Comparative immunogenicity of two peste des petits ruminants (PPR) vaccines in South Indian sheep and goats under field conditions

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Abstract Peste des petits ruminants (PPR) is an economically important endemic viral disease of sheep and goats in India, where several different homologous PPR vaccine candidates have been developed. We evaluated the serological response to two vaccine strains, Arasur/87 and Sungri/96, in South Indian cross-bred and native sheep and goats reared under organized and unorganized settings. Animals seronegative (percent inhibition or PI <40) by competitive enzyme-linked immunosorbent assay (c-ELISA) were immunized with either of the vaccine strains or placebo. Sera collected on 21, 60 and 90 days post-vaccination were subjected to c-ELISA and serum neutralization test (SNT). Seropositivity (PI >40), seroconversion

(fourfold increase in SNT titres) and seroprotection (SNT titre of ≥ 8 deemed to be protective) ranged from 66.7 to 84.0 %, 56.0 to 69.2 %, and 60.0 to 76.0 %, respectively. However, no significant difference was observed between responses to the two vaccine strains. These results support the premise that the two vaccine strains are equally efficacious.

Keywords Peste des petits ruminants · Homologous vaccine · Comparative evaluation · Humoral immune response · Field testing

Introduction

Peste des petits ruminants (PPR) is endemic to Sub-Saharan Africa, Arabian Peninsula, Middle East and parts of Asia, including India [2–4, 7, 12, 15, 24–26, 34]. The high morbidity and mortality associated with PPR, and the impracticability of culling animals infected with PPR are major concerns for the sustenance of meat and wool industry. Thus, PPR poses serious threat to small ruminant livestock production.

PPR is caused by a virus, PPRV. Although the global PPRV strains form a single serogroup, four lineages have been identified based on the sequences of the *F* gene [2, 25]. Lineages I, II and III are mostly limited to Africa whereas lineage IV is mostly restricted to Asia, although some incursion of lineages I, III and IV into other regions have been reported [6–8, 13, 37, 39]. It is probable that any vaccine strain could protect against viruses belonging to all the lineages, but concerns of development of virulent mutants and chances of co-circulation of mixed lineages have been raised for the use of heterotypic strains (e.g., the use of lineage IV strain Nigeria 75/1 in India) [24]. Hence, indigenous isolates have been developed as vaccine strains in

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India. These include the conventionally attenuated strains Sungri/96, Arasur/87 and Coimbatore/97 as well as the thermo-adapted strains Sungri/96, Jhansi/2003 and Revati/2006 [2, 17, 20, 21, 23, 32]. These isolates were obtained from outbreaks in South (Arasur, Coimbatore) or North (Sungri, Jhansi, Revati) India, and from sheep (Arasur, Revati) or goats (Sungri, Coimbatore, Jhansi). An earlier study, which compared the three conventionally attenuated strains Arasur, Sungri and Coimbatore in captive North Indian sheep and goats showed that all afforded protection against challenge [34]. In the present study, we report the evaluation of the immune response of South Indian sheep and goats to Sungri and Arasur strains of PPRV under field conditions.

Materials and methods

Cells and vaccines

Vero cells (American Type Culture Collection, USA) were propagated in minimum essential medium with Earle's salts (Invitrogen, USA) supplemented with 6 % foetal bovine serum (Biological Industries, Israel) and antibiotics (Alembic, India, and Ranbaxy, India). Vaccines were produced in Vero cells maintained in stationary cultures. Sterility, identity and quality of the vaccines were ascertained to conform to required standards through control tests as described earlier [11, 16].

Animals and immunization

The study was carried out to compare the immunogenicity and the ability to elicit protective antibodies by Arasur and Sungri vaccine strains in sheep and goats reared in organized or unorganized setting under field conditions. The organized sector comprised of a private sheep breeding farm and a government livestock breeding and training centre where both sheep and goats were maintained. The

unorganized sector encompassed two villages, one from Bellary District in North Karnataka and the other from Bengaluru Rural District in South Karnataka.

In order to select animals for vaccination, 190 sheep and 134 goats (Table 1) with no previous history of vaccination were screened by competitive enzyme-linked immunosorbent assay (c-ELISA), as described [27, 28]. Briefly, serially diluted sera were mixed with a constant dilution of a monoclonal antibody (MAb) directed against the hemagglutinin protein of Sungri/96 strain, and applied to wells coated with Sungri/96 virus antigen before detecting binding of the MAb to the antigen. Percent inhibition (PI) was calculated as $[1 - (\text{absorbance of the test wells} / \text{absorbance of the reference antibody control wells}) \times 100]$.

Seronegative animals were grouped into in-contact control ($n = 16$), Arasur vaccinates ($n = 39$), and Sungri vaccinates ($n = 38$). Each animal was vaccinated with 10^3 TCID₅₀ of the respective vaccine strain, or sterile phosphate buffered saline (placebo). Animals were monitored for any abnormality until the end of the study. Blood was collected on the day of vaccination, and on days 21, 60 and 90 following vaccination, and separated serum was stored at -20°C until further use.

Assessment of immune response

Antibody response and seropositivity were studied using c-ELISA as described above, and the protective ability of the antibodies was studied by employing serum neutralization test (SNT). For SNT, heat-inactivated sera were serially diluted, incubated for 60 min with 100 TCID₅₀ of Sungri/96 virus and then inoculated on to Vero cells in 96-well plates. Cytopathic effect was scored at 5–6 days post-inoculation and neutralization index was calculated as described previously [5]. A titre of 1:8 and above was considered to be protective [1].

The PI values were subjected to one-way analysis of variance and analyzed using GraphPad Prism software

Table 1 Serological status of animals screened for the study

Field setting	Sheep			Goats		
	Breed	No. screened	Seronegative ^a	Breed	No. screened	Seronegative ^a
Organized Farm-I	Rambouillet × Deccani	82	36	NA ^b	0	0
Organized Farm-II	Rambouillet × Deccani	30	21	Jamnapari × Nandidurga	34	20
Unorganized (village)	Local breed	78	0	Non-descript	100	16
Total number of animals screened	–	190	57	–	134	36

^a As defined by PI value of <40 in c-ELISA

^b Not applicable (no goats maintained in the farm)

Fig. 1 Immune response of sheep and goats as assessed by c-ELISA. Seronegative sheep and goats in farm or village setting were inoculated with 10^3 TCID₅₀ of either Arasur (square) or Sungri (triangle) vaccines, or with buffer (diamond). Sera collected at 0, 21,

60 and 90 days post-inoculation were evaluated by competitive ELISA using Sungri virus antigen. Error bars represent standard error of the mean. For controls, the errors are too small and hence not visible

version 4.01. The SNT titres were subjected to Chi square (χ^2) test using a 2×2 contingency table at 95 % confidence interval.

Results

Bearing in mind that large scale outbreaks occurred recently (2004–2005) in the State of Karnataka [12], 190 sheep and 134 goats reared in organized farms and villages (unorganized sector) were screened in order to identify seronegative animals. As can be deduced from Table 1, 49.1 % of the sheep and 41.2 % of the goats in organized farms and 84 % of the goats and 100 % of the sheep in village setting were seropositive.

Seronegative animals were grouped into three in each of the farms or villages and immunized once with 10^3 TCID₅₀ of vaccine virus per animal or equivalent volume of buffer. None of the vaccinated or control animals showed any clinical sign, including fever, confirming attenuation of both the vaccine strains. Further, the absence of clinical signs in in-contact animals for up to 90 days of commingling with vaccinated animals supported the safety of the vaccine strains. Both the vaccine strains were equally immunogenic in both sheep and goats, irrespective of whether the setting was organized or unorganized (Fig. 1).

Serum samples collected 21, 60 and 90 days post-vaccination revealed a 100 % correlation between seropositivity as assessed by c-ELISA (PI >40) and protective SNT titres (≥ 8). The PI values were similar in all groups (Fig. 1). Sheep vaccinated with either vaccine strain showed seropositivity between 75 and 87.5 % in farm setting, at 21 days post-vaccination (Table 2). Goats vaccinated with Arasur and Sungri strains showed seropositivity of 66.7 and 71.4 % in the farm setting, and 71.4 and 83.3 % in village setting, respectively, at 21 days post-vaccination (Table 2). The seropositivity levels were maintained at 60 and 90 days post-vaccination in all cases (Table 2). All the c-ELISA positive samples produced SNT titres of ≥ 8 . The titres ranged from 8 to 64, but were somewhat lower with the village (unorganized setting) goats than other groups (Fig. 2). Considering a four-fold increase in SNT titres, seroconversion rate in sheep ranged from 53.3 to 75.0 and 43.8 to 77.8 % to Arasur and Sungri strains, respectively, in farms (Table 2). Seroconversion in goats was somewhat variable. While it was 66.7 and 71.4 % in farm setting for Arasur and Sungri strains, respectively, in village setting, the range was 33.3–50.0 % for Arasur strain and 50.0–66.7 % for Sungri strain (Table 2).

Immune responses in terms of PI values were significantly different between control animals and Arasur or Sungri vaccinates in both sheep and goats ($P < 0.001$ for Rambouillet crossbred sheep, $P < 0.05$ for Nandidurga

Table 2 Immune response parameters (% of animals immunized) for vaccinated sheep and goats in farm or village setting

Setting	Species	Day	Sero-positive ^a	Sero-converted ^b	Protective ^c
Arasur/87 strain vaccine					
Farm-I	Sheep (<i>n</i> = 15)	21	80.00	53.33	66.67
		60	73.33	53.33	66.67
		90	80.00	60.00	66.67
Farm-II	Sheep (<i>n</i> = 8)	21	75.00	62.50	75.00
		60	75.00	75.00	75.00
		90	75.00	62.50	75.00
	Goats (<i>n</i> = 9)	21	66.67	66.67	66.67
		60	66.67	66.67	66.67
		90	66.67	66.67	66.67
Village	Goats (<i>n</i> = 6)	21	71.43	42.86	57.14
		60	66.67	50.00	50.00
		90	66.67	33.33	50.00
Sungri/96 strain vaccine					
Farm-I	Sheep (<i>n</i> = 16)	21	87.50	43.75	75.00
		60	87.50	50.00	75.00
		90	87.50	62.50	75.00
Farm-II	Sheep (<i>n</i> = 9)	21	77.78	77.78	77.78
		60	77.78	77.78	77.78
		90	77.78	77.78	77.78
	Goats (<i>n</i> = 7)	21	71.43	71.43	71.43
		60	71.43	71.43	71.43
		90	71.43	71.43	71.43
Village	Goats (<i>n</i> = 7)	21	83.33	66.67	66.67
		60	83.33	66.67	66.67
		90	83.33	50.00	50.00

^a Defined by PI >40 in c-ELISA^b Defined as fourfold increase in SNT titre^c Defined by SNT titre of ≥ 8

crossbred goats and $P < 0.05$ for non-descript goats for both vaccines). The Sungri strain appeared to be marginally better than the Arasur strain, although the differences were not significant ($P > 0.05$ for sheep or goats of any breed). Further, the increase in antibody titres was dependent on vaccination, and the responses were independent of the vaccine virus strain in χ^2 test.

Discussion

PPR is endemic throughout India, with seroprevalence and mortality rates of 30–45 and 15–30 %, respectively. Outbreaks are more common in sheep in South India and in goats in North India [4, 19, 20, 29, 36]. To control the disease, two vaccine strains, Arasur and Sungri, were initially characterized, and the latter has been

commercialized. This vaccine is safe to animals, including pregnant ewes and does, and protects vaccinates for at least six years [20, 24, 29].

It is well established that PPR disease is more severe in goats than in sheep [26, 31, 33, 35, 38]. In controlled studies [15, 26], some North Indian goat isolates of PPRV were shown to cause severe disease in goats as compared to sheep. In addition, at least one goat isolate required serial passage to even induce clinical signs in sheep (Dr. Muthuchelvan, Indian Veterinary Research Institute, personal communication). In India, the higher rate of slaughter and fecundity in goats results in production of naive population every year, presumably leading to greater susceptibility of the goat population [26]. On the other hand, we have found more pronounced clinical signs and severe disease in sheep than in goats in flocks where sheep and goats are reared together in some parts of Karnataka. It is possible that species selection of strains and further amplification may lead to strain adaptation, resulting in more severe disease in the adapted species. In general, the recovery rate in goats is comparatively less than that in sheep. Besides species, some breeds of sheep appear to be more susceptible to PPR than others. The Guinean as well as British breeds have been reported to be highly susceptible whereas Sudanese breeds failed to develop characteristic clinical symptoms [14, 33]. In West Africa, acute form of the disease was observed in WAD goats while WALL breed developed only mild form of the disease [9]. No reports exist on the differential clinical presentation of different breeds of the sheep or goats in India (Dr. Balamurugan, Project Directorate in Animal Disease Monitoring and Surveillance, Indian Council of Agricultural Research, personal communication), although reported differential expression of PPRV receptor on peripheral blood mononuclear cells [18] suggests that this could very well be the case. Nevertheless, susceptibility to disease when exposed to or challenged with a wild type virus may not necessarily translate to post-vaccinal immune responses. There is therefore a need to explore whether sheep and goats, and different breeds of each respond differently to different vaccine strains of PPRV.

The two Indian vaccine strains, Arasur and Sungri, differ in several ways. The strains were isolated from sheep in South India and goats in North India, respectively, which agrees with the prevalence of PPR in these species. In vitro, the Arasur strain grows without a noticeable eclipse phase and produces rapidly spreading, degenerating cytopathic effect (CPE) with high titres ($\sim 10^8$ TCID₅₀/mL) by 3–4 days post-infection [30], and has the ability to agglutinate erythrocytes from several species [10]. On the other hand, the Sungri strain grows with a distinct eclipse phase, followed by slowly spreading, syncytial CPE with comparatively lower titres ($\sim 10^6$ TCID₅₀/mL) by 7–8 days

Fig. 2 Immune response of sheep and goats as assessed by SNT. Seronegative sheep and goats in farm or village setting were inoculated with 10^3 TCID₅₀ of either Arasur (*square*) or Sungri (*triangle*) vaccines, or with buffer (*diamond*). Sera collected at 0, 21,

60 and 90 days post-inoculation were evaluated by SNT using 100 TCID₅₀ of Sungri virus. Error bars represent standard error of the mean. For data points where error bars are not visible, the errors are too small to be depicted

post-infection, and fails to show hemagglutination [30]. In addition, while Sungri strain is clearly detected in sandwich ELISA with the MAb 4G6, the Arasur strain is only weakly positive at best. Also, MAb 4B11 produces a high neutralization index with Sungri strain whereas the index is more than 100-fold lower with Arasur strain. Similarly, MAb's to N protein also react better with Sungri than Arasur strain. While these observations may be attributed to the fact that all the MAb's were produced against Sungri strain, the patterns cannot be generalized since other MAb's to HN and P proteins react better to Arasur strain, supporting antigenic relatedness between the two strains [30].

In a recent study, the Arasur and Sungri strains were compared for immunogenicity and protection in sheep and goats of the sub-Himalayan region and the adjoining plains of North India [22]. Seropositivity as assessed by c-ELISA varied but the animals were protected against challenge with either strain. In the present study, immunogenicity and the ability to elicit protective antibodies was extended to sheep and goats from farm and village settings in the southern State of Karnataka. However, the two studies differ in two major ways. First, Saravanan and colleagues [27] immunized and challenged free ranging animals which were maintained under captive and isolated conditions, a setting which is required for challenge with virulent strains. By contrast, we used sheep and goats in farms or villages without relocating them, and did not perform challenge studies as

this could pose the risk of spread of virulent virus. The two challenge strains used by Saravanan et al. [22] differed in virulence: the Sungri strain was highly virulent, producing severe clinical signs by day seven followed by death whereas Arasur strain was moderately virulent, producing mild clinical signs by day nine followed by recovery. Moreover, for challenge, 10 % spleen suspension from infected animals was used without titrating, and prepared at passages seven and 75, respectively, for Sungri and Arasur strains. In addition, Arasur strain is a South Indian sheep isolate and the challenge Arasur virus was prepared in North Indian sheep. It is not clear whether different results would have been obtained with viruses prepared at similar passage level and at similar titres; however, both the vaccine strains protected against both challenge viruses in both sheep and goats.

During field evaluation of the Sungri vaccine, >90 % of sheep and goats seroconverted, with SNT titres varying from 8 to 256, the average peak titre being 32 in sheep and 64 in goats [23]. In the present study, seropositivity was 66.7–87.5 % and seroconversion rate was 43.8–77.8 % in farm setting and 33.3–66.7 % in village setting, with SNT titres ranging from 8 to 64 in both sheep and goats. It is worth noting that Sungri virus was used in SNT and was therefore heterologous for sera obtained from Arasur virus vaccinated animals. Immunogenicity of both the vaccines was slightly poor in village setting, possibly due to pre-existing disease, poor management, malnutrition, parasitism, or undetectable levels of pre-existing antibodies. In

any case, both the vaccines induced seroconversion efficiently in both sheep and goats.

In conclusion, both Arasur and Sungri strains are equally effective in eliciting protective antibodies in field setting. Whereas production of higher virus titres in shorter period of time by Arasur strain may be beneficial for bulk manufacturing purposes, it is not clear whether the same attributes could prove to be disadvantageous and counterproductive in vivo. Long-term evaluation following vaccination with both vaccine strains and comparing in farms, villages and nomadic flocks should provide important insights into the efficacy and effectiveness of the two vaccine strains.

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