Protective immune response of live attenuated thermo-adapted peste des petits ruminants vaccine in goats

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Received: 30 December 2013 / Accepted: 12 March 2014 / Published online: 29 March 2014
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Abstract Virulent isolate of peste des petits ruminants virus (PPRV) of Indian origin (PPRV Jhansi 2003) initially adapted in Vero cells was further propagated in thermo-adapted (*Ta*) Vero cells grown at 40 °C for attaining thermo-adaption and attenuation of virus for development of *Ta* vaccine against PPR in goats and sheep. The virus was attenuated up to 50 passages in *Ta* Vero cells, at which, the virus was found sterile, innocuous in mice and guinea pigs and safe in seronegative goats and sheep. The developed vaccine was tested for its immunogenicity in goats and sheep by subcutaneous inoculation of 100 TCID₅₀ (0.1 field dose), 10³ TCID₅₀ (one field dose) and 10⁵ TCID₅₀ (100 field doses) of the attenuated virus along with controls as per OIE described protocols for PPR

vaccine testing and were assessed for PPRV-specific antibodies 7–28 days post vaccination (dpv) by PPR competitive ELISA and serum neutralization tests. The PPRV antibodies were detected in all immunized goats and sheep and goats were protective when challenged with virulent PPRV at 28th dpv along with controls for potency testing of the vaccine. The attenuated vaccine did not induce any adverse reaction at high dose (10⁵ TCID₅₀) in goats and sheep and provided complete protection even at low dose (10² TCID₅₀) in goats when challenged with virulent virus. There was no shedding and horizontal transmission of the attenuated virus to in-contact controls. The results indicate that the developed PPR *Ta* attenuated virus is innocuous, safe, immunogenic and potent or efficacious vaccine candidate alternative to the existing vaccines for the protection of goats and sheep against PPR in the tropical countries like India.

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Keywords PPR virus · Thermo-adapted · Vaccine ·
Efficacy · Goats and sheep

Introduction

Peste des petits ruminants (PPR), also known as “Goat Plague” is one of the most economically important dreadful diseases of small ruminants affecting primarily the sheep and goats. The clinical symptoms include fever, necrosive stomatitis and pneumoenteritis, lasting more often, than not in the death of the animal. Of late, three groups of scientists have developed Vero cell line based live attenuated PPR vaccines around the world using the different lineage of PPR virus (PPRV) of either goat or sheep origin for prophylaxis of disease [9, 21, 23, 24]. However, as with many modified live vaccines the

attenuated PPR vaccine is no exception in thermal inactivation and hence maintenance of cold chain is a must to preserve the quality of the vaccine. Maintenance of cold chain is a major constraint during the storage and transport of vaccine in any tropical region like India. Therefore, thermostable vaccines are preferred in such countries, wherein, the viability of the vaccine viruses would be ensured.

Despite strict control measures including statutory regulations along with the availability of scores of vaccines and diagnostics, this infection still remain as a constant threat to livestock industry [8]. In India, the control strategy is the large scale vaccination with a live attenuated PPR vaccine and assessment of sero-conversion using PPR competitive ELISA [26]. Vaccination has become a recommended tool for the control and eradication of this disease with an effort to limit the economic losses. Though the live attenuated PPR vaccines offer solution to these problems, these vaccines necessitate maintenance of cold chain from production till delivery. Therefore, it is desirable to develop PPR vaccine with a better thermo-resistance or thermo-tolerance.

In order to achieve intrinsic thermo-resistance in a virus, the native virus is to be grown successively for many passages at higher temperatures and it may result in an intrinsically thermo-resistant virus clonal population. Such a adapted thermo-resistant virus clonal population can be very well grown at a higher temperature than an usual temperature of 37 °C in thermo-adapted (*Ta*) cell line growth at 40 °C and that are relatively temperature tolerant. In this approach, the results are largely empirical as they depend on the capability of the virus to resist detrimental effect of high temperature. Selection and subsequent propagation of the virus clonal population, their stability, and maintenance of their immunogenic potential need to be assessed before considered them as a vaccine candidate.

In a tropical country like India, one of the main hurdles in achieving reliable vaccine coverage is lack of or has poor infrastructure for maintenance of cold chain at field and under these conditions, it is difficult to maintain a cold chain to ensure the preservation of vaccine potency. This drawback can be overcome through the development of a thermo-tolerant vaccine with freeze-drying in the presence of a cryoprotectant as described earlier [27]. Further, PPR control will benefit greatly from the use of this type of thermostable form of the attenuated vaccine in tropical countries. Hence, the present work has been undertaken with objective of developing a *Ta* PPR vaccine by using intrinsic *Ta* PPR vaccine virus by attenuation in *Ta* Vero cells to overcome limitations associated with conventional vaccines and also to assess its protective immune response (efficacy) in goats and sheep.

Materials and methods

PPR vaccine virus and cell lines

The isolated PPR virus (PPRV Jhansi/2003-goat origin-belong to lineage IV) from outbreak of PPR in goats and sheep flocks at Jhansi, Uttar Pradesh, India [5], is being maintained in the virus repository of the Division of Virology, IVRI, Mukteswar, Uttarakhand and was used in the study for development of *Ta* PPR vaccine in the *Ta* Vero cell (grown at 40 °C) line. Initially, Vero cells (CCL-81) were grown at 37 °C with suitable growth media like Eagle's minimum essential medium (EMEM), Iscove's Modified Dulbecco's Medium (IMDM), (Sigma, USA) containing serum ranging from 10 to 20 % with an increment of temperature given at intervals of 1 °C for every passage levels in 25 cm² tissue culture flask for adaption. *Ta* Vero cells between 20 and 30th passages were propagated in EMEM containing 10 % fetal bovine serum (FBS) and for maintenance, EMEM with 2 % FBS was used for production of vaccine in bulk as well as for virus titrations.

Thermo-adaptation of the vaccine virus

Attenuation of the PPRV Jhansi/2003 was carried out by passaging it at 37 °C in Vero cells for initial 25 passages. Once stable Vero cell line (growing at 40 °C with EMEM containing 10 % serum) was obtained, the cells were split in the ratio of 1:2 and maintained their sustainable growth at 40 °C. *Ta* Vero cell lines that grow at 40 °C were cryopreserved as per standard procedure and stored in liquid nitrogen until use. Further, this virus was adapted to grow in *Ta* Vero cells at 40 °C for additional 25 passages [using 0.1 m.o.i (multiplicity of infection) for each passage] to generate candidate *Ta* vaccine virus following standard virological procedures by selection of clonal virus population.

Preparation of the PPR vaccine

Thermo-adapted Vero cells were seeded into roller culture bottles (1,700 cm²) (Corning Inc, NY, USA) with a cell density of 2.5×10^7 cells. Two days after cell seeding, an even, confluent monolayer was produced, and the bottles were immediately infected (at a m.o.i. of 0.05) with vaccine virus. Infected bottles were incubated at 40 °C in the roller apparatus with a change of maintenance media at every alternative day and cells were observed for CPE regularly under microscope. After 6–7 days post-infection (dpi), when, more than 80–90 % cytopathic effect (CPE) was observed, virus was harvested from the infected cells by a cycle of freezing and thawing. To maintain the uniformity in the virus titre, virus harvest from all the roller

bottles were pooled after the first thaw and were divided into aliquots. The aliquots were preserved at -80°C until lyophilized.

Virus titration

Virus titration for estimation of *Ta* vaccine virus titres using liquid virus as well as freeze-dried preparations was carried out in *Ta* Vero cells. Serial ten-fold dilutions of virus suspension were made in maintenance medium and the viruses were titrated in monolayers of *Ta* Vero cells grown in 96-well microtiter plates using four replicates per dilution (100 μL /well). The plates were incubated in the presence of 5 % CO_2 for 6 days with a change of maintenance media at every alternative day and cells were observed for CPE regularly under microscope. Virus infectivity titre was quantified by estimating the 50 % tissue culture infectivity doses (TCID_{50}) and end points were calculated as described earlier [14]. After visual observation of PPRV specific CPE on the sixth day, final reading of the micro titre plates was re-confirmed by cell-ELISA to detect the presence of virus as described earlier [1, 15].

Briefly, after fixation, microtiter plates were air-dried and blocked (200 μL /well) with blocking buffer (PBS with 1 % gelatin, 0.5 % fetal calf serum, and 0.1 % Tween-20) and incubated for 1 h. In each step, the plates were incubated at 37°C for 1 h under constant shaking. After incubation, the wells were washed three-times with washing buffer (0.002 mol/L PBS $\downarrow$ 0.05 % Tween-20). After incubation and washing, PPRV-specific anti-nucleoprotein (N) monoclonal antibody (MAb) at 1:20 dilution (100 μL /well) was added, and the plates were incubated. An anti-mouse horseradish peroxidase (HRPO) conjugate diluted 1:1,000 in blocking buffer without gelatin was added (100 μL /well) and the plates were incubated at 37°C for 1 h. The substrate (orthophenylene diamine, OPD) solution was added at 100 μL /well. The color was allowed to develop for 15 min, after which the reaction was stopped and Absorbance (A492) was read in an ELISA reader. The cutoff value with two-times the absorbance compared with uninfected cells was set for declaring a well positive.

Characterization/identity of *Ta* PPR vaccine

In order to determine the identity of the attenuated *Ta* PPR vaccine virus, monoclonal antibody based PPR sandwich ELISA (s-ELISA) kit [26], RT-PCR [the nucleocapsid (N), the matrix (M), the fusion (F) and the haemagglutinin (H) gene] assays [2, 3, 7, 10] and neutralization tests [6, 16] were carried out as described earlier. For this purpose anti-PPR serum raised in rabbit/goat with a serum neutralization titre of at least 1:256 was used at 1:10 dilution to neutralize the virus in the vaccine.

Sterility and innocuity test

The vaccine was tested for bacterial, fungal contamination by employing standard sterility tests according to OIE protocols [12]. The sterility tests were carried out in Fluid thioglycollate medium and Soyabean casein digest medium-Tryptone soya broth in 5 mL volume and incubated the inoculated tubes along with controls for 7 days at $30\text{--}35^{\circ}\text{C}$ for detection of bacteria and $20\text{--}25^{\circ}\text{C}$ for fungi and other psychrotrophic organism.

The innocuity test was carried out in rodents to detect any non-specific toxicity associated with the vaccine as per the procedure laid out in OIE manual [12]. Freshly reconstituted 0.5 mL of PPR vaccine (mixed contents of five vials in recommended diluents) injected intramuscularly (i/m) and intraperitoneally (i/p) to two guinea pigs each and 0.1 mL intraperitoneally into six Albino mice along with two guinea pigs and four mice as uninoculated controls. The animals were observed for 3 weeks for any signs including death that can be attributed to a living organism in the vaccine. The vaccine was considered to be satisfactory if at least 80 % of animals remain in good health during the period of observation, and no significant postmortem lesion was found. Further reversion to virulence for live attenuated vaccine in goats and sheep was carried out for demonstration of non-reversion to virulence of the attenuated vaccine strain after three back needle passages in natural host using the blood samples and the animals were observed for any apparent clinical symptoms and shedding of virus in the secretion (nasal/ocular) of goats and sheep by employing the PPR s-ELISA kit [25] during serial natural needle passaging.

Immunogenicity of *Ta* PPR vaccine

Animals

Goats/sheep of either sex, aged 6–12 months were procured from sub-Himalayan regions, Uttarakhand (India) and maintained at the quarantine animal facility of the institute. The animals were de-wormed against gastrointestinal helminths using albendazole and rectal temperature was recorded to identify infection, if any prior to experiment. The Animal Ethics Committee norms were followed for the experiment as a part of routine PPR vaccine testing being carried out in goats/sheep. All the healthy animals were housed separately with adequate containment and screened for sero-negativity to PPRV antibodies by PPR c-ELISA [26] and serum neutralization test (SNT) [6, 16].

Vaccination and sampling

Goats/sheep were used for experiment to evaluate the *Ta* vaccine virus safety, immunogenicity and potency as per

OIE protocols for PPR vaccine testing [12]. The mixed contents of five vials are reconstituted in the diluent to give solutions of, 1 dose/mL, 100 doses/mL and 0.1 dose/mL. Six goats and six sheep, which were free from PPRV antibodies were used. Animals were randomly divided and two of each animals immunized with different doses [10^2 TCID₅₀ (0.1 dose), 10^3 TCID₅₀ (1 field dose) and 10^5 TCID₅₀ (100 field doses)] of attenuated virus at passage 50 (P50) in 1 mL volume by subcutaneous route in the neck region along with two controls, which were inoculated with reconstituent diluents, 0.85 % NaCl₂. All the vaccinated goats/sheep along with two controls were observed daily for thermal reaction (rectal temperature) and development of any symptoms associated with clinical disease up to 28 days post-vaccination (dpv). The vaccinated goats and sheep were sampled (blood collected through jugular vein puncture) periodically at different weekly intervals (0, 7, 14, 21, 28 dpv) as per an earlier study [18] for demonstration of sero-conversion. The vaccine was considered as safe if no abnormal clinical signs were observed in the vaccinated animals, in particularly those which have received the higher dose.

Assessment of immune response

PPR vaccine sero-monitoring i.e. serum antibody response in different groups of goats and sheep was carried out and measured by PPR c-ELISA [26] as per described methods. The ELISA reactivity was expressed as percentage inhibition (PI) values and serum samples showing PI > 40 were considered as positive. Briefly, ELISA plates were coated with the PPR virus antigen. After incubation, at 37 °C for 1 h, the wells were washed three times with 0.002 mol/L phosphate-buffered saline (PBS). Next all the wells of the plates received 40 µL of blocking buffer (PBS with 0.2 % PPR-negative goat serum and 0.1 % Tween 20). The test serum samples (20 µL) were added to duplicate sets of well followed by addition of 40 µL of monoclonal antibody in each well (except conjugate control wells) at a final dilution of 1:500. Anti-mouse–HRPO conjugate diluted 1:1,000 in blocking buffer was added to each well (50 µL/well) after incubation. Finally, substrate OPD solution was added in each well and colour reaction was developed for 10 min before stopping the reaction with 1 mol/L H₂SO₄ and OD was measured at a wavelength of 492 nm.

For the detection of neutralizing antibodies to PPRV and to determine the serum neutralization (SN) titer, a micro-assay was carried out in Vero cells as described earlier [6, 16]. Briefly, serum samples were diluted two-fold serially (from 1:2 to 1:256) in a 96-well tissue culture plate and incubated with 100 TCID₅₀ of PPR vaccine virus Sungri-96 strain in a 5 % CO₂ humidified incubator at 37 °C for 2

h. Vero cell suspension (10^5 cells in 100 µL) was added to each well and the plates were further incubated at 37 °C in a humidified CO₂ incubator for 7–8 days under regular observation until the CPE were evident with change of maintenance media on every alternative day. The appropriate cell, virus and serum controls were included for each plate. After visual observation of PPRV-specific CPE on the sixth day, final readings of the microtitre plates were re-confirmed by the cell-ELISA method [1, 22] to detect the presence of virus as described earlier.

The SN titers were calculated as the reciprocal of the highest dilution of serum that shows no CPE in 50 % of wells. The protective levels of antibodies in goats and sheep serum samples were analyzed and the SN titers of 1:8 and above were considered as protective [16, 18].

Challenge study

In the challenge study, all the goats after 28 dpv along with other two sero-negative control animals were experimentally infected with 2 mL of goat-adapted highly virulent challenge PPRV (Izatnagar/94 isolate-needle passage of virus in natural host for preparation of challenge virus for PPR vaccine testing) subcutaneously at two different sites in the form of 10 % splenic suspension of needle passaged virus in goats as described previously [18]. The amount of nucleocapsid (N) protein in 1 mL of splenic suspension was estimated by anti-PPRV N protein MAb-based sandwich-ELISA (s-ELISA) [25], which is equivalent to the amount of N protein present in 1 mL of PPR vaccine (titer of $10^{5.5}$ TCID₅₀/mL).

Briefly, the s-ELISA assay was carried out using an anti-PPRV rabbit capture antibody at 1:4,000 dilution (100 µL) in PBS in 96-well flat-bottomed polystyrene microtitre plates. At each step the plates were incubated at 37 °C for 1 h with constant shaking. After incubation, the wells were washed three times with a washing buffer. Then 50 µL of a blocking buffer (PBS + 0.1 % Tween-20 + 0.5 % PPR-negative serum) was added to each well. Clinical samples (50 µL per well) were added in duplicate. Positive (PPRV) and negative (uninfected Vero cell supernatant) controls were included. Rest all the steps were similar to Cell-ELISA procedures described above. The animals were observed for the development of any clinical signs specific to PPR, if any. Vaccine efficacy was proven if all the vaccinated animals resist the challenge while the in-contact control animals remain susceptible to the challenge virus.

From this experiment, the nasal, ocular and oral swabs were collected periodically [from day 1 to 20 days post challenge (dpc)] from all the challenged goats during the course of infection. The collected samples were tested for detection of either PPRV antigen (swab materials were directly squeezed or extracted with 0.5–1 mL phosphate

buffered saline, PBS) or nucleic acid sequences (viral RNA was extracted from the swab materials by using QIAamp viral RNA kit) by s-ELISA [25] and M and N gene based RT-PCR assays [2, 3, 7] respectively. M and N genes have been amplified from viral RNA extracted from the swabs samples. Sheep were not challenged due to non-availability of the sheep adapted highly virulent PPRV strain and however, the protective PPRV-specific antibodies response was assessed by SNT and cELISA as described earlier [16, 18, 26].

Results

Characterization of the Ta vaccine virus

The novel intrinsically *Ta* PPRV at P49 was used as *Ta* seed virus and stored as seed vaccine for immunological evaluation in homologous hosts, sheep and goats. On testing the identity of the *Ta* vaccine virus PPRV Jhansi-2003 (p-50) and standard Sungri-96 vaccine strain employing PPR s-ELISA, OD values of 0.602 and 0.620 were obtained, respectively. On verifying, the candidate vaccine virus was neutralized by the rabbit hyper immune serum (HIS) at a titer of 1:32 in virus neutralization test. Further, the amplification and sequence analyses of structural gene sequences identified the vaccine virus as Asian lineage IV PPRV. The virus at passages 45, 49 and 50 were grown in bulk, titrated [virus titre $10^{5.0}$ TCID₅₀/mL (P45), $10^{5.5}$ TCID₅₀/mL (P50)], lyophilized and stored at -80°C either in liquid or freeze-dried form in a 50 or 100 doses (titre $10^{4.5-5.0}$ TCID₅₀/mL).

Sterility and innocuity test

On testing the vaccine for bacterial and fungal contamination by employing standard tests, it was found free of these contaminants. The vaccine seed passed the innocuity test in rodents (mice and guinea pigs) and safety test in goats and sheep. Lack of any apparent clinical symptoms and no shedding of virus in the secretion (not detected PPRV antigen in nasal/ocular swab by sELISA) of goats and sheep during three needle passage of the virus.

Efficacy of Ta vaccine

All the animals remained apparently healthy without any adverse reactions and signs of illness on immunization with PPR vaccine. No marginal rise in temperature (no pyrexia) was evident in the immunized goats and sheep after inoculation. PPRV antibody response in different groups of goats and sheep was measured by c-ELISA at weekly intervals and the levels of antibodies were expressed as PI

values, which ranged from 42 to 80 (Fig. 1a) and 45 to 82 (Fig. 1b) in goats and sheep, respectively from 2nd to 4th week pv. The vaccine was safe as evidenced by the absence of clinical signs including pyrexia even after inoculation with 100 vaccine doses (10^5 TICD₅₀). The vaccine was found to elicit immune response even at lower dose (10^2 TICD₅₀). The SN antibody titer ranged between 1:8 and $\leq 1:256$ was observed from 14 to 28 dpv in various groups in goats and sheep. The SN titers of $\geq 1:8$ were considered as protective in case of PPR [16, 18], which was observed after 14 dpv in all the vaccinated goats and sheep. No sero-conversion was detected in the control animals and the SN titers of 1:2 to $\leq 1:4$ with the c-ELISA PI values ranged from 18 to 31 was observed in sheep and goats.

All the vaccinated animals survived upon challenge without any apparent clinical infection. One out of two control goats succumbed to PPR on day 9 pc. None of the PPR vaccinated goats showed presence of virus in both nasal and ocular swabs and oral swabs collected at regular intervals following virulent PPRV challenge (Fig. 2). Further, PPRV antigen could be demonstrated in both nasal and ocular swabs of control goats on day 7–12 dpc by s-ELISA (Fig. 2). The two control challenged goats exhibited signs of pyrexia, dullness, ocular and nasal discharges, oral lesion, coughing, etc., from day 4 pc and diarrhea from day 9–11 pc. One of these goats, showed only dullness and anorexia accompanied with rise in rectal temperature (40.2°C on day 7 and 8 pc) and died on 9 dpc.

Discussion

Modifying or improving the existing vaccine or developing *Ta* or thermo-stable vaccine in terms of its stability at ambient temperature is warranted in tropical countries like India to avoid the vaccination failure due to breakdown in the maintenance of cold-chain. Worrall et al. [27], developed thermo-tolerant vaccine with freeze-drying in the presence of a cryoprotectant containing trehalose, which was relatively stable at higher temperature with minimal loss of potency and suitable in tropical countries. In this study, *Ta* PPR vaccine candidate virus [PPRV Jhansi/2003] has been developed from virus which was isolated from PPR outbreak [5] and were subsequently adapted and attenuated (up to 50 passages) gradually in *Ta* Vero cells grown at higher temperature (40°C) following the method described elsewhere [13], which resulted in *Ta* PPR vaccine candidate virus. Therefore, this research work would largely contribute to address the problems of deterioration of vaccine quality under higher temperature conditions. The thermo-stability of this vaccine virus using extrinsic stabilizers and its genetic characterization was carried out earlier [4, 15]. In the present study, the protective immune

Fig. 1 Immune response of PPRV specific antibodies in animals vaccinated with thermo-adapted vaccine (PPRV Jhansi/2003) by PPR c-ELISA. **a** Goats. **b** Sheep. The percentage inhibition (PI) value is

calculated [(mean reactive of the test serum/monoclonal control) $\times$ 100 – 100] and the samples showing PI > 40 and PI < 40 were considered as positive and negative respectively

response (efficacy) of *Ta* PPR vaccine in goats was assessed in in-house trial and was found safe, potent and efficacious.

Sequence analyses of structural gene sequences identified the vaccine virus as Asian lineage IV PPRV (Genbank Accession Nos GU014571; GU014572; GU014573) as described earlier [8]. Further, on analyses of N, M, F and H gene sequences of vaccine strains of PPRVs showed considerable sequence homology among the lineage IV viruses, which showed no significant changes in the functional or structural surface protein-coding gene sequences. This indicates the stability of the surface proteins of PPRV TS vaccine strains developed at high temperature (at 40 °C) as reported earlier [8]. However, it is necessary to find the differences (mutations in a sequences alignment) and which and how they contribute to deteriorate the vaccine quality under higher temperature conditions in the subsequent studies.

Lack of any apparent clinical symptoms and no shedding of virus in the secretion of goats and sheep during three needle passages indicated that the virus has been stably attenuated and the possible reversion to virulence is unlikely. Experimental observation in this study was consistent with the previous studies on other PPR vaccines in the laboratory [18]. There was no shedding of virus as determined by testing of nasal and ocular swabs in s-ELISA for 3 weeks regularly following vaccination, which confirmed further the safety of the vaccine. Further, study showed that the attenuated virus (*Ta* PPRV Jhansi/2003) is safe even at higher dose (10^5 TCID₅₀) without any side effects, and conferred protection even at lower dose (10^2 TCID₅₀) against virulent virus challenge.

The developed vaccine efficacy is in concurrence with the earlier homologous Sungri 1996 vaccine [19] and Nigeria75/1 vaccine developed by Diallo et al. [9], which is efficacious and no clinical signs were detected in

Fig. 2 Detection of PPR virus antigen in the swab materials collected from the challenged goats by PPR sandwich ELISA. The cut-off value is calculated as the twice mean reactive OD value of the controls, and

the samples showing value above and below the cut off line were considered as positive and negative, respectively

challenged animals and resistance of the in-contact sheep on challenge. In addition, sheep and goats which have recovered from a PPR infection appear to be protected against a subsequent infection for the rest of their lives [12]. It might possible that resistance in sheep is attributed to the host and might be unrelated to the virus [11]. The animals which showed typical PPR clinical signs and lesions, indicated that the needle passage adapted virus in goats maintained the virulence in goats. After completion of the challenge experiment, all the survived goats were rehabilitated.

After ensuring the safety and efficacy of the vaccine at in-house trials, the experimental vaccine was supplied to field veterinarians in limited level to ensure its safety and potency in sheep and goats. The feedback from the field indicated that there was no untoward effect following vaccination, implying the safety of the PPR *Ta* vaccine at the field level. However, further, extensive systematic experimental field trial has to be carried out in order to determine the sero-conversion and efficacy of *Ta* vaccine at the field level in order to control the disease at the time of outbreak flare-up.

In conclusion, *Ta* PPR vaccine was developed following attenuation of a well characterized PPR virus that grew well in *Ta* Vero cells at 40 °C. The present safety and immunogenic studies of *Ta* PPR vaccine showed the protective immune response (efficacy) in goats and sheep in successful in-house trial and was found safe, potent and efficacious. However, extensive field trials are warranted for *Ta* PPR vaccine before being considered it as an adjunct to in-use PPR vaccine. In India, decrease in the

number of PPR outbreaks and the severity of the disease in the recent past is an indicative of the effectiveness of in-use PPR vaccine, timely vaccination of susceptible flock and stability of the virus. Currently available conventional PPR vaccines [17, 19, 20, 24], deuterated PPR vaccine [20] and *Ta* PPR vaccine would be adequate to contain PPR in India.

Acknowledgments The authors thank the Director, Indian Veterinary Research Institute for providing necessary facilities to carry out this work and the staff of Rinderpest and Allied Disease Laboratory and Animal Sheds, Division of Virology, Mukteswar, for their valuable assistance and timely help in carrying out this work. The study in the form of Ad-hoc Scheme (F.No.11-3/2007-GA-II and 1-1/2007-ASR-IV) was funded by Indian Council of Agricultural Research, Government of India, New Delhi, India.

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