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Seroprevalence of PPR Virus in Pre- and Post-Vaccinated Sheep and Goats of Saurashtra Region of Gujarat

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A B S T R A C T

The seroprevalence study of peste des petits ruminants (PPR) in sheep and goats was carried out in 5 districts of Saurashtra region of Gujarat state. A total of 828 (prevaccinated) and 423 (postvaccinated) serum samples from sheep and goats were screened for PPR specific antibodies using PPR specific c-ELISA. Overall seroprevalence of PPR was found to be 53.86 (446/828) and 72.34 (306/423) % in pre- and post-vaccinated small ruminants, respectively. Overall pre-/post-vaccinated seroprevalance rates were highest in Bhavnagar district (83.89/96.30 %), followed by Surendranagar (73.61/77.78 %), Amreli (56.25/83.33 %), Jamnagar (32.22/43.33 %) and Rajkot (27.78/64.44 %) districts. Pre and post vaccination seropositivity was noted higher in goats (59.90/78.75 %) than in sheep (35.75/52.43 %). Males in both the species (goats and sheep) showed higher seroprevalence (60.11/89.01 %) as compared to their female counterparts (52.09/67.77 %). Higher prevaccinated seroprevalence was recorded among age group of 1 to 2 years (63.41 %), followed by below 1 year age group (53.99%) and above 2 years of age group (44.20%).

Introduction

As per 20th livestock census, population of goats and sheep in Gujarat are 4.96 million and 1.71 million, respectively. Peste des petits ruminants (PPR) is a highly contagious, acute, febrile viral disease in goats and sheep, characterized by fever, anorexia, depression, nasal discharge, ocular discharge, anorexia, abortion, erosion on nasal mucosa, stomatitis, coughing and depression. Some of the states in India practiced focused vaccination (within a radius of 3-10 km) in the outbreaks situation for the control of the PPR since 2002 (Singh *et al.*, 2009). However, the strategic mass vaccination program (entire small ruminants population >4 months of age and subsequent biannual/annual

vaccination of young and unvaccinated population) was implemented in some of the states since 2014 through the national control program on PPR (PPR-CP) for the control and eradication of the disease even before the global framework was planned (Balamurugan *et al.,* 2016). Nevertheless, neither a surveillance plan nor systematic post-vaccination monitoring and/or evaluation was initiated to assess the effectiveness of the vaccination and its strategies. Several PPR outbreaks go unrecorded due to under-reporting or non-reporting due to poor surveillance system in India. Further, outbreaks are being reported regularly in some of the states despite focus vaccination and a few sporadic outbreaks were also reported in mass vaccination program implemented states. Moreover, studying the prevalence and generating evidence of the level of PPRV antibodies in the target population is paramount importance to formulate and implement a proper strategic disease control vaccination program in a particular geographical area with a long-term plan to eradicate PPR by 2030.

The disease is most effectively controlled by vaccinating sheep and goats with live attenuated vaccines that provide lifelong immunity. However, the current vaccines and serological tests are unable to differentiate between naturally infected and vaccinated animals (DIVA). This factor precludes meaningful assessment of vaccine coverage and epidemiological surveillance based on serology, in turn reducing the efficiency of control programs. Considering these facts and importance of disease in economics of sheep and goat farming, the present research was undertaken to study the seroepidemiology of PPR Virus in pre- and post-vaccinated sheep and goats of Saurashtra region of Gujarat by competitive ELISA''.

Materials and Methods

The serum samples were collected from 828 pre-vaccinated animals (goats 621 and sheep 207) and 423 post-vaccinated animals (goats 320 and sheep 103) from 5 districts (Jamnagar, Rajkot, Surendranagar, Amreli and Bhavnagar) of Saurashtra region of Gujarat. At the time of serum sample collection, anamnesis details (like species, breeds, age, sex etc.) were recorded along with clinical findings, if any (Fig. 1). The samples were subjected to competitive ELISA (C-ELISA) using c-ELISA kit for PPR antibodies developed by IVRI, Mukteswar. The wells were coated with purified recombinant PPR nucleoprotein (NP) antigen. Anti-NP monoclonal antibodies raised against an attenuated PPR virus were used as competitive antibodies. The test protocol was used as per the instructions of manufacturer.

The plate (Fig. 2) was read at 492 nm in ELISA reader (Multiskan plus, LabSystem) using EDI software (approved by OIE/IAEA). The mean OD values of well showing 50 % inhibition were considered as positive for PPR antibodies as per the formula provided by the manufacturer. The test is rapid, simple and has a high sensitivity (Singh *et al.,* 2004).

The data was analyzed by standard statistical procedures. Chi-square test was used according to WEB AGRI STAT PACKAGE software developed by Ashok Kumar, ICAR research complex, Goa and Statistical Packages for social Science (SPSS) software (version 17).

Results and Discussion

Overall Seroprevalence Pre-Vaccination

The findings on district, species, age and sex wise seroprevalence of PPR in pre-vaccinated small ruminants overall, and in sheep and goats separately are presented in Table 1 and 2, respectively.

Out of 828 prevaccinated sera samples tested for PPR in the goats and sheep of the five districts of Saurashtra region of Gujarat under study, 446 (53.86 %) were found positive for PPR antibodies. The sero-positivity was noted as 59.90% (372/621) in goats and 35.75 % (74/207) in sheep. It was significantly higher in goats than the sheep. Our finding corroborated with the report of Chauhan *et*

al., (2012), who found 51.86 % (503/970) seroprevalence of PPR in sheep and goats maintained under different management conditions. Chauhan *et al.,* (2011) found seropositivity level in Saurashtra as 43.08%, and it was higher (112/176, 63.64 %) in migratory/village flocks of sheep and goats of Kutchh region of Gujarat. Chandrahas *et al.,* (2011) recorded overall seroprevalence of PPRV infection in sheep and goats of North Gujarat as 46.26%, while Tiwari *et al.,* (2004) and Ranaware *et al.,* (2013) recorded lower seropositivity of 33.33% in South Gujarat and 37.09 % in Gujarat state, respectively. Similarly, Thakor *et al.,* (2016) and Patel *et al.,* (2017) recorded 25.70% and 47.14% overall seroprevalence in goats of South Gujarat.

Even at national level seroprevalance was found lower than our results. Balamurugan *et al.,* (2011) found overall prevalence of 43.56 % in different states of India between 2003 and 2009. Reports from different countries also indicate higher seroprevalecne in their region 47.5 (114/240) and 35.23 (2500/7096) % overall seroprevalence was observed in Ethiopia and Pakistan, respectively, by Afera *et al.,* (2014) and Nizamani *et al.,* (2015).

District-wise Seroprevalence

In district-wise study, we found highly significant difference (P<0.00001), the overall seroprevalence was found higher in Bhavnagar (83.89%) and Surendranagar (73.61%), lower in Rajkot (27.78%) and Jamnagar (32.22%) districts, whereas almost average percent positivity was observed from Amreli district (56.25%). Very similar trend was also observed for sheep and goats separately, with lower prevalence in sheep than goats in all five districts (Table 1, 2, Fig. 3).

The percent positivity of Rajkot district was in accordance with Ranaware *et al.,* (2013) and that of Amreli district in accordance with Chandrahas *et al.,* (2011). The reason of higher seroprevalance in Bhavnagar and Surendranagar districts may be explained on the basis of its location as small ruminants migration tract, as substantiated by Chandrahas *et al.,* (2011) in north Gujarat also. Karelewad *et al.,* (2007) and Devi *et al.,* (2016) recorded overall prevalence of 58.78% (435/740) in Latur District of Maharasthra and 28.67% (166/579) in different parts of the Assam, respectively. In Pakistan, Khan *et al.,* (2007) and Zahur *et al.,* (2011) found 43.33 % prevalence in 24 districts of Punjab and 45.5% from different villages of 27 randomly selected districts.

Species-wise Seroprevalence

On the basis of species, sero-positivity was noted as 59.90% (372/621) and 35.75 % (74/207) in goats and sheep, respectively, with an extreme statistically significant difference (P<0.0001) between species, the PPRV infection being more prevalent in goats (Table 1, 2). The results concurred with the findings of earlier workers (Tiwari *et al.,* 2004; Zahur *et al.,* 2011; Balamurugan *et al.,* 2011; Rahman *et al.,* 2017; Sakhare, 2019). In general goats are more susceptible than sheep, therefore have a higher probability of developing PPRV antibodies (Kumar *et al.,* 2014). However, reports of Singh *et al.,* (2004) and Chandrahas *et al.,* (2011) from India, Khan *et al.,* (2007) and Nizamani *et al.,* (2015) from Pakistan have showed higher seroprevalence in sheep than goats.

Sex-wise Seroprevalence

The sex-wise seroprevalence of PPR observed in small ruminants was non-significantly higher (60.11%, 110/183) in male than that in female (52.09%, 336/645). Very similar trend was observed within sheep and goats also (Table 1, 2). The higher prevalence in males may be because the outbreaks were observed during the festive season during which demands for male was more, particularly bucks. This was in agreement with the findings of Thakor *et al.,* (2016) and Sakhare (2019) from Gujarat. Karelewad *et al.,* (2007) also recorded higher prevalence in male (73.28%) as compared to female (55.22%) goats in Maharasthra, and similarly Mahajan *et al.,* (2012) found significantly higher seroprevalence in males (33.33%) than the females

(24.53%) in J & K. On the contrary, Patel *et al.,* (2017) observed higher seroprevalence of PPR in female than male in South Gujarat. Nizamani *et al.,* (2015) and Rahman *et al.,* (2017) also observed higher seroprevalence of PPR in female than male.

The reports published in other countries by Swai *et al.,* (2009) showed males as a most susceptible gender to PPRV infection. The true host and pathogen basis of difference could not be found but fact can be attributed to that the high demands of male animals for meat purpose driven them to the market and contribute to the higher infection rate than in females which are relatively maintained at home for breeding purpose.

Age-wise Seroprevalence

Age-wise significantly (P<0.05) higher seroprevalence was observed among small ruminants in 1 to 2 years age group (63.41%) followed by <1 year of age group (53.99 %), while it was lowest (44.20%) in age group >2 years. In sheep and goats also, a very similar trend was observed for age-wise prevalence of PPR (Table 1, 2). These findings were in agreement with previous reports from Gujarat and J&K by Chauhan *et al.,* (2012) and Mahajan *et al.,* (2012). The former recorded Maximum seroprevalence of 55.65 per cent in the age group of > 3 years, followed by 2 to 3 years (52.00 %), 1 to 2 years (31.91 %) and < 1 year (27.87%) in goats, and later author noted significantly ($p < 0.05$) higher seroprevalence in >12 months age group (39.58%) followed by 8–12 months (26.38%) and 4–8 months (20.83%) age group. The factual explanation of the present findings has been justified by the fact that sheep and goats exposed to natural infection to PPRV at a very young age may carry antibodies for 1-2 year following exposure and might be carried for life long time (Singh *et al.,* 2004). Singh *et al.,* (2004) investigated 33 % overall antibody prevalence of PPRV in sheep and goats in age >6 months, while Karelewad *et al.,* (2007) found highest prevalence (83.03%) 0n age group 4-12 months followed by 0-3 months (62.99%) and >12 months (48.66%) of age. However, Begum *et al.,* (2016) and Devi *et al.,* (2016) reported higher seroprevalence rate in above 6 months of age than below 6 months. In other countries, Nizamani *et al.,* (2015) and Rahman *et al.,* (2017) reported higher seroprevalence in adult than young animals. Contrary to this, Afera *et al.,* (2014) reported higher seroprevalence in younger than the adults.

Seroprevalence Post-Vaccination

In the present study, overall out of 423 sera samples from post-vaccinated sheep and goats of Saurashtra region tested using PPR competitive ELISA, 306 (72.34%) were found positive for PPR antibodies. Among the species, post-vaccination seropositivity noted was 78.75% (252/320) in goats and 52.43% (54/103) in sheep. It was significantly (P<0.0001) higher in goats than the sheep (Table 3).

For the district and sex wise post-vaccination seroprevalence studied, we found statistically significant difference between district (P<0.0001) and sex (P<0.05). Among small ruminants, the highest district wise overall post-vaccination seroprevalence was recorded in Bhavnagar (96.30%) followed by Amreli (83.33%), Surendranagar (77.78%), Rajkot (64.44%) and Jamnagar (43.33%) districts (Fig. 3). The male small ruminants showed numerically higher seroprevalence (89.01%) as compared to females (67.77%). Within goats and sheep also almost same trend was noted for district-wise prevalence of PPR (Table 3).

Table.1 Overall seroprevalence of PPR in pre-vaccinated small ruminants using c-ELISA

Factor	Attributes	No. of tested	No. of positive	% positive
Region	Saurashtra	**828**	**446**	**53.86**
Districts	Jamnagar	180	58	32.22
	Rajkot	180	50	27.78
	Surendranagar	144	106	73.61
	Amreli	144	81	56.25
	Bhavnagar	180	151	83.89
	χ^2= 171.439** (P<0.00001)			
Species	Goat	621	372	59.90
	Sheep	207	74	35.75
	χ^2= 36.445** (P<0.00001)			
Sex	Male	183	110	60.11
	Female	645	336	52.09
	χ^2= 3.687 (P>0.05)			
Age	<1 year	276	149	53.99
	1-2 years	276	175	63.41
	>2 years	276	122	44.20
	χ^2= 20.479* (P<0.05)			

Table.2 Seroprevalence of PPR in pre-vaccinated goats and sheep using c-ELISA

Factor	Attributes	Goats			Sheep		
		No. of tested	No. of positive	% positive	No. of tested	No. of positive	% positive
Region	Saurashtra	**621**	**372**	**59.90**	**207**	**74**	**35.75**
Districts	Jamnagar	135	48	35.56	45	10	22.22
	Rajkot	135	41	30.37	45	9	20.00
	Surendranagar	108	89	82.41	36	17	47.22
	Amreli	108	71	65.74	36	10	27.77
	Bhavnagar	135	123	91.11	45	28	62.22
		χ^2= 161.384** (P<0.00001)			χ^2 = 25.234* (P<0.05)		
Sex	Male	139	92	66.19	44	18	40.91
	Female	482	280	58.09	163	56	34.36
		χ^2= 2.944 (P>0.05)			χ^2= 0.648 (P>0.05)		
Age	<1 year	207	127	61.35	69	22	31.88
	1-2 years	207	144	69.57	69	31	44.93
	>2 years	207	101	48.79	69	21	30.43
		χ^2= 18.866* (P<0.05)			χ^2 = 3.828 (P>0.05)		

Table.3 Seroprevalence of PPR in post-vaccinated goats and sheep using cELISA

Factors	Goat		Sheep		Total	
District	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**
Jamnagar	69	32 (46.38)	21	7 (33.33)	90	39 (43.33)
Rajkot	69	50 (72.46)	21	8 (38.10)	90	58 (64.44)
Surendranagar	56	49 (87.50)	16	7 (43.75)	72	56 (77.78)
Amreli	69	66 (95.65)	21	9 (42.86)	90	75 (83.33)
Bhavnagar	57	55 (96.49)	24	23 (95.83)	81	78 (96.30)
	χ^2= 69.905**(P<0.0001)		χ2=24.183* (P<0.05)		χ2=70.382**(P<0.0001)	
Sex						
Male	69	62 (89.86)	22	19 (86.36)	91	81 (89.01)
Female	251	190 (75.70)	81	35 (43.21)	332	225 (67.77)
Total	**320**	**252 (78.75)**	**103**	**54 (52.43)**	**423**	**306 (72.34)**
	χ^2= 6.482*(P<0.05)		χ^2= 12.917*(P<0.05)		χ^2= 16.103*(P<0.05)	

Note: Figures in parentheses indicate percentage.

Fig.1 Nasal discharge in goats and sheep affected with PPR

Fig.2 C-ELISA plates pre- and post-vaccination of PPR in small ruminants

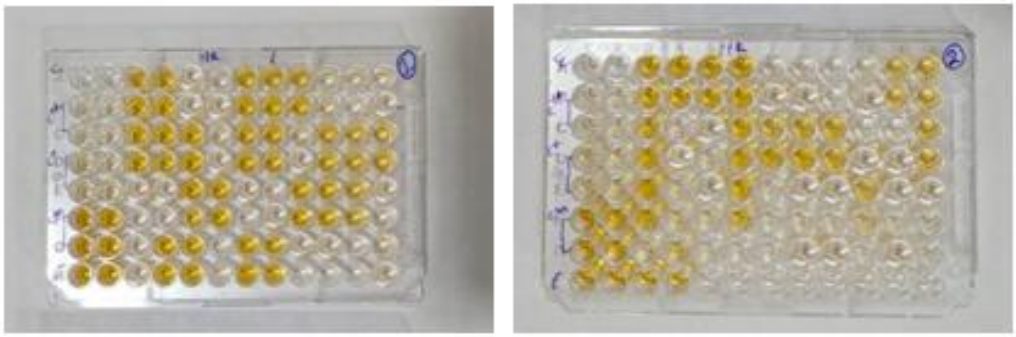
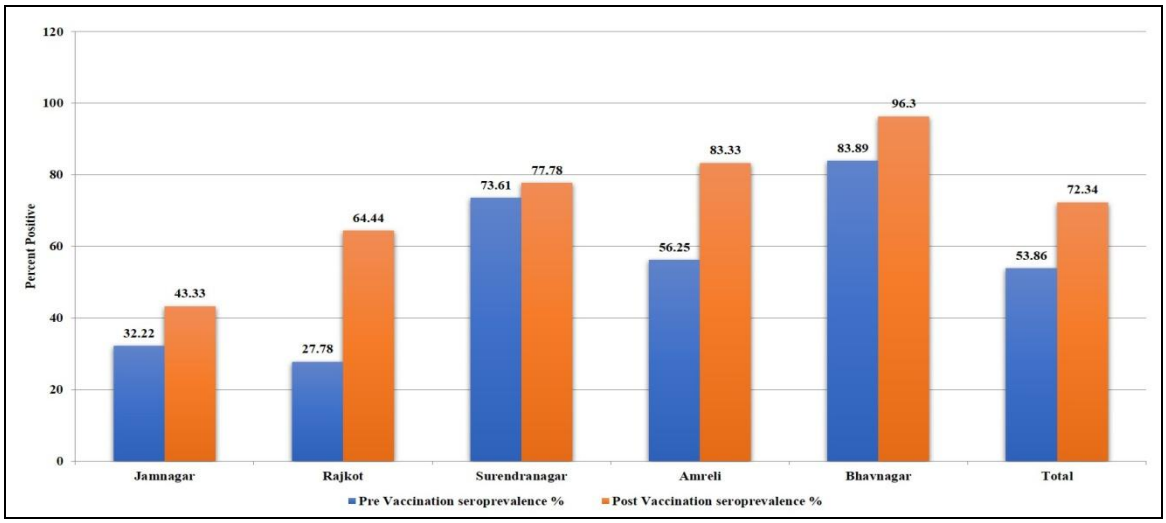


Fig.3 Pre- and post vaccination seroprevalence of PPR antibodies in small ruminants of different districts of Saurashtra region



Similar to our result Balamurugan *et al.,* (2020) observed overall post-vaccination seroprevalence of antibodies as 65.39% with higher seroprevalence of 79.39% (104/131) in sheep than 59.29% (166/280) in goats of Uttar Pradesh. It indicated mass vaccination was not practiced in all districts of UP as per strategic plan. While lower than our result was recorded by Lakeshar (2017), who found that 42 % of vaccinated small ruminant population were protected against PPRV with highest post-vaccination seroprevalence of PPR in Chittorgarh (55 %) and lowest in Banswara (12%) district of Rajasthan with an overall seroprevalence of PPR virus antibodies in vaccinated goat and sheep as 58.13% and 20.00%, respectively. Balamurugan *et al.,* (2020) observed overall seeroprevalence of antibodies in 55.22 % animals, with seroprevalence of 57.37% (179/314) in sheep and 54.03% (302/559) in goat of Punjab state which could be due to mass vaccination of small ruminants.

Seronegative titre of vaccinated sheep and goats may indicate improper competence due to malnutrition, heavy parasitic overload, bad management, overcrowding, nomadism and improper vaccination or not follow proper cold chain (Balamurgan *et al.,* 2014). Seropositivity in apparently healthy vaccinated flock is a caveat to initiate comprehensive steps to contain identification of vulnerable animals through sero-surveillance and vaccination can be focused in sensitive area to block epidemic cycle of virus (Singh *et al.,* 2009).

In spite of regular vaccination being done in small ruminant population, outbreaks of PPR have been reported. This may be due to intermixing of vaccinated and unvaccinated animals and incomplete coverage of population for vaccination (Lakeshar, 2017). Although there are few reports about seroprevalence of PPR antibodies in different areas of Saurashtra region of Gujarat state, clinical finding of this study confirmed the circulation of PPR virus among population of sheep & goats in the study area, which should be kept in mind while deciding vaccination strategy for control of disease. This study showed varying antibody level in affected districts reflecting infection and vaccination profile of herds.

The study alarmed of significant virus activity in Saurashatra region of Gujarat as determined by seroprevalence rate of 53.86% (446/828) of PPR in pre-vaccinated and 72.34% (306/423) in post-vaccinated flocks of sheep and goats. The pre- and post-vaccination prevalence was significantly higher in goats than sheep, and in males as well as 1-2 years of age group over young and older age groups of both the species. The district wise difference was

also highly significant, Bhavnagar and Surendranagar having highest prevalence and Amreli the lowest. Further studies are needed to provide more insights on the epidemiology of PPR and phylogenetic characterization of circulating viruses in an attempt to determine origin, spread and distribution of various virus lineages and risk factors in Saurashtra region of Gujarat State of India.

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Conflict of interests

The authors declare that they have no competing interest.

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