ORIGINAL ARTICLE



Towards eradication of peste des petits ruminants: postvaccination evaluation in sheep and goats in Southern Peninsular India

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Abstract The cross-sectional seroprevalence study of the peste des petits ruminants (PPR) in sheep and goats was carried out in the Southern Peninsular region of India to ascertain the prevalence of PPR virus (PPRV) antibodies at the epidemiological units (epi-units) level in the small ruminant population. The serum samples were collected from various epi-units (villages) in the different states and union territory (UT) in Southern Peninsular region using a stratified random sampling methodology from August 2017 to March 2018. A total of 6643 serum samples [sheep (n = 2785) and goats (n = 3858)] were collected from 360 epi-units and were screened by PPR competitive ELISA kit for the detection of PPRV antibodies. The results revealed that the seroprevalence of PPR in small ruminants in Telangana, Andhra Pradesh, Karnataka, Tamil Nadu, and Kerala states, and Puducherry UT was 87.0%, 66.4%, 64.3%, 47.8%, 11.4%, and 50.4%, respectively in the studied region. Further, the results of the chi-squared test revealed that the PPRV antibodies across different states and UT in the region were associated (sheep- $\chi^2 = 218.8$, p < 0.01; goats- $\chi^2 = 827.1$, p < 0.01), as all the states and UT adopted the PPR vaccination programme. The study

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also implies that the small ruminants in some of the epiunits (n = 102) had < 30% seroprevalence, which necessitates comprehensive intensive vaccination and active surveillance programmes to make this region as PPR free zone.

Keywords Cross-sectional study \cdot Post-vaccination \cdot PPR \cdot Seroprevalence \cdot Sheep and goats \cdot Southern Peninsular India

Introduction

Peste des petits ruminants (PPR) otherwise known as 'Goat Plague', is a contagious economically important and a world organization for animal health (OIE) notifiable transboundary viral disease of domestic (sheep and goats) and wildlife small ruminants. The disease is caused by the small ruminant morbillivirus (SRMV) (formerly known as PPR virus-PPRV), a member of the genus Morbillivirus of the family Paramyxoviridae (http://ictvonline.org/vir usTaxonomy.asp). Clinically, PPR is characterized by high fever, oculonasal discharges, necrotizing and erosive stomatitis, gastroenteritis, diarrhea, and bronchopneumonia [7]. PPR primarily affects sheep and goats and causes major constraints in augmenting the productivity of the small ruminants in enzootic sub-Saharan Africa, the Arabian Peninsula, the Middle East, and Central and Southeast Asia. The disease has huge potential to cause high economic losses and it significantly impacts the livestock sector especially small ruminants in enzootic countries [18]. Due to the vast economic impacts of PPR, after the eradication of rinderpest, a global consensus was extended on the need to eradicate PPR with the adoption of a PPR-Global Control and Eradication Strategy (PPR-GCES) to

make the world free from PPR by 2030 [23]. European Commissioner for International Cooperation and Development, Food and Agriculture Organization (FAO) and OIE jointly launched an international strategic plan for control and eradication to gather all stakeholders behind the PPR Global Eradication Programme (PPR-GEP) and mobilized the additional support required for the eradication [23]. Therefore, FAO and OIE, launched PPR-GEP for the period from 2017 to 2021, into action with the adoption of a PPR-GCES.

In India, sheep and goats are an important productive asset of settlers, landless, marginal, and small landholder farmers and it generates income and employment and supports their livelihood throughout the year. Several PPR outbreaks have occurred in the past and now being occurring regularly, round the year throughout India, as the disease is enzootic [7]. PPR control and eradication depend mainly on disease reporting, understanding of the epidemiology of the disease, rapid and accurate diagnosis, surveillance or monitoring, and implementation of the vaccination programme. The success of the national rinderpest eradication programme (NPRE) in India has provided the confidence and impetus to launch a similar programme for PPR. India practiced focused vaccination (vaccination limited to the place of the outbreak with the radius of 3-10 km to contains the disease spread) in PPR outbreak places in some states since 2002 [25] and the vaccination programme mode (mass vaccination covering the entire small ruminants population above the age of 4 months old and subsequent vaccination of naïve young population and leftover unvaccinated animals) in some states since 2010-2011 even before the global framework was planned [6]. Department of Animal Husbandry and Dairying (DAHD), Government of India (http://www.dahd.nic.in) implemented a national control programme on PPR (PPR-CP) during 2010–2011, with a sum of INR 432.5 million in the first phase in a time-bound manner (http://dahd.nic.in) following the eradication pathway of OIE [6] to control and eradicate the disease from India.

In the first phase of the control programme, the states and union territories (UTs) in the Southern Peninsular region of India namely Karnataka, Andhra Pradesh, Telangana, Tamil Nadu, Kerala, Maharashtra, Goa and Lakshadweep, Daman and Diu, Dadra and Nagar Haveli, Puducherry, and Andaman and Nicobar Island were included in the vaccination programme (www.dadf.nic.in). The disease has been brought under control in some of the states and PPR outbreaks threat reported declined progressively and substantially in the continuous vaccination practiced states [6] and benefits outweigh the cost of a vaccination programme [19]. The states, where vaccination is being adopted, disease outbreaks are being reported sporadically. However, in India, several outbreaks of PPR in sheep and goats have not been recorded properly, owing to inadequate animal disease surveillance and reporting systems [9]. Studying the prevalence of PPRV antibodies in sheep and goats from different geographical areas with varying agro-climatic conditions may help to devise effective appropriate disease control strategies towards the eradication of PPR. Nevertheless, the presence of PPRV specific antibodies indicated either the subclinical or inapparent infection [9, 13, 10] or naturally animals exposed to the virus and recovered in the unvaccinated areas or animals' immune response to the vaccine in the vaccinated areas. The cross-sectional study on seroprevalence after PPR vaccination in small ruminants have been reported from different endemic countries in the world [1-3, 15, 16]. However, systematic seroepidemiological surveys for PPR for the state or region have not been conducted, despite the implementation of PPR-CP in the Southern Peninsular India since 2010, especially the prevalence of PPRV antibodies level at epidemiological units (epi-units)/villages. Further, neither a surveillance plan nor a systematic seromonitoring was initiated to assess the effectiveness of the vaccination programme. Furthermore, in India, no such a systematic cross-sectional epidemiological study except a few studies [8, 12] has been undertaken so far from different geographical areas for formulating disease control strategies. Therefore, a cross-sectional serological survey (2017-2018) is being applied here to establish the seroprevalence of PPR at the epi-unit level in the target population at a given period to determine PPRV antibodies status, as post-vaccination evaluation in sheep and goats towards the eradication of PPR after implementing the vaccination programme in the Southern Peninsular region.

Materials and methods

Study area

Southern India includes five Indian states (Andhra Pradesh, Karnataka, Kerala, Tamil Nadu, and Telangana) as well as the three union territories (Lakshadweep, Andaman and Nicobar Islands, and Puducherry) covering the southern part of the Peninsular Deccan Plateau. The Southern Peninsular region was purposively selected as the PPR-CP initiated in the region during 2010 (http:// www.dahd.nic.in/) and further, the reported outbreaks have been substantially reduced since 2011 [6]. In this study, PPR vaccination adopted states (Telangana, Andhra Pradesh, Karnataka, Kerala, Tamil Nadu) and UT (Puducherry) were included. Moreover, the Lakshadweep and the Andaman and Nicobar Islands were excluded due to the meager animal populations, unique niche geographical island areas, as well as no PPR outbreaks, were reported (http://dahd.nic.in).

Sampling designs

A cross-sectional seroprevalence study was conducted by the Indian Council of Agricultural Research (ICAR)- National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) during 2017-2018 to ascertain the status of PPRV antibodies in sheep and goat populations in the control programme implemented Southern Peninsular region. The working hypothesis was the homogeneous occurrence of PPRV antibodies in the target populations in the epi-units in the different states/UT of the studied region. The village is considered as an epi-unit in the study area as described earlier [12]. Therefore, a list of villages in various blocks/tehsils in different districts in the state/UT and their sheep and goat populations was prepared. To have a sizeable population, when approached for sampling, villages having more than 200 animals were shortlisted (with inclusion and exclusion criteria), which accounted for the sampling frame. The sample size was determined to the finite population as per Cochran (1963) formula $N = Z^2$ [p $(1 - p/e^2)$], where N = sample size, Z = 95% confidence level, p = 30% proportion (animal unitlevel prevalence of 30% was considered as per GCES [22] as well as based on the prevalence of PPR before the implementation of vaccination in India [27], e is the precision level (5%). Based on these inputs a total sample size of 323, were determined by using the epitools (http://epitools.ausvet.com. au/content.php?page=1Proportion), for the finite large populations. However, after considering the attrition rate of 10%, the total sample size arrived was 356. The multistage stratified random sampling method was adopted for collecting samples from different states/UT in the studied region. In the first stage, the states in the study region were stratified and in each stratum (State/UT), 60 sampling primary epi-units (villages) were equally allocated randomly using R software (R_Core_Team 2014). In the next stage in each of the selected epi-units, the number of secondary animal units samples was calculated by the hypergeometric distribution as per GCES guidelines by considering the animal unit-level prevalence of 30% [22] in small ruminants and a maximum of 11 samples to be collected was determined in each epi-units. Thus, a maximum of 1320 secondary animal units [660 for each of the target (sheep or goats) species] samples to be sampled arrived from each state/ UT at a given large infinite target (sheep and goats) population by using epi-calculator (https://www.nivedi.res.in/Nadres_ v2/Epical/stratified/random_sampling.php.).

Serum samples

In each epi-unit, a total of 22 serum samples were collected as per the sampling method with a maximum of 11 samples for each species, through All India Co-ordinated Research Project on Animal Disease Monitoring and Surveillance (AICRP on ADMAS), a collaborating center of ICAR-NIVEDI, in the respective states/UTs. In the selected epiunit, where only either goats or sheep species reared, 11 samples of either species were only collected. The sample surveyed villages in the states/UT of the studied region are depicted in GIS Map (Fig. 1) based on their geo-coordinates using QGIS Software 2.18.6 version. The collected blood samples were labelled and separated sera were transported in an ice-cool shipment box to ICAR-NIVEDI, Bengaluru, and the samples upon received were stored at - 20 °C until further use.

Testing of samples

The collected serum samples were tested by an indigenously developed PPR competitive ELISA [28] kit (has 92.4% sensitivity and 98.4% specificity), which is being employed for serosurveillance or seromonitoring in India [28] for the detection of PPRV specific antibodies, which were measured in terms of percentage inhibition (PI) according to the protocol described by [28]. In this c-ELISA, the binding of the H protein-specific monoclonal antibodies (MAb) to the PPRV antigens coated wells of the plate was selectively inhibited in the presence of PPRV specific antibodies (both IgM and IgG) in the test serum samples (i.e., competition occurs between the known MAb and PPRV antibodies). Further, the level of inhibition of MAb binding is directly proportional to the presence of PPRV antibodies concentration in the test sera, which indicates the specificity of the inhibition of PPRV antibodies. Samples with a PI of > 40% were considered positive for the presence of PPRV specific antibodies and the overall percentage positivity or seroprevalence was calculated with 95% confidence intervals (CI).

Statistical analysis

The seroprevalence was estimated as per Thrushfield [31] based on the number of positive animals versus numbers of tested animals. The chi-squared test (χ^2) was carried out in Microsoft office Excel 2013 as per the method described by Snedecor and Cochran [29] to understand the association of the presence of PPRV antibodies in sheep and goats across states and districts as well as between the sheep and goats within each state in the study region.

Further, annual growth rate (GR) of the prevalence of PPRV antibodies in the different states in the region was assessed to predict the number of years of vaccination required to achieve desired 70% prevalence level of antibodies [22, 23] for the control and eradication of disease

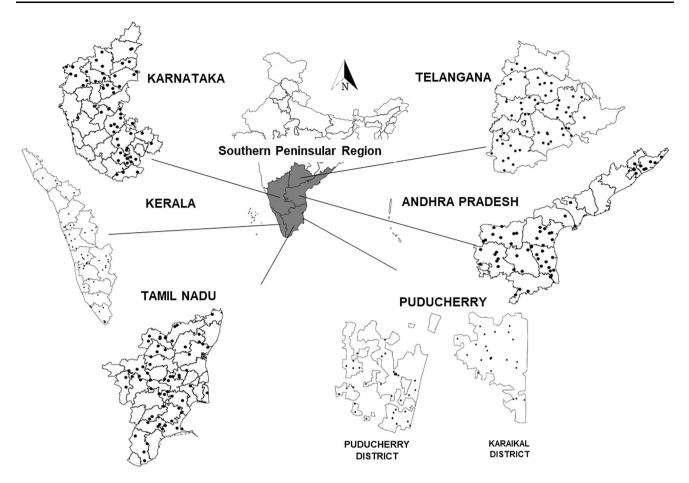


Fig. 1 The surveyed epi-units (villages) location are depicted (as filled square a dot) in the GIS Map of the studied States/Union Territory in Southern Peninsular India

using the mathematical formula [GR = $[(b - a)/a] \times 100/N$] as described earlier [8], Where, b = Initial value, a = first value, which was taken as ~ 30% base level of seroprevalence [27], N = No. of years. Even though vaccination started since 2011 the number of years considered for growth assessment was three due to the vaccinated populations would have turned over by then as sheep and goat typical lifespan is three years. Further, keeping in view the turnover of the sheep and goat populations (i.e. slaughtering and fecundity of animals, resulted in the appearance of approximately 30% naïve population every year), the calculated growth was discounted by 30% each year [27].

Results

The observed percentage prevalence of PPRV antibodies in small ruminants in the studied region was 87.0, 66.4, 64.3, 47.8, 11.4, and 50.4%, in Telangana, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, and Puducherry, respectively with overall 59.0% seroprevalence in small ruminants with 67.1% in sheep and 52.1% in goats. State/ UT-wise details of serum samples screened for PPRV antibodies from the studied region and their percent positivity with seroprevalence levels are presented in Table 1 and Fig. 2. The results of the chi-squared test revealed that the prevalence of PPRV antibodies in sheep $(\chi^2 = 218.8, p < 0.01)$ and goats $(\chi^2 = 827.1, p < 0.01)$, across different states / UT of this studied region were associated, as most of the states adopted the PPR vaccination programme. Further, the observed chi-squared value ($\chi^2 = 130.3$, p < 0.01) between the sheep and goats species in the studied region was also indicated the association of the presence of PPRV antibodies in the species. Furthermore, the percentage prevalence of the PPRV antibodies in various epi-units of the different states/UT is shown in Fig. 3. The annual GR of the prevalence of PPRV antibodies was assessed at different states/UT levels separately if regular vaccination is being in practice. The calculated year-wise annual GR by the mathematical model to achieve the desired 70% prevalence level of PPRV antibodies for the studied states/UT are tabulated (Table 2).

Discussion

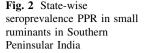
The present study assessed the prevalence status on PPRV antibodies in sheep and goats separately at each state/UT level in the Southern Peninsular region as a part of postvaccination evaluation towards the eradication of PPR and generated evidence on the status of seroprevalence, which is paramount important for devising effective control strategies. By employing the presently available PPR c-ELISA [28], it is not possible to distinguish the immune response due to vaccination and infection, as the DIVA vaccine was not being used in the PPR-CP in India. However, the earlier population surveys in the non-outbreaks reported Chhattisgarh state indicated above 50% prevalence of PPRV antibodies implies vaccination is being implemented in the small ruminants population [8]. Further, the base-line seroprevalence of disease in the small ruminants before the implementation of the vaccination was also reported in India, which varied from 32.4 to 46.11% [9, 24, 27].

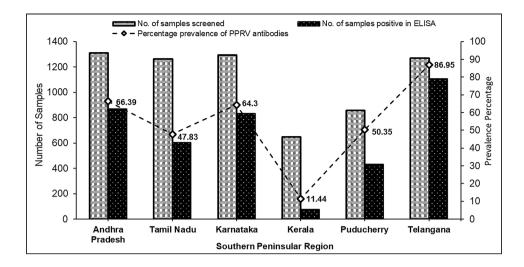
Telangana and APstates followed focused vaccination to contain the outbreaks and reduced the epidemic level by 95% [26]. In Telangana state, the observed prevalence level of PPRV antibodies was 87.0% [1106/1272] in small ruminant population with association of 86.8% in sheep $(\chi^2 = 86.4, p < 0.01)$ and 87.1% in goats $(\chi^2 = 43.4, p < 0.01)$ p < 0.01) (Table S1), as regular vaccination is being practiced in all the districts in the state since implementation of vaccination programme. Similarly, in AP state, the seroprevalence of 66.4% (869/1309) was observed with 69.1% in sheep and 63.7% in goats (Table S2). Moreover, the chi-squared test revealed that immune protection levels between districts were not associated with sheep $(\chi^2 = 8.18, p > 0.05)$, which might be due to variation in the vaccination coverage level during two to three years preceding the sampling survey period (2017-2018). AP state-initiated 'mass vaccination programme' during 2007-2008 covering 82% of small ruminants population followed by annual vaccination since 2010. This annual vaccination is to cover the newborn young stock above five months of age and unvaccinated animals in the previous vaccination campaign, which resulted in the marked decline in the PPR outbreaks [30]. Further, in consonance with national PPR-CP since 2011, State continued vaccination on a half-yearly basis during the predesignated period. This resulted in limited outbreaks [25] with the flock immunity in vaccinated animals ranged from 81 to 95.6% [26]. With a strategic vaccination campaign, the disease has been kept under control in these states, it may eventually lead to complete control and eradication from the state and subsequently from the region or country [6]. Moreover, the proportion of epi-units with > 70% of

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Table 1

Name of the state/union territory No. of tehsils/b	/ No. of No. of vi tehsils/blocks /epi-units	No. of villages /epi-units		No. of samples screened		No. of positive	No. of samples positive in ELISA	SA	Percentage prev (confidence into	Percentage prevalence of PPK viru (confidence interval value at 95%)	Percentage prevalence of PPR virus antibodies (confidence interval value at 95%)	Percentage seroprevale in epi-units	Percentage seroprevalence status in epi-units (No.)	status o.)
			Total	Total Sheep Goats Total Sheep Goats	Goats	Total	Sheep		Total	Sheep	Goats	< 30	< 30 30-70 > 70	> 70
Andhra Pradesh	57	09	1309 654	654	655	869	452	417	66.39 (64-69)	66.39 (64-69) 69.11 (66-73) 63.66 (60-67) 5	63.66 (60-67)	5	25	30
Tamil Nadu	54	60	1265	629	636	605	338	267	47.83 (45–51)	47.83 (45–51) 53.74 (50–58) 41.98 (38–46)	41.98 (38-46)	18	29	13
Kamataka	46	60	1294	649	645	832	429	403	64.3 (62–67)	64.3 (62–67) 66.1 (62–70) 62.48 (59–66)	62.48 (59–66)	٢	21	32
Kerala	36	60	647	I	647	74	I	74	11.44 (9–14)	I	11.44 (09–14)	53	5	7
Puducherry	5	60	856	210	646	431	91	340	50.35 (47-54)	50.35 (47–54) 43.33 (37–50) 52.63 (49–56)	52.63 (49-56)	18	28	14
Telangana	58	60	1272	643	629	1106	558	548	86.95 (85-89)	86.95 (85–89) 86.78 (83–89) 87.12 (84–90)	87.12 (84–90)	1	4	55
Total	256	360	6643	2785	3858	3917 1868	1868	2049	58.96 (58-60)	58.96 (58–60) 67.07 (65–69) 52.11 (52–55) 102	52.11 (52-55)	102	112	146

Between sheep and goats = $(\chi^2 = 130.3, p < 0.01)$





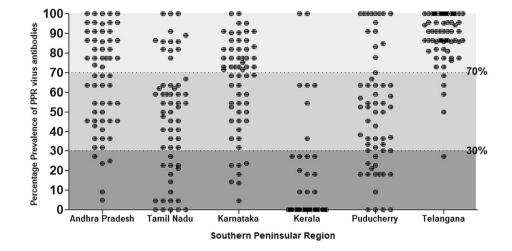


Fig. 3 Distribution of epi-units based on percent positivity levels of PPR virus antibodies in the studied region

animals to be protected as per PPR-GCES guidelines [22]. The present study in APstate, 30 out of 60 epi-units covering the 57 blocks in 10 districts, had shown > 70%cluster level prevalence as per OIE recommendation, whereas, in Telangana state, 55 out of 60 epi-units had >70% level prevalence of PPRV antibodies. Therefore, PPR vaccination strategies need to be revisited in Telangana, if sporadic outbreaks are not reported frequently as desired > 70% levels of PPRV antibodies already attained in the population. The vaccination to be continued for a few more years to achieve the desired levels in all the epiunits at a given period, so that, further vaccination may be restricted to the area adjoining with borders, the animals in the migratory route and border areas as well as places of sporadic outbreaks if any. Thus it will reduce the cost of PPR control and facilitates the state to divert the available funds and manpower to other livestock health activities.

Karnataka state started "focused vaccination" during 2003 [20] and controlled the outbreaks tremendously. In

consonance with PPR-CP, the state continued mass vaccination campaigns in the target population since 2011–2012 and the disease has been kept under control [6]. The observed PPRV antibodies prevalence level was 64.3% (832/1294) in small ruminants with 66.1% in sheep ($\chi^2 = 130.1$, p < 0.01) and 62.5% in goats ($\chi^2 = 64.1$, p < 0.01) (Table S3) being associated as regular vaccination is being practiced in all the districts in Karnataka. Moreover, only 32 out of 60 epi-units covering 46 blocks in 17 districts, had > 70% desired prevalence level of PPRV antibodies.

Even though Kerala state initiated PPR vaccination practice during 2005 (focused vaccination) and 2011 (mass vaccination), the overall mass vaccination coverage was very poor for the last three years preceding the sampling period (2017–2018) i.e. from 5.6% in 2017–2018 to 10.4% in 2015–2016. The seroprevalence of 11.4% (74/647) in goats ($\chi^2 = 71$, p < 0.01) (Table S4) was observed with none of the tested 60 epi-units (except Mazhuvannoor and

territory	Population as per the 19th Livestock Census ^a (20th Livestock Census ^a)	sr the 19th Is ^a (20th S ^a)	Vaccination type and implemented year	ype and year	Percentage of sero prevalence	Calculated prevalence	Calculated annual growth rate for achieving the desired 70% prevalence level of PPR virus antibodies (Immune population)	rate for achi irus antibodi	eving the desi es (Immune p	red 70% ppulation)
	Sheep	Goats	Focus vaccination	Strategic vaccination	2017-2018	2018–2019	2019–2020 2020–2021	2020–2021	2021–2022	2022-2023
Andhra Pradesh ^b	13.52 M (18.80 M)	4.4 M (5.53 M)	2002-2003	2007–2008	66.4	74.78	103.08	131.38	159.69	187.99
Tamil Nadu	4.79 M (4.53 M)	8.14 M (9.91 M)	2003–2004	2011–2012	47.5	47.35	61.22	75.08	88.95	102.82
Karnataka	9.58 M (11.15 M)	4.79 M (6.24 M)	2003–2004	2011–2012	64.3	71.69	98.37	125.04	151.72	178.40
Kerala°	1446 (1979)	1.25 M (1.46 M)	2005–2006	2011–2012	11.4	I	I	I	I	I
Puducherry	1601 (3421)	54,950 (73,786)	2003–2004	2011-2012	49.1	51.07	66.90	82.73	98.56	114.38
Telangana	12.87 M (19.55 M)	4.67 M (49.43 M)	2002–2003	2014-2015	87.0	105.16	149.45	193.75	238.04	282.34
Southern Peninsular Region as total	40.77 M (54.04 M)	23.31 M (28.16 M)	I	I	54.28	I	I	I	I	I
^a M-million; as per the recent release of provisional key results of the 20th Livestock Census, 2019, India has 148.88 million goats and 74.26 million sheep during 2019 (http://dahd.nic.in/ division/provisional-key-results-20th-livestock-census accessed on 25th October 2019). The small ruminants population has increased by 10.14% goat and 14.13% sheep when compared to the 19th Livestock Census, 2012 (http://www.dahd.nic.in) and they contribute to livestock population by 28.7% goat and 13.8% sheep ^b Undivided Andhra Pradesh (AP), which include Telangana and AP states and divided into AP and Telangana for administrative purpose as a separate state, during 2014 ^c For Kerala state annual growth rate could not be carried out due to the prevalence of PPRV antibodies during 2017–2018 was below the base level 30% seroprevalence employed for the	nt release of provis ults-20th-livestock 2 (http://www.dahu t (AP), which inclu owth rate could noi	sional key results census accessed i d.nic.in) and they ide Telangana and t be carried out d	of the 20th Li on 25th Octobe · contribute to 1 d AP states and lue to the preva	vestock Census, 2 r 2019). The small livestock population I divided into AP alence of PPRV ar	s of the 20th Livestock Census, 2019, India has 148.88 million goats and 74.26 million sheep during 2019 (http://dahd.nic.in/ 1 on 25th October 2019). The small ruminants population has increased by 10.14% goat and 14.13% sheep when compared to the sy contribute to livestock population by 28.7% goat and 13.8% sheep and AP states and divided into AP and Telangana for administrative purpose as a separate state, during 2014 due to the prevalence of PPRV antibodies during 2017–2018 was below the base level 30% scroprevalence employed for the	llion goats and s increased by .8% sheep istrative purpo 18 was below	174.26 millior 10.14% goat an se as a separa the base level	a sheep durin nd 14.13% sh te state, durir 30% seropre	g 2019 (http:/ eep when con 1g 2014 .valence emplo	dahd.nic.in/ pared to the yed for the

Puthur villages, which had 100% positivity level of antibodies), have > 70% prevalence level of PPRV antibodies, as Kerala state has not adopted mass vaccination across the districts as per PPR-CP plan for the past three years.

Tamil Nadu (TN) stateinitiated focused vaccination in the area of the outbreak, during 2003-2004 through Assistance to States for Control of Animal Diseases (ASCAD) scheme and programme mode vaccination was continued since 2011-2012, however, like Kerala state, the overall vaccination coverage was very poor since the inception, i.e., from 40% in 2011-2012 to 9.8% in 2013-2014; 46% in 2014-2015 and for the last three years preceding the sampling period it was negligible. The observed prevalence of PPRV antibodies was 47.83% (605/ 1265) in small ruminants with association of 53.7% in sheep $(\chi^2 = 115.5, p < 0.01)$ and 42.0% in goats $(\chi^2 = 170.8, p < 0.01)$] (Table S5). However, only 13 out of 60 epi-units had > 70% desired prevalence level of antibodies as TN state was not adopted a mass vaccination programme across the districts as per the PPR-CP plan for the past three years. Similarly, Puducherry followed focused vaccination to contain the outbreaks as and when required in regular vaccination practice. The seroprevalence of 50.4% (431/856) in small ruminants was observed with 43.3% in sheep and 52.6% in goats (Table S6), as it might be due to non-adoption of the regular mass vaccination programme in the state as per PPR-CP strategic plan. Moreover, only, 14 out of 60 epi-units covering the five blocks in two districts had > 70% cluster level prevalence of PPRV antibodies.

The annual GR was used for calculating the number of years vaccination need to be continued to reach the desired minimum 70% levels [22] in all the epi-units in different states/ UT of the studied region (Table 2) for the control and eradication of the disease. Moreover, for the control of the disease, Fournié [17] stated that viral spread could be prevented if the proportion of immune small ruminants is kept permanently above 37% in at least 71% of the village population in an endemic setting by fitting a metapopulation simulating model. However, due to the high turnover of sheep and goats, maintaining the fraction of the immune population above this threshold would require high vaccine coverage within villages and vaccination campaigns to be conducted regularly. In this study, this estimate corresponded with the observed results of the current study in Telangana, Karnataka and Andhra Pradesh were 98.3%, 83.3%, 83.3% of epi-units respectively, which showed the restricted spread of the virus in these mass vaccination programme implemented states, as there were no frequent outbreaks have been reported in these state for the past three preceding years of the survey period. For Kerala state, it will take 4 to 5 years (2022-2023) to achieve the desired 70% cluster level prevalence as envisaged in PPR-

CP, because it was below the base level of 30% prevalence. For that, the mass vaccination programme with three to four cycles of vaccination needs to be adopted in the lines of the OIE eradication pathway of PPR control strategies. Each cycle of vaccination should cover the entire target population initially, subsequently bi-annual vaccination of covering the naïve young population.

Further, the present studied region covered two zones out of 15 agro-climatic zones of India [21] viz., Sothern plateau and hills, and West coastal plateau and hills zones covering major states in southern peninsular India (TN, Karnataka, AP, Kerala, and Telangana). The prevalence of PPRV antibodies in these states varied significantly due to variation in risk population, disease incidence, and extent of vaccination coverage in the PPR-CP. Further, the observed prevalence of antibodies level in the states in the Southern Peninsular covering two zones was high (population immunity) due to continuous vaccination coverage in the states due to PPR-CP implementation since 2010-2011 (www.dahd.nic.in) when compared to non-vaccination implemented states of India, where baseline 30% prevalence was observed [13]. Similarly, the earlier reported seroprevalence in other agroclimatic zones/states was also varied. The reported seroprevalence from the Eastern Himalayan zone (covering north-eastern states of India) showed 34.3%, 10.3%, 4.7%, 15.7%, 14.7%, and 5.5%, in small ruminants in Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura, respectively [13], whereas the Central and Western plateau and hills and Western Dry as well as Gujarat plains and hills zones covering the central and western India, showed seroprevalence of 34.4%, 20.8%, 51.6%, 74.1% 68.3%, and 64.8% in Madhya Pradesh, Goa, Chhattisgarh, Maharashtra, Gujarat, and Rajasthan states respectively [4, 5]. Similarly, Western Himalayan zone and Upper and Trans Gangetic Plains zones covering North Indian states showed that seroprevalence of 57.32%, 55.22%, 65.69%, 37.09%, 32.73%, and 29.35% in small ruminants in Haryana, Punjab, Uttar Pradesh, Himachal Pradesh, Jammu and Kashmir, and Uttarakhand states, respectively [14]. The East coast plains and hills and Middle Gangetic Plains and Western plateau and hills zones covering Eastern India, and island zone of Andaman and Nicobar, showed the seroprevalence of 30.91% and 54.20% in Bihar and Odisha states, respectively and 1.28% in the Andaman Islands [11, 12]. Overall, the reported prevalence of PPRV antibodies in small ruminants in India as a whole in a large scale study varied between 33.0 to 43.6% [9, 27] and 43.7% [4, 5], which indicated the need for vigorous vaccination among small ruminants in the country to be continued to achieve desired population immunity status for the eradication of PPR from India. Therefore, PPR-CP mass vaccination programme needs to be continued for a few more years to achieve the

desired 70% cluster level of antibody prevalence in Sothern India. Nevertheless, this cross-sectional study needs to be visualized with certain limitations, such as the host factors (age, sex, etc.), and animal vaccination status for the individual animals was not available for further multifactorial regression analysis.

Conclusion and perspectives

The present survey provides information on the prevalence of PPRV antibodies in sheep and goats, as the samples analyzed were a true representation of the target sheep and goats population in Southern Peninsular India. There exists variation in the prevalent levels of antibodies among the PPR-CP implemented states in the studied area since the vaccination pattern was not uniform across the states/districts. Further, in some states, the timely vaccination was not adopted or in some states in a few rounds of vaccination has not been taken up due to administrative reasons. Hence, to achieve the desired cluster level immunity as envisaged in PPR-CP, the mass vaccination programme in the designated period with two to three cycles of vaccination to be carried out to reach 70-80% level prevalence of antibodies or immunity status. Further, vaccination may be restricted to bordering districts, animal markets, and check posts only, if the state reached the desired 70% cluster level PPRV antibodies prevalence (immunity levels) in all the tested epi-units without the occurrence of PPR outbreaks. The study also implies that the small ruminants population in some of the epi-units in the studied region were having less than 30% seroprevalence. This necessitates comprehensive intensive vaccination and active surveillance programmes to make Southern India as PPR free zone. Therefore, zoning the PPR risk regions and initiating vaccination program at a specified period with complete vaccination coverage of all the risk population in the identified zone is of paramount importance along with monitoring and surveillance. At the time of declaring, India is provisionally free from PPR, surveillance needs to be carried out as per OIE guidelines to support the freedom from PPR in unvaccinated sheep and goats populations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The manuscript does not contain animal experimental trials. No ethical clearance is required for collecting small volumes of blood samples required for seroepidemiological studies, as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines. Moreover, samples were collected by well-trained veterinarians concerning animal welfare regulations.

References

- Acharya N, Poudel SP, Acharya KP. Cross-sectional seroprevalence study of Peste des Petits Ruminants (PPR) in goats of Syangja and Kaski districts of Nepal. Virusdisease. 2018;29:173–9.
- Al-Majali AM, Hussain NO, Amarin NM, Majok AA. Seroprevalence of, and risk factors for, Peste des Petits Ruminants in sheep and goats in Northern Jordan. Prev Vet Med. 2008;85:1–8.
- Almeshay MD, Gusbi A, Eldaghayes I, Mansouri R, Bengoumi M, Dayhum AS. An epidemological study on Peste des petits ruminants in Tripoli Region, Lybia. Vet Ital. 2017;53:235–42.
- Anonymous ICAR-NIVEDI. Annual report 2018–2019. Bengaluru: ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI); 2019. p. 17–18. https://nivedi.res. in/reports.
- Anonymous ICAR-NIVEDI. Annual report 2019. Bengaluru: ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI); 2020. p. 16. https://nivedi.res.in/reports.
- Balamurugan V, Govindaraj GN, Rahman H. Planning, implementation of peste des petits ruminants control programme and strategies adopted for disease control in India. Br J Virol. 2016;3:53–62.
- Balamurugan V, Hemadri D, Gajendragad MR, Singh RK, Rahman H. Diagnosis and control of peste des petits ruminants: a comprehensive review. Virusdisease. 2014;25:39–56.
- Balamurugan V, Muthuchelvan D, Govindaraj G, Roy G, Sharma V, Sowjanya Kumari S, Choudhary D, Mohanty BS, Suresh KP, Rajak KK, Hemadri D, Roy P. Serosurvey for assessing PPR vaccination status in rural system of Chhattisgarh state of India. Small Ruminant Res. 2018;165:87–92.
- Balamurugan V, Saravanan P, Sen A, Rajak KK, Bhanuprakash V, Krishnamoorthy P, Singh RK. Sero-epidemiological study of peste des petits ruminants in sheep and goats in India between 2003 and 2009. Rev Sci Tech. 2011;30:889–96.
- Balamurugan V, Saravanan P, Sen A, Rajak KK, Venkatesan G, Krishnamoorthy P, Bhanuprakash V, Singh RK. Prevalence of peste des petits ruminants among sheep and goats in India. J Vet Sci. 2012;13:279–85.
- Balamurugan V, Varghese B, Muthuchelvan D, Kumar KV, Govindaraj G, Suresh KP, Kumar P, Hemadri D, Roy P. Seroprevalence of peste des petits ruminants in sheep and goats in Eastern India. VirusDisease. 2020. https://doi.org/10.1007/ s13337-020-00574-2.
- Balamurugan V, Varghese B, Muthuchelvan D, SowjanyaKumari S, Kumar KV, Suresh KP, Govindaraj G, Sunder J, Hemadri D, Roy P. Cross-sectional seroprevalence study of peste des petits

ruminants in goats in Andaman and Nicobar Islands, India. Small Rum Res. 2019;178:111–16.

- Balamurugan V, Varghese B, Muthuchelvan D, SowjanyaKumari S, Vinod Kumar K, Dheeraj R, Govindaraj G, Suresh KP, Hemadri D, Roy P. Seroprevalence of Peste des petits ruminants in small ruminants in the North Eastern Region of India. Vet Ital. 2020;56:55–60.
- 14. Balamurugan V, Varghese B, Vinod Kumar K, Muthuchelvan D, Dheeraj R, Govindaraj G, Suresh KP, Hemadri D, Roy P. Seroprevalence study of peste des petits ruminants in sheep and goats in the northern region of India. Vet World. 2020;13:1573–80.
- Dayhum A, Sharif M, Eldaghayes I, Kammon A, Calistri P, Danzetta ML, Di Sabatino D, Petrini A, Ferrari G, Grazioli S, Pezzoni G, Brocchi E. Sero-prevalence and epidemiology of peste des petits ruminants in Libya. Transbound Emerg Dis. 2018;65:e48-54.
- Faris D, Yilkal A, Berhe G, Kelay B. Seroprevalence and seroconversion after vaccination against Peste des Petits Ruminants in sheep and goats from Awash Fentale District, Afar, Ethiopia. Prev Vet Med. 2012;103:157–62.
- Fournié G, Waret-Szkuta A, Camacho A, Yigezu LM, Pfeiffer DU, Roger F. A dynamic model of transmission and elimination of peste des petits ruminants in Ethiopia. Proc Natl Acad Sci India. 2018;115:8454.
- Govindaraj GN, Balamurugan V, Rahman H. Estimation of economic loss of PPR in sheep and goats in India: an annual incidence based analysis. Br J Virol. 2016;3:77–85.
- Govindaraj GN, Roy G, Mohanty BS, Balamurugan V, Pandey AK, Sharma V, Patel A, Mehra M, Pandey SK, Roy P. Evaluation of effectiveness of mass vaccination campaign against peste des petits ruminants in Chhattisgarh state, India. Transbound Emerg Dis. 2019;66:1349–59.
- Hegde R, Gomes AR, Muniyellappa HK, Byregowda SM, Giridhar P, Renukaprasad C. A short note on peste des petits ruminants in Karnataka, India. Rev Sci Tech. 2009;28:1031–5.
- MeitY. Agro Climatic zones in India. Hyderabad, India: Center for Development of Advanced Computing, Ministry of Elecronics and information Technology (MeitY), Government of India.

2020. https://vikaspedia.in/agriculture/crop-production/weather-information/agro-climatic-zones-in-inida.

- 22. OIE FAO. Annexe 3.4. Post vaccination evaluation tool. 2015. p. 1–32. http://www.fao.org/3/a-i4460e.pdf.
- OIE FAO. Manual on global strategy for the control and eradication of Pesti des petits ruminants. 2015. http://www.fao.org/3/ a-i4460e.pdf.
- 24. Raghavendra AG, Gajendragad MR, Sengupta PP, Patil SS, Tiwari CB, Balumahendiran M, Sankri V, Prabhudas K. Seroepidemiology of peste des petits ruminants in sheep and goats of southern peninsular India. Rev Sci Tech. 2008;27:861–7.
- 25. Singh RK, Balamurugan V, Bhanuprakash V, Sen A, Saravanan P, Pal Yadav M. Possible control and eradication of peste des petits ruminants from India: technical aspects. Vet Ital. 2009;45:449–62.
- 26. Singh RP, Bandyopadhyay SK. Peste des petits ruminants vaccine and vaccination in India: sharing experience with disease endemic countries. Virusdisease. 2015;26:215–24.
- Singh RP, Saravanan P, Sreenivasa BP, Singh RK, Bandyopadhyay SK. Prevalence and distribution of peste des petits ruminants virus infection in small ruminants in India. Rev Sci Tech. 2004;23:807–19.
- Singh RP, Sreenivasa BP, Dhar P, Shah LC, Bandyopadhyay SK. Development of a monoclonal antibody based competitive-ELISA for detection and titration of antibodies to peste des petits ruminants (PPR) virus. Vet Microbiol. 2004;98:3–15.
- Snedecor GW, Cochran WG. Statistical methods (Indian Edition). Oxford: IBH Publishing; 1967.
- Srireesha S, Krishna Jyothi Y, Jyothi N, Hanmanth GR. Strategic control of PPR disease in Andhra Predesh. In: National confrence on PPR disease: "control of PPR diseases: challenges and opportunities". 2014.
- Thrushfield M. Veterinary epidemiology. 3rd ed. Ames: Blackwell Publishing; 2005.

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