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ORIGINAL ARTICLE

Prevalence of *Peste-des-petits-ruminant virus* antibodies in cattle, buffaloes, sheep and goats in India

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Abstract The present study describes the prevalence of *Peste-des-petits-ruminant virus* (PPRV) antibodies in cattle, buffaloes, sheep and goats carried out during the period 2011 using the serum samples randomly collected from different villages of five states of India. A total of 1,498 serum samples [$n = 605$ (cattle); $n = 432$ (buffaloes); $n = 173$ (sheep); $n = 288$ (goats)] were collected from 52 districts in five states (Andhra Pradesh, Gujarat, Jammu and Kashmir, Maharashtra and Rajasthan) of India and were screened for PPRV-specific antibodies by using PPR monoclonal antibody-based competitive ELISA kit. Analysis of 1,498 samples indicates that an overall seroprevalence of 21.83 % with 11.07 % in cattle, 16.20 % in buffaloes, 45.66 % in sheep and 38.54 % in goats. This report presents the results of PPRV-specific antibodies in situations where the subclinical, inapparent or nonlethal or recovery of infection was suspected in cattle, buffaloes, sheep and goats. The presence of PPRV antibodies demonstrate that bovines are exposed to PPRV infection and it implies the importance of cattle and buffaloes as subclinical hosts for the virus besides widespread presence of the disease in sheep and goats. Further, the study showed that the prevalence of PPRV antibodies in apparently healthy livestock under natural situation, 21.83 % of the animals were protected from PPRV re-infection. This inturn help in the implementation of disease control strategies such as vaccination in that particular geographical area.

Keywords PPRV · Antibodies · Cattle · Buffaloes · Sheep · Goats

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Introduction

Peste des petits ruminants (PPR) is a highly contagious, notifiable to World Organization for Animal Health (WOAH-OIE) and economically important transboundary viral disease of sheep and goats. The causative agent, PPR virus (PPRV) belongs to the *Morbillivirus* genus of *Paramyxoviridae* family. There is a single serotype of PPRV, but genetically grouped into four distinct lineages (I, II, III, and IV) based on partial sequence analysis of Fusion (F) gene [22]. Clinically, the disease resembles rinderpest (RP) in cattle and is characterized by pyrexia, ocular and nasal discharges, necrotic stomatitis, catarrhal inflammation of the ocular and nasal mucosa, enteritis, diarrhoea and bronchopneumonia followed by either death or recovery from the disease [10]. Mortality and morbidity of disease are high when occurring in naive sheep and goat populations. The mortality ranges from 50 to 90 % and sometimes can be nil and morbidity can be 10–100 %, even lower than 10 % depending on circumstances [2]. PPRV primarily affects goats and sheep; cattle and buffaloes are asymptomatically infected with seroconversion while other wild ruminants and camels may exhibit clinical signs and mortality [3].

PPR was first reported in the Ivory Coast, West Africa [10], and later from other parts of the world namely sub-Saharan Africa, the Middle East and the Indian subcontinent [22, 23]. Spread of disease to a number of new countries in Africa and Asia with the involvement of various lineages of PPRV is a cause of global concern especially recent introduction of Asian lineage in some African countries and presence of PPR in Europe through Western Turkey [3, 7, 15]. This transboundary nature of the disease is one of the main constraints in augmenting the productivity of small ruminants in enzootic regions in the world.

In India, PPR was first recorded in 1987 from Tamil Nadu [21] and it continues to be present in the Southern India until 1994. Later, a number of PPR outbreaks were reported from the northern states of India [18] with a solitary report in Indian buffalo in southern state [11]. Now, PPR is enzootic in India as outbreaks occur in small ruminants regularly throughout the country [24] and is a major constraint in small ruminant production incurring huge economic losses [estimated to be INR 1,800 million (US$ 39 million)] annually in terms of morbidity, mortality, productivity losses with trade restriction [24, 30].

Information on the prevalence of PPRV antibodies in cattle, buffaloes, camels, wild ruminants etc., is available from a number of countries in which the disease has been reported [1, 12, 14, 17, 30]. Majority of the reports from India except few indicated only the regional data from various states about the PPR seroprevalence in small ruminants and bovines [5, 6, 20, 24]. The prevalence of PPRV antibodies in unvaccinated sheep and goats indicated not only the subclinical or inapparent infection but also, non-lethal clinical infection or in other words, recovered infected animals, which could be of epidemiological significance. Efficient and sensitive diagnostic assays/tests are of great help in quickly providing evidence that PPRV is not circulating in a free population based on serological investigation. Monoclonal antibody-based competitive ELISA (c-ELISA) [25] is the currently employed assay for serosurveillance/monitoring of PPR in India.

For the control of PPR, there is need for base line epidemiological data on the disease prevalence in population, strong support of diagnostic methods and proper, timely vaccination of the susceptible population. Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS) is a premier research institute under the Indian Council of Agricultural Research (ICAR) carrying out research in the field of animal disease monitoring and surveillance, epidemiology and diagnostics. Therefore, the present study was undertaken with an objective of generating the baseline data on prevalence of PPRV antibodies in cattle, buffaloes, sheep and goats using random samples collected from different villages in five states of India during 2011 surveys.

Materials and methods

Clinical samples

Seroprevalence of PPR in cattle, buffaloes, sheep and goats investigated in the present study was recorded from 52 districts in five states (Andhra Pradesh, Gujarat, Jammu and Kashmir, Maharashtra and Rajasthan) of India. Serum samples of unknown antibody status {$n = 1{,}037$ [bovine-$n = 432$ (buffaloes); $n = 605$ (cattle)]; $n = 173$ [sheep]; $n = 288$ [goats]} were collected by AICRP (All India Co-ordinated Research Project) collaborating centre on ADMAS as per the random sampling frame designed by the PD_ADMAS from different villages in the selected districts in various states during 2011 sample surveys. Five to ten samples from each species from each village were collected using random sampling technique. All the serum samples mentioned above were available in the National Livestock Serum Repository of PD_ADMAS. These samples were stored at −20 °C upon receipt from field and used for further analysis as and when required. The details of the species from which the samples were collected during 2011 with the area of origin are presented in Table 1.

Competitive ELISA

PPR c-ELISA kit was used for detection of PPRV antibodies in terms of percentage inhibition (PI) as per the method described earlier [25]. Samples with PI of ≥40 % were considered positive for the presence of PPRV antibodies. Briefly, ELISA plates (NUNCMaxisorp, Hamburg, Germany) were coated with the PPRV antigen (50 µl/well). After incubation, at 37 °C for 1 h, the wells were washed three times with 0.002 mol/L phosphate-buffered saline (PBS). Then, 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 MAb in each well (except conjugate control wells) at a final dilution of 1:500. Anti-mouse–HRPO conjugate (Dako, Glostorp, Denmark) diluted 1:1,000 in blocking buffer was added to each well (50 µl/well) after incubation. Finally, substrate solution (orthophenylene diamine, OPD) was added in each well and colour reaction was developed for 10 min before stopping the reaction with 1 mol/L H_2SO_4 and OD was measured at a wavelength of 492 nm.

Statistical analysis

The estimation of apparent prevalence with 95 % confidence interval and data analysis were carried out as per standard statistical method [26] using Statistical Analysis

Table 1 Details of the samples screened for PPRV antibodies in cattle, buffaloes, sheep and goats during 2011 survey in India

State name	No.of districts covered	Name of districts	Cattle	Buffaloes	Sheep	Goats	Total
Andhra Pradesh	10	Adilabad, Prakasam, Nizamabad, Medak, West Godavari, Chittoor, Kadappa, Rangareddy, Warangal and Mehabubnagar	70	70	70	70	280
Gujarat	11	Amreli, Jamnagar, Narmada, Surat, Patan, Vadodara, Rajkot, Junagadh, Panchmahal, Bhavnagar and Surendranagar	90	87	37	80	294
Jammu and Kashmir	8	Pulwama, Samba, Kathua, Jammu, Reasi, Udhampur, Doda and Kupwara	299	148	–	–	447
Maharashtra	14	Amaravati, Nanded, Yavatmal, Satara, Buldhana, Nagpur, Sangli, Chandrapur, Raigarh, Jalgoan, Pune, Sindhdurg, Aurangabad, and Bid	95	75	40	75	285
Rajasthan	9	Ganganagar, Dungarpur, Chittore, Barmer, Bhilwara, Tonk, Alwar, Jaipur and Chuou	51	52	26	63	192
Total	52		605	432	173	288	1,498

System (SAS) software version 9.3 package (SAS India Ltd., Mumbai). The apparent prevalence and true prevalence were also estimated as per the following formula [29]. (i) Apparent prevalence = number of positive animals/number of tested animals. (ii) True prevalence = [apparent prevalence + (specificity − 1)]/[(sensitivity + specificity) − 1]. True prevalence rate was calculated based on the sensitivity and specificity of the c-ELISA employed in the study, which is having high relative specificity (98.4 %) and sensitivity (92.4 %) when compared with virus neutralization assay [25].

Results

The percent positivity of PPRV antibodies in cattle, buffaloes, sheep and goats with apparent prevalence are presented in Table 2. The overall true prevalence of 24.02 % with 12.18 % in cattle, 17.83 % in buffaloes, 50.27 % in sheep and 42.43 % in goats was observed. Analysis of 1,037 serum samples from bovine indicated 13.21 % (137/1,037) prevalence with no significance difference between cattle and buffaloes. Similarly, analysis of 461 serum samples from small ruminants indicated 41.21 % (190/461) prevalence with no significance difference between sheep and goats. The percentage prevalence of PPRV antibodies in bovine and small ruminants were 11.43 and 55.71; 9.60 and 25.64; 5.82; 35.29 and 36.52; 17.48 and 44.94 in Andhra Pradesh, Gujarat, Jammu and Kashmir, Maharashtra and Rajasthan, respectively. High percent prevalence (35.3 %) in cattle and buffaloes from Maharashtra was observed, where as, in Jammu and Kashmir, only 5.82 % of the cattle and buffaloes was positive for PPRV antibodies. The sheep and goats in Andhra Pradesh showed high seroprevalence of PPR followed by Rajasthan. However, there was no significant difference observed between the various states of India in the PPRV antibodies and different species of animals studied.

The PI values (range from 25 to 100) obtained in c-ELISA from tested bovine and small ruminant samples were depicted in a graph (Fig. 1) as four categories. Most of the serum samples from different species showed PI values between 60 and 80 reactivity in c-ELISA representing the weak positive level. There were 1,059 samples (495, 328, 82 and 154 samples from cattle, buffaloes, sheep and goats, respectively) fall in the range of −25 to +25 PI. In the distribution of the PPRV-specific antibodies in positive cattle and buffaloes, 44 base level positive samples were having the PI range from 40 to 60, 32 weak positive samples with PI values of 60–80 and more number of positive cases ($n = 61$) fall between 80 and 100 PI values, which indicates the prevalence of PPRV antibodies in bovines at positive level.

Discussion

Measurement of the PPRV antibodies in livestock in different geographical area of the country with varying agro-climatic conditions may be helpful in knowing the prevalence status under natural situation and inturn help in disease control strategies for implementation of vaccination programme. Organized serological investigation or survey using random samples has not been conducted against PPR in India in different livestock species. The present study has provided baseline information on prevalence of PPRV antibodies in bovine and small ruminants population in situations where the sub-clinical, non-lethal, inapparent or recovered infection status was suspected in different districts in various states of India.

In this study, the prevalence rate of 13.21 % was observed in bovines on screening of 1,037 serum samples

Table 2 Prevalence of PPRV antibodies in cattle, buffaloes, sheep and goats during 2011 survey in India

State/species	Cattle		Buffaloes		Sheep		Goats		Total	
	P	PP	P	PP	P	PP	P	PP	P	PP
Andhra Pradesh	8	11.43	8	11.43	43	61.43	35	50.00	94	33.57
Gujarat	8	8.89	9	10.34	13	35.14	17	21.25	47	15.99
Jammu and Kashmir	17	5.69	9	6.08	–	–	–	–	26	5.82
Maharashtra	31	32.63	29	38.67	10	25.00	32	42.67	102	35.79
Rajasthan	3	5.88	15	28.85	13	50.00	27	42.86	58	30.21
Total	67	11.07	70	16.20	79	45.66	111	38.54	327	21.83
Mean ± SE*	11.07 ± 5.03		16.20 ± 6.25		45.66 ± 10.58		38.54 ± 9.18		21.83 ± 5.75	
Confidence interval at 95 % level	1.90–20.94		3.96–28.44		24.92–66.39		20.53–56.54		10.56–33.10	

P number of samples positive, *PP* percent positive

* Standard error was calculated by dividing standard deviation by square root of total number of samples

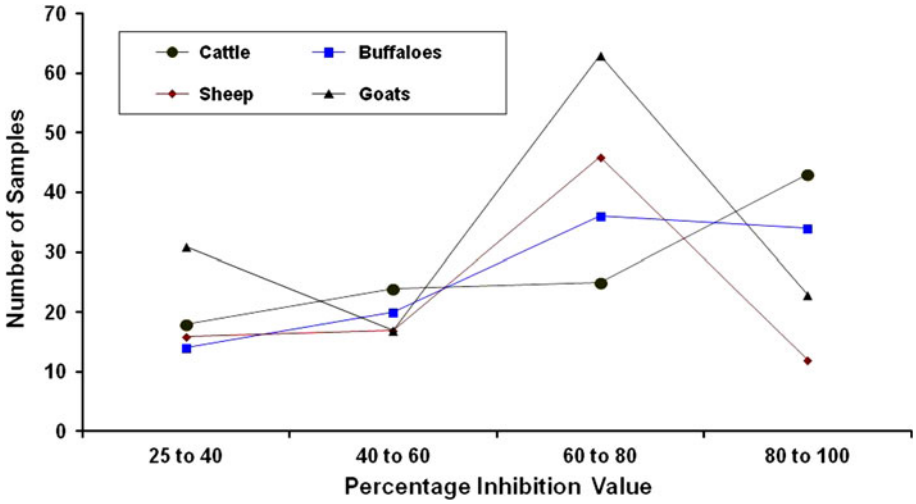

Fig. 1 Distribution of the PPRV-specific antibodies in cattle, buffaloes, sheep and goats

for PPRV antibodies. Different prevalence rates of PPRV antibodies in cattle and buffaloes have been reported from various regions and countries [5, 20, 31]. In Ethiopia, PPRV antibodies were reported in 9 % of cattle when analysed with 910 samples [1]. Similarly, few seropositive cases of cattle have also been reported from Kazakhstan [17]. In addition, a high seroprevalence rate of 67.42 % in buffalo and 41.86 % in cattle with a significant difference was reported from Pakistan [14]. In our earlier study, Balamurugan et al. [5] reported an overall 4.58 % prevalence of PPRV antibodies in cattle and buffaloes on analysis of 2,159 bovine serum samples collected from selected different farms in Southern Peninsular of India. The increase of positive samples is important for which actual reason could not be given. However, high prevalence in bovine may be due to the random samples collected from the villages, where cattle, buffaloes, sheep and goats were reared together (can be called as one epidemiological unit). The presence of PPRV antibodies in bovines indicating that the population was exposed to PPRV infection in naturally either directly or indirectly. Further, this indicates possible serological evidence for the natural transmission of PPRV from small ruminants to bovine and subsequent virus adaption without showing clinical signs under natural conditions, which may lead to a change of virulence of the strain circulating in that particular geographical area as reported earlier [1, 5, 14]. It implies the importance of bovines as sub-clinical hosts for the virus besides widespread presence of the disease in sheep and goats.

High percent prevalence in cattle and buffaloes in Maharashtra may be due to the coexistence of infected sheep and goat population with bovines as reported earlier that PPRV infection needs close contact between infected and susceptible animals to spread [4]. Where as, low prevalence in Jammu and Kashmir may be due to the rearing of bovine in isolation rather than with small ruminants in farming system or topology of region, where restricted movement of animals with reduced transmission of the virus between animals. These factors may account

for the low PPRV antibodies prevalence and also concurrence with previous reports in sheep and goats [24]. However, transboundary migration of animals has to be monitored for proper management of disease especially in the border states of India.

The prevalence rate of 41.21 % was observed in small ruminants on screening of 461 serum samples. The prevalence rates are in concurrence with reports from other countries too namely, in Africa, PPRV antibodies were reported in 55 % of Nigerian sheep and goats [16] and 46.5 % of sheep and goats from the Cameroon [9]. Seroprevalence study of PPR in sheep and goats was also reported from the Sudan, Saudi Arabia and Ethiopia [1, 19]. Recently significant higher seropositive cases in goats than in sheep (49.5 vs. 39.8 %) with the overall seroprevalence of 45.8 % have been reported from Tanzania [28]. Earlier studies in India, Singh et al. [24], have recorded an average prevalence of 33 % in Indian states with higher prevalence in sheep (36.3 %) than in goats (32.4 %) while studying the countrywide prevalence using random/suspected serum sample mostly from northern India. In contrast to this, Raghavendra et al. [20] reported a higher prevalence of antibodies in sheep (41.35 %) than in goats (34.91 %) while screening the random samples collected from field with opinion that prevalence rate comparable to the population in southern states. The susceptibility of PPR varies with the breeds of the sheep and goats, which also play an important role in epidemiology of PPR. In this study, susceptibility of sheep and goat breeds could not be assessed due to most of the samples obtained from the local non-descriptive animals in the villages from different geographical locations.

High percent positivity in Andhra Pradesh could be correlated with the population of sheep and the circulation of sheep adapted virus in that region of intensive husbandry practices. Further, this could be attributed to the greater recovery rate (lower case fatality rate) rather than increased susceptibility of sheep to PPRV and retention of sheep for meat and wool purposes for larger duration in comparison to goats in India as reported earlier [24]. This may also be due to vaccination of sheep and goats under the National Control Programme on PPR (NCPPPR) implemented during 2011. The regional difference in the prevalence of antibodies based on the relative population has also been reported earlier [24]. Chavan et al. [8] also reported an overall seroprevalence of disease in goats was 46.01 % with ranges from 42.30 to 52.94 at different places in Parbhani region of Maharashtra. Hinshu et al. [13] also reported 53.6 % prevalence in sheep besides some goats and buffaloes had shown PPRV specific antibody while analysing the samples from Rajkot district of Gujarat during 1996. In an another study from Kerala, 45.8 % of sheep, and 0.93 % of goats tested positive to PPRV antibodies with no positivity in cattle. These animals were mostly in the migration from the neighbouring states of Tamil Nadu and Karnataka as reported earlier [27]. These variations in seroprevalence could be due to differences in sample size, age, prevailing management practices, humidity and season as reported earlier by other researchers.

As there was no specific cutoff value adapted in the c-ELISA kit based on the negative and positive distribution of PPRV antibodies in the bovine population, the same cutoff value determined for sheep and goats was used as per earlier study [5]. However, the samples having the PI value between 25 and 40 may not be the true negative, as the standard cutoff value in the negative control panel lies between -25 and $+25$ (as per OIE or internationally reputed kits), and hence, these samples may be described as suspicious cases [$n = 77$-cattle (43) and buffaloes (34)] at this stage. In general, the percent positivity of the antibodies indicates enzooticity of the disease in the country, which is attributed to variations in the husbandry practices within different geographical regions, the agro-climatic conditions, the topography of different states, the socio-economic status of individual farmers and the migration of small ruminants.

This study also indicated an extensive endemicity of the disease in various states of India. Although, a high proportion of cattle and buffaloes have antibodies to PPRV, implications of these antibodies are still unknown at this stage. However, these results suggested natural transmission of PPRV among cattle, buffaloes, sheep and goats under field situation in village. The prevalence of PPRV antibodies apparently healthy animals showed 21.83 % of the animals are protected form re-infection of PPRV. However, NCPPPR implemented during 2011 by Department of Animal Husbandry, Dairying & Fisheries (DADF), Ministry of Agriculture, Government of India, will drastically change the epidemiology of PPR in India. Hence, the change in virus virulence i.e., virus adaption in the bovines will also help to increase the recovery rates of the small ruminants, which in turn restrict the spread of disease in that particular area. These aspects needs further, confirmative study, based on the survival of the virus, host susceptibility, genetic mutation of the organism or change in the virulence of organism, change in the disease pattern or change in the exhibiting the prominent clinical signs etc., Further, the role of PPR in the control of rinderpest (RP) might have also helped to eradicate the disease due to the possibility of seroconversion of PPRV antibodies in cattle. It is hoped that PPR is the next animal disease in the direction of RP will be eradicated in India within a decade or more. This goal is expected to be achieved by identifying areas of infection through comprehensive surveillance and then implementing intensive vaccination campaigns in those areas.

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