}{}_{G}$	$2.15^{a}{}_{BCDEH}$	$2^{a}{}_{BCDEFH}$
2	$0^{a}{}_{A}$	$2.05^{a}{}_{B}$	$2^{b}{}_{BC}$	$2.3^{b}{}_{D}$	$2.25^{b}{}_{BDE}$	$2.1^{b}{}_{BCDEF}$	$1.9^{b}{}_{BCFG}$	$1.85^{b}{}_{BCGH}$	$1.8^{b}{}_{CGH}$	$2.25^{b}{}_{BDEF}$
3	$0^{a}{}_{A}$	$2.1^{a}{}_{B}$	$2^{b}{}_{BC}$	$1.8^{c}{}_{CD}$	$1.35^{c}{}_{E}$	$1.3^{c}{}_{EF}$	$1.25^{c}{}_{EF}$	$2.15^{c}{}_{BCG}$	$2.05^{a}{}_{BCGH}$	$1.9^{a}{}_{BCDH}$
1	$0^{a}{}_{A}$	$1.95^{a}{}_{B}$	$2.10^{a}{}_{BC}$	$2^{a}{}_{BCD}$	$1.90^{a}{}_{BCDE}$	$1.80^{a}{}_{BDEF}$	$1.4^{a}{}_{G}$	$1.35^{a}{}_{G}$	$2.15^{a}{}_{BCDH}$	$1.95^{a}{}_{BCDEFH}$
2	$0^{a}{}_{A}$	$2^{a}{}_{B}$	$1.9^{b}{}_{BC}$	$2.25^{a}{}_{D}$	$2.15^{b}{}_{BDE}$	$2^{b}{}_{BCEF}$	$1.85^{b}{}_{BCFG}$	$1.8^{b}{}_{BCFGH}$	$1.75^{b}{}_{CGH}$	$2.2^{b}{}_{BDEF}$
3	$0^{a}{}_{A}$	$2.05^{a}{}_{B}$	$1.95^{b}{}_{BC}$	$1.75^{c}{}_{CD}$	$1.3^{c}{}_{E}$	$1.25^{c}{}_{E}$	$1^{c}{}_{F}$	$2.1^{c}{}_{BCG}$	$1.95^{c}{}_{CDGH}$	$1.8^{c}{}_{CDH}$

Figure in column and row bearing a common superscript and subscript respectively do not differ significantly.

antibody titre was 50% against type O and 33.33% both against type A and Asia-1 (Table 1). Again, following revaccination (on 210 dppv) antibody titre was restored to the higher level at 240 dppv. Percentages of animals that showed protective antibody titre was also restored to 100% level against all the 3 serotypes of FMDV (Table 1). The antibody titre maintained at higher level on 270 dppv. In animals of group 1, statistically, there was no significant difference between antibody titre from 30 dppv to 150 dppv against all the FMD virus types, however it was observed that there was significant difference between antibody titre of 150 dppv and 180 dppv and also between 210 dppv and 240 dppv against all the 3 serotypes of FMDV. However there was no significant difference of antibody titre between 240 dppv and 270 dppv (Table 2).

In animals of group 2, which received a booster dose at 60 dppv showed a peak titre at 90 dppv (Table 1). Similar findings were reported by Francis *et al.* (1983) who observed a rise in neutralizing antibody titre in serum of cattle after 7 to 21 days following vaccination either with oil emulsion or aqueous vaccines. He also observed a further rise in titre following revaccination at 63 days. Cloete *et al.* (2008) also reported that following booster at 8 weeks will help to rise the neutralizing titre to maximum level by 12 weeks. Protective antibody titre in this group was maintained up to 240 dppv except a slight fall against type Asia-1. Percentage of animals showed protective antibody titre at 240 dppv was 83.33% against all the 3 serotype of FMD virus (Table 1). Spath *et al.* (1995) also reported similar type of observation using oil adjuvanted quadrivalent (O1, A79, A87 and C85 FMDV strain) vaccine in calves devoid of maternally derived antibody. They observed protective antibody titre up to 4 months after vaccination and additional second vaccination at 60 dppv ensure high antibody levels for longer duration. This finding also corroborated with the findings of Doel (2005), who observed similar type of antibody response in cattle against type A following vaccination with oil adjuvanted aluminium hydroxide/saponin vaccine containing A_{22} strain. He reported that boostering at 2 month interval following primary vaccination showed excellent titre of antibody for a sustained period in contrast to the primary course of vaccination, where the intervals were shorter. Cloete *et al*. (2008) also reported that booster immunization at 8 week post primary vaccination using oil adjuvanted FMD vaccine elicited a long lasting protective immune response in cattle which persisted up to 32 to 40 weeks post primary vaccination. In group 2, again antibody titre was restored at higher level at 270 dppv (following revaccination at 240 dppv). Statistically, there was significant difference of antibody titre between 60 dppv and 90 dppv, however there was no significant difference of antibody titre of 30 dppv and 60 dppv against all the three serotypes of FMDV. Again there was no significant difference of antibody titre between 240 dppv and 270 dppv (Table 2).

However, the animals of group 3 which were not boosted but revaccinated at 180 dppv showed higher level of antibody titre up to 60 dppv (Table 1). In this group lower protective antibody titre persisted up to 90 dppv. Percentage of animals showing protective antibody titre at 90 dppv was 33.33% against all the serotypes of FMD virus. The antibody titre then declined steadly and it remained below protective level up to 180 dppv. Similar finding was also reported by Bandyopadhyay *et al*. (2009) who observed protective antibody titre up to 90 days after primary vaccination in yaks. Das (2007) and Mahanta (2007) reported similar observation in cattle. They found that following vaccination with BEI inactivated FMD vaccine protective antibody titre appear at 30 dppv and persisted up to 90 dppv against FMDV type O, A and Asia-1. Similar type of declining trend in antibody response was also reported by Awad *et al.* (1979) and Mathur *et al.* (1981). At 120 dppv a drastic fall of antibody titre against all the 3 serotypes was observed in group 3 and this fall in titre continued up to 180 dppv (Table 1). None of the animals showed protective antibody titre at 180 dppv.

Following revaccination (180 dppv) antibody titre was restored to higher level at 210 dppv and animals remained protective till the last observation period (270 dppv). Auge de Mello and Gomes (1977) also observed that, following revaccination at 5 months apart with oil adjuvanted vaccine containing type O, A and C, the antibody titre showed higher level at 60 days post revaccination and the titre was maintained up to 180 days post revaccination. Statistically, there was no significant difference of antibody titre between 60 dppv and 90 dppv whereas significant difference of antibody titre was observed between 90 dppv and 120 dppv and between 180 dppv and 210 dppv. However no significant differences were observed in antibody titre between 240 dppv and 270 dppv, 60 dppv with 240 dppv and 270 dppv (Table 2).

Analysis of variance showed that, during the whole period of study, highly significant difference ($P<0.01$) in antibody response between the groups at each time of post vaccination. Result of critical difference table showed significant difference in overall mean antibody titre among groups 1, 2 and 3 against all the 3 serotypes irrespective of days post vaccination.

The overall mean antibody titre was highest in group 2 followed by group 1, while group 3 showed the lowest level of antibody response as ditected by LPBE.

In group 1, which received a booster dose of vaccine at 30 dppv showed a higher and long lasting antibody response compred to group 3. But it was lower and shorter duration in comparision to group 2. While comparing the 3 schedules of vaccination against FMD in yaks it was observed that the schedule followed in group 2 is most efficient for maintenance of protective antibody titre for a longer period of time.

SUMMARY

Immune response against an oil adjuvanted binary ethylenimine inactivated trivalent FMD vaccine containing virus types O, A and Asia-1 were evaluated in 3 groups of yak, viz. 1, 2 and 3 with subsequent boostering after 1, 2 and 6 months respectively. Animals of group 1 and 3 showed antibody titre above protective level up to 150 dppv and 90 dppv respectively, indicating that protective antibody titre did not persist up to the next revaccination period (210 dppv and 180 dppv respectively) in both these 2 groups. Highest antibody titre was detected against FMDV type O followed by type A and Asia-1. With oil adjuvanted trivalent FMD vaccine, a booster dose at 60 day post primary vaccination is more effective in yaks which were not vaccinated earlier to maintain them in disease free status for a longer duration.

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