EVALUATION OF PESTE DES PETITS RUMINANTS (PPR) CELL CULTURE VACCINE IN GOATS AND SHEEP IN INDIA

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ABSTRACT
Peste des Petits Ruminants (PPR) is a highly contagious viral disease of ruminants. The disease has high impact on small ruminants market, especially in Africa and Middle East. India has a large population of sheep and goats, having significant part in world ruminant population. Prevention and control programs by vaccines are necessary parts of ruminants business. PPR vaccines are successfully used by small and large farmers in various parts of India. Researches have been done to investigate the efficacy of PPR vaccines on sheep and goats, but few data are available on sero-conversion in the bodies. In present study, sheep and goats were vaccinated with Sungri/96 strain and serum collection was done up to one year. Antibodies levels were measured with competitive ELISA. Antibody levels reached to protective levels within 21 days of vaccination, which continued up to one year. Sheep responded to vaccine slightly better than goats. Further studies are required to investigate total duration of protection by PPR vaccine in small ruminants.

Keywords: PPR, India, antibody, vaccine, immune

INTRODUCTION
Peste des Petits Ruminants (PPR) disease is a viral disease of sheep and goats having significant economic concern all over the world. PPR virus (PPRV) is a member of the genus Morbivirus of family Paramyxoviridae. The disease has 3-6 days of incubation period, followed by high fever, ocular nasal discharge, pneumonia, stomatitis and inflammation of gastrointestinal tract leading to severe diarrhoea followed by death or recovery (Zahur et al., 2008; Sen et al., 2010; Balamurugan et al., 2014). The virus spread through close contact between infected and healthy animals. Primarily it is transmitted through respiratory route. PPR disease was first recorded in West Africa in 1940s by Gargadennec and Lalanne (1942). Prevalence was also found in other parts of Africa and Middle East (Rahim et al., 2010; Aamer et al., 2014, Banyard et al., 2014). The disease was reported in India in 1990s, first in southern and then northern part (Shaila et al., 1996; Kumar et al., 1999, Taylor et al., 2001). PPR has huge economic concern worldwide as well as India (Muthuchelvan et al., 2015). The morbidity of the disease may be up to 100% and severe fatality in acute cases (OIE, 2013). India has a small ruminant population of 200 million and estimates show that PPR causes economic losses up to US$ 39 every year in India. In past years, there has been several outbreaks of PPR recorded in India (Singh, 2011). Controlling PPR seems to be comparatively easier than other economically important fatal viral diseases like foot and mouth disease and bluetongue. Effective vaccines are also available to control the prevalence of the disease. Several researches reveal that PPR vaccines of different strains have protected small ruminants like sheep and goats (Awa et al., 2003; Rashid et al., 2010; Aamer et al., 2014). Most researchers have preferred ELISA method to detect presence of specific antibodies against PPRV. It is easy, rapid, reliable and less time consuming method for the experiment. Looking at present scenario of PPR disease in India and control programs in the urban as well as rural animal husbandries, present study was designed to investigate specific antibody response of PPR vaccine of Indian market on sheep and goats.

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MATERIALS AND METHODS

Grouping and rearing of goats
45 healthy Jamunapuri goats and 45 healthy Marwari sheep of one year of age were selected for the study and divided into four groups:
Group 1: 35 goats (vaccinated with PPR live vaccine (Sungri/96 strain), procured from Hester Biosciences Limited, India)
Group 2: 10 goats (unvaccinated control)
Group 3: 35 sheep (vaccinated with PPR live vaccine (Sungri/96 strain), procured from Hester Biosciences Limited, India)
Group 4: 10 sheep (unvaccinated control)

Vaccinated goats and sheep from group 1 and 3 were kept separately at Merda-Adraj village, Gujarat, India. Unvaccinated goats and sheep from group 2 and 4 were kept at Jetpura village, Gujarat, India. All animals were subjected to free supply of feed and water and observed daily throughout study.

Vaccination, blood collection and testing
Animals from group 1 and 3 were vaccinated subcutaneously in cool atmosphere with one dose of PPR live vaccine (Sungri/96 strain), procured from Hester Biosciences Limited, India. Blood samples were collected from 10 animals/group at 0, 7, 14, 21, 28, 35, 42, 49 and 56 days; followed by 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months. Serum was separated and stored at -20 °C.

All sera samples were subjected to ELISA test using ELISA kit (ID.vet innovative diagnostics, France) at Hester Biosciences Limited, Anand laboratory. The ELISA was run after collection of all the blood samples and it was run as per the manufacturer's protocol and instruction. Before use, all the samples and reagents were allowed to room temperature and homogenized by gentle mixing. 25 µl of dilution buffer was added to each well. 25 µl of the positive control was added to wells A1 and B1. 25 µl of the negative control was added to wells C1 and D1. 25 µl of each sample to be tested was added to the remaining wells. Plate was incubated for 45 min at 37 °C. Each well was washed with 300 µl wash solution 3 times. Conjugate 1X was prepared by diluting 10X conjugate to 10 times with dilution buffer. Further, 100 µl 1X conjugate was added to each well. Plate was incubated for 30 min at 21 °C. Each well was washed with 300 µl wash solution 3 times. 100 µl of substrate solution to each well was added. Again it was incubated for 15 min. at 21 °C. Finally, 100 µl of stop solution was added to each well to end the reaction. Then O.D. at 450 nm was read. The unit of measurement was S/N percentage. S/N percentage of ≤ 50% was considered positive, while that of 50-60% was considered doubtful. >60% S/N percentage was considered negative for presence of antibodies against PPRV. All the results were recorded as Mean ± S.E.M.

Data analysis
All data were entered into Microsoft Office Excel Worksheet (2013, Microsoft Corporation). The data were analyzed by single factor – analysis of variance method and p < 0.05 was considered as significant difference between the groups.

RESULTS
The initial antibody titres of goats were 229.0 ± 3.2 (Group 1) and 238.3 ± 6.4 (Group 2). The initial titres of sheep were 245.1 ± 7.8 (Group 3) and 253.9 ± 8.4 (Group 4). S/N percentage decreased gradually in both vaccinated groups (Group 1 and 3). Vaccinated goats’ (Group 1) S/N percentage was decreased drastically and significantly up to 21 days (24.6 ± 1.3), which continued to change at smaller differences thereafter up to one year. Same scenario of results was observed in the results of vaccinated sheep also (Group 3) (Figure 1). It was also observed that there was slightly less S/N % in vaccinated sheep as compared vaccinated goats (Figure 2). There was consistent antibody level observed in both unvaccinated groups of goats and sheep (Group 2 and 4) throughout the year.
Figure 1. S/N percentage of group 1 and 2 (Vaccinated and unvaccinated goats)
The data are expressed as mean ± SEM
* p < 0.05, significantly different as compared to 0 days

Figure 2. S/N percentage of group 3 and 4 (Vaccinated and unvaccinated sheep)
The data are expressed as mean ± SEM
* p < 0.05, significantly different as compared to 0 days
DISCUSSION
In present study, unit of measurement was S/N percentage, using calculation referred by manufacturer of ELISA kit. Rashid et al. (2010) reported the antibody levels with percentage inhibition values, which was considered directly proportional to the antibody levels. In present study, it was assumed that lesser the S/N %, higher the antibody levels. Before vaccination, blood was withdrawn from sheep and goats and screening was done for the presence of antibodies, which was found negative, as all samples had > 200 % S/N ratio. After vaccination, within 7 days only, S/N % decreased drastically to around 80 in both the species. But, the titre reached to protective level (<50%) at 21 days interval after vaccination. Similar observation was reported by Rashid et al., 2010, where % inhibition reached to >50 in both vaccinated species i.e., sheep and goat. Other researchers also found similar results after vaccination to sheep and goats (Chaudhary et al., 2009; Gelagay et al., 2012; Fakri et al., 2015). After 21 days of vaccination, the titre remained protective up to the end of the study in sheep and goats as well (Rashid et al., 2010; Aamer et al., 2014; Zeidan et al., 2016). It was also observed that sheep responded better to the vaccine than goats, but the data were not significantly different. Further investigations can be done to understand the protective behavior of the vaccine extended than one year.

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