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Incidence of Peste Des Petits Ruminants Virus infection in Small Ruminants of Saurashtra Region of Gujarat State

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ABSTRACT

A total of 119 different clinical samples (nasal swab, conjunctival swabs, oral swabs and tissue samples) from sheep and goats were collected from the area under study for detection of PPR antigen by Sandwich-ELISA. Out of 119 clinical samples, 37 samples were found positive in small ruminants by S-ELISA, giving an overall incidence rate of 31.09 % (37/119). In sheep 33.33% and goats 30.52 % samples were detected positive. District wise incidence of PPRV in small ruminants differed non significantly. It was recorded in Bhavnagar (33.90%), Amreli (29.41%) and Rajkot (26.92%) districts. Month wise incidence of PPRV in small ruminants differed non significantly. It was recorded in month of October (29.41%), November (31.58%) and December (33.33%). Age wise incidence of PPRV in small ruminants differed non significantly. It was recorded in below 1 year of age group (39.29%), 1 to 2 year (28.95%) and above 2 years of age (16.00%). Sex wise incidence of PPRV in small ruminants differed non significantly. It was recorded in male (30.43%) and female (31.51%). Breed wise incidence of PPRV in small ruminants differed non significantly. It was recorded (36.62%) in nondescript breed and (22.92%) in descript breed. Out of 119 clinical samples, 37 samples including 13 Nasal swabs, 3 conjunctival swabs, 7 oral swabs and 14 tissue were found positive. PPRV antigen was detected by S-ELISA in tissue (66.67%), oral swab (43.75%), nasal swab (20.97%) and conjunctival swab (15.00%). Most suitable sample for virus isolation was tissue and oral sample.

Keywords

Peste Des Petits Ruminants (PPR), Sandwich-ELISA (S-ELISA), Small Ruminants

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Introduction

At present, PPR is enzootic in India and out break occur regularly among small ruminants throughout the country and the overall

prevalence of PPRV was reported within the range of 20-60% (Singh *et al.*, 2004). The reports documented the presence of PPRV in Rajasthan in the north (Kataria *et al.*, 2007) and Maharashtra in the west (Santhosh *et al.*,

2009) and across the southern peninsula (Raghavendra *et al.*, 2008). But, presence of PPR was studied and confirmed from Gujarat by Hinsu *et al.*, (2001) and later on by other workers (Kanani *et al.*, 2006; Chauhan *et al.*, 2009; Sharma *et al.*, 2015; Patel *et al.*, 2017; Sakhare, 2019).

Conventional serological tests like Agar Gel Immunodiffusion Test (AGID), Counter Immuno Electrophoresis test (CIEP) etc. often fail to differentiate PPRV and RPV infections. The techniques available to differentiate PPR from RP are virus neutralization test (VNT), Complementary DNA (cDNA) probes (Diallo *et al.*, 1989), Virus specific monoclonal antibodies in an immunocapture enzyme linked immunosorbent assay (ELISA) (Libeau *et al.*, 1995) and haemagglutination using piglet or chicken red blood cells (Shaila *et al.*, 1996).

The lower relative sensitivity observed in RT-PCR might be attributed to nature of PPRV genome which shows higher rate of transcription of N gene. Thus, the abundance of nucleoprotein, which is targeted in sandwich-ELISA, may result in relatively higher sensitivity of the ELISA as compared to F gene and N gene RT-PCR. (George, 2002). Moreover, studying the prevalence 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.

Considering the above facts and importance of disease on economics of sheep and goat farming, a present research study "Detection of Peste Des Petits Ruminants Virus in Sheep and Goats of Saurashtra region of Gujarat by sandwich -ELISA" was undertaken with overall, location wise, species wise, sex wise and age wise incidence and sample wise positivity of PPRV.

Materials and Methods

Samples Collection

A total of 119 samples (goats -95 and sheep -24) including swabs (nasal, oral and conjunctival swabs) and tissue (lungs, Intestine, spleen, etc.) samples were collected from clinically ailing animals showing symptoms suggestive of PPR in viral transport medium (VTM) i.e. Dulbecco's Modified Eagle's Medium (DMEM) from different districts of Saurashtra region of Gujarat (Table 1 and Fig. 1&2).

Sandwich-ELISA Kits

PPR Sandwich-ELISA kit for PPRV antigen detection along with the user manual was obtained from Division of Virology, IVRI, Mukteswar and supplied by Animal Disease Investigating Laboratory, Ahmedabad.

Test Protocol

Dispensed 100 µl of diluted capture antibody (1:4000) in all the wells of ELISA module supplied with the kit. Covered the plate with a lid and incubated for one hour at 37°C in an ordinary incubator with continuous shaking on orbital shaker.

At the end of incubation period, contents of the plate were discarded by inverting the plate over sink and jerking it down with a single motion of hand. The plate was washed three times with wash buffer (PBS diluted four times with distilled water containing 0.05% Tween-20) and dried by gently tapping over filter paper.

After washing, following reagents were added very carefully step by step:

50 µl of blocking buffer in all the wells.

50 µl of additional blocking buffer to antigen blank (B) wells (A1-H1).

50 µl of clinical samples in vertical duplicates as per the template provided with the kit (A3/B3, C3/D3 and so on).

50 µl of positive reference (C+) antigen in four designated wells (A2/B2, C2/D2).

50 µl of negative reference (C-) antigen in four designated wells (E2/F2, G2/H2).

Contents of the well were mixed by gently tapping the sides of the plate. The plate was covered with a lid and incubated at 37 °C for one hour on an orbital shaker with continuous shaking at moderate speed.

At end of incubation repeated the discard and washing procedures as in step

Diluted detection antibody (100 µl) was added in all the wells using multichannel pipette. The plate was covered with a lid and incubated at 37°C for one hour on an orbital shaker with continuous shaking at moderate speed.

At the end of incubation, the plate was taken out of the incubator and discard and washing was performed as described in step b).

Diluted (1:1000) anti-mouse conjugate (100 µl) was added in all the wells. The plate was covered with a lid and incubated at 37°C for one hour on an orbital shaker with continuous shaking at moderate speed.

At the end of incubation, the plate was taken out of the incubator and discard and washing was performed as described in step b).

A freshly prepared OPD substrate solution (100 µl) was added in each well and the plate was incubated for 10 to 20 min at 37°C without shaking or till the colour developed in positive reference (C+) wells.

Stopping solution (100 µl) was added to all the wells and the plate was gently tapped to mix

the fluids. The plate was read in an ELISA plate reader (Multiskan Plus, Lab System) at 492 nm.

Interpretation of test results

Cutoff

For calculation of cutoff point, four antigen blank wells (B) having extreme OD values (two wells of lowest OD values and two wells of highest values) were excluded. The remaining four wells having intermediate OD values were considered. Cut off was taken as two times the mean OD of these intermediate wells. Samples having more OD than the cut off were taken as positive, while samples having less OD than the cut off were taken as negative. Further, a sample positive in both the duplicate wells was taken as positive. A sample positive in one well and negative in other duplicate well was retested before recording the results.

Results and Discussion

A total of 119 different clinical samples (nasal swabs, conjunctival swabs, oral swabs and tissue samples) from goats and sheep were collected from Jamnagar, Rajkot, Amreli, Surendranagar and Bhavnagar districts of Saurashtra region of Gujarat for detection of PPR antigen by Sandwich ELISA. The district-wise, month-wise, age wise and sex wise details are depicted in Table2.

Overall incidence

Clinical samples from goat and sheep were available from 3 districts of Saurashtra like Rajkot, Amreli and Bhavnagar. Goat clinical sample number (95) were from Bhavnagar (45), Amreli (28) and Rajkot (22) districts. Whereas, sheep clinical samples, which were relatively a less number (24) were only from Bhavnagar (14), Amreli (6) and Rajkot (4)

districts (Table 1). Out of 119 clinical samples, 37 samples were found positive in small ruminants by S-ELISA, giving an overall incidence rate of 31.09 percent. In Goat, 30.52% (29/95) samples were detected positive whereas in sheep 33.33% (8/24) cases were confirmed as positive.

Our findings of goats are lower than findings of earlier reports where antigens could be detected in 66.7%, 60%, 55.55%, 54.54% and 61.32% cases by Malik *et al.*, (2011), Tiwari (2004), Mahajan *et al.*, (2013) and Sakhare (2019) respectively. Significantly higher antigen detection was reported by Nagraj (2006) and Chaudhary *et al.*, (2009) in Gujarat (India) and their values were 81% and 83.33%, respectively. In our finding low incidence rate of PPR was noted due to PPR control programme in Gujarat state by vaccination.

Species wise incidence of PPRV antigen

Species wise incidence was observed statistically non-significant. Species wise analysis of data revealed higher incidence of PPR infection in sheep than goat. In Goat 30.52% (29/95) were positive whereas in sheep 33.33% (8/24) cases were confirmed as positive by S-ELISA (Fig. 3).

Similar to our results, some reports also indicate high incidence in sheep like Nagraj (2006) found (83.33%) sheep and (80%) goats and Chaudhary (2009) recorded (100%) sheep and (92.4%) goats positive for PPRV by S-ELISA in Gujarat. In contrast to our study Tiwari (2004) found higher incidence of PPR in Goat than Sheep in Gujarat. Chauhan (2009) founded that 9.61% incidence rate of PPR in sheep at Saurashtra region (Rajkot).

In India, Mahajan *et al.*, (2013) also noted a higher incidence of PPR infection in goats than sheep. Similarly, Abubakar *et al.*, (2008)

reported that outbreaks of PPR in Pakistan were more severe in goats than in sheep.

Lefevre and Diallo, (1990) who opined that goats are severely affected while sheep generally undergo a mild form. In some outbreaks, goats are not affected, while sheep succumb with high rates of mortality and morbidity (Yesilbag *et al.*, 2005). However, reports detailing an increased susceptibility of sheep and goat population and outbreaks affecting sheep and goat populations have been equally reported (Chauhan *et al.*, 2009).

Conclusively, there is no indication of the existence of PPRV variants more adapted to one than to another small ruminant species (Diallo, 2003). Absence of disease in sheep can be explained on the basis of a particular resistance of the local species and/or a loss of virulence of the PPRV strains for sheep. However, a systemic study involving large number of samples from sheep and goats coupled with well-designed experimental study may yield a scientific clue to the species wise more or less incidence of PPR in sheep and goat.

District wise incidence of PPRV

District wise incidence of PPRV was statistically non-significant. The incidence in case of goats was higher in Bhavnagar 33.33% (15/45) followed by Amreli 28.57% (8/28) and Rajkot 27.27% (6/22). While, in case of sheep antigen was detected in Bhavnagar 35.71% (05/14) followed by Amreli 33.33% (2/6) and Rajkot 25.00% (1/4) (Table 1).

Tiwari (2004) studied variation of PPR incidences at two locations namely, Patan (55%) and Vadodara (80%) of Gujarat state. Also, Choudhary (2009) recorded highest incidence (100%) of PPR virus in both Bhavnagar and Gandhinagar districts, whereas

least incidence (60%) was reported in Rajkot district. Sakhare (2019) recorded highest incidence of PPR in goat at Navsari (66.94%) than Surat (50.00%) district.

Location wise variation in morbidity rate (29.2-80%) observed by Kulkarni, *et al.*, (1996) during outbreaks of PPR at 9 different locations in Maharashtra.

Month wise incidence of PPRV

In the present study, month wise incidence of PPRV was statistically non-significant. Percent PPR positive goat cases observed were highest in the month of November(19) followed by October(8) and December (2). Percent PPR positive sheep cases observed were highest in the month of November(5) followed by October(2) and December (1).

Overall small ruminant percent PPR positive cases observed were highest in the month of November(24) followed by October(10) and December (3)

These findings can be compared with the report of Balamurugan *et al.*, (2012) who reported the seasonal influence on PPR outbreaks in India i.e. PPR outbreaks are most frequent during cold dry months (Oct. to Dec.). Sakhare (2019) recorded that PPR outbreak in South Gujarat during August to February month.

The highest frequency of PPR outbreaks in Pakistan during the first and last quarter of the year with highest in the month of March. Additionally, PPR can also occur mostly during the cool, dry season in most endemic areas of Africa. The reason for the high incidence of PPR in these months may be the climatic factors that are favorable for the survival and spread of the virus may also contribute to the seasonal occurrence of PPR outbreaks (Abubakar *et al.*, 2017).

PPR outbreaks have been linked to the introduction of new animals into flocks since the animals are usually under stress due to traveling over long distances (Balamurugan, *et al.*, 2014). During their migration, these animals frequently infect local populations along the migration route and may be one of the reasons for the higher frequency of PPR outbreaks with increased susceptibility (Singh *et al.*, 2004). The infected animals help to maintain viral circulation throughout the year via frequent animal to animal transmission.

Confinement and restricted movement of the animals, due to rainy seasons in tropical countries, may affect the nutritional status of the animals and hence predispose them to PPRV infection (Munir *et al.*, 2015).

Age wise incidence of PPRV

In present study age wise incidence of PPRV antigen positivity was statistically non-significant among the different age groups. The viral antigen was detected in goat 38.64% (17/44) in below 1 year of age, followed by 29.03% (9/31) in 1-2 years of age and 15.00% (3/20) in above 2 years of age group. The viral antigen was detected in sheep 41.67% (5/12) in below 1 year of age, followed by 28.57% (2/7) in 1-2 years of age and 20.00% (1/5) in above 2 years of age group. The overall viral antigen was detected in small ruminant 39.29% (22/56) in below 1 year of age, followed by 28.95% (11/38) in 1-2 years of age and 16.00% (4/25) in above 2 years of age group.

The findings of present study are in the line of previous reports published by Tiwari (2004) who found out of the 25 animals, five, eight, nine and three animals belonged to age groups of 0-24, 24-48, 48-72, and >72 months, respectively, showing the respective incidence of PPRV as 100, 62.50, 44.44 and 33.33 per cent in Gujarat as per age grouping.

Mahajan *et al.*, (2013) from India recorded highest incidence of PPR (83.33%) in young sheep/goat having age of 0-6 months, followed by 6-12 months (66.66%) and lowest (31.35%) in adults having age more than 12 months in Jammu & Kashmir.

Similar to our results, highest case fatality rate was observed by Mahajan *et al.*, (2017) in goats of 3-6 months age group as compared to adults in two districts of Punjab (India) state affecting migratory flocks of goats.

Corroborating reports was also reported from other countries of higher incidence in <6 months of age and severe disease in sheep and goat by El-Rahim *et al.*, (2010) in Kalubia province of Egypt.

Sex wise incidence of PPRV antigen

In the present study incidence of PPRV viral antigen was statistically non-significant between sexes. Accordingly, 30.77% (12/39) male goats and 30.36% (17/56) female goats showed positivity against PPRV antigen. Whenever, 35.29% (6/17) female sheep and 28.57% (2/7) male sheep showed positivity against PPRV antigen. Overall, 31.51%

(23/73) female small ruminant and 30.43% (14/46) male small ruminant showed positivity against PPRV antigen.

The present study finding of high incidence rate in male corroborate with Sakhare (2019) from South Gujarat observed 69.23% male goat were positive as compare to 56.96% female goat. Opposite to our finding, Mahajan *et al.*, (2013) from India observed 80 per cent females were positive as compared to 54.54 per cent males in case of goat.

Our findings of nearby equal incidence in male & female corroborate with Afera *et al.*, (2014) who reported nearly equal percent prevalence of the disease in male and female goats which was 47.4 per cent (28/59) and 47.5 per cent (86/181), respectively in selected sites of Ethiopia. However, data from Pakistan reported no important relationship between males and females, with respect to susceptibility to gender (Kozat and Sepehrizadeh, 2017).

Male Goats are sold for meat purpose and retain female for future breeding this may be reason for low incidence in male (Mohapatra, 2015).

Table.1 District wise detection of PPR antigen by S-ELISA

Sr. No.	Place of sample collection	Species of the animals					
		Goat		Sheep		Total	
		Tested	Positive	Tested	Positive	Tested	Positive
01	Rajkot	22	6 (27.27)	4	1 (25.00)	26	7 (26.92)
02	Amreli	28	8 (28.57)	6	2 (33.33)	34	10 (29.41)
03	Bhavnagar	45	15 (33.33)	14	5 (35.71)	59	20 (33.90)
Total		95	29 (30.52)	24	8 (33.33)	119	37 (31.09)
		$\chi^2 = 0.328(P>0.05)$		$\chi^2 = 0.160(P>0.05)$		$\chi^2 = 0.473 (P>0.05)$	

Note: Figures in parentheses indicate percentage

Table.2 Months, Age and Sex wise PPR virus prevalence in goats and sheep.

Particulars	Species of the animals				Total		
	Goat		Sheep		Tested	Positive	
	Tested	Positive	Tested	Positive			
1. Months							
October	27	8 (29.63)	7	2 (28.57)	34	10 (29.41)	
November	62	19 (30.65)	14	5 (35.71)	76	24 (31.58)	
December	6	2 (33.33)	3	1 (33.33)	9	3 (33.33)	
Total	95	29 (30.52)	24	8 (33.33)	119	37 (31.09)	
		$\chi^2 = 0.033 (P>0.05)$			$\chi^2 = 0.107 (P>0.05)$		$\chi^2 = 0.074 (P>0.05)$
2. Age							
<1 Year	44	17 (38.64)	12	5 (41.67)	56	22 (39.29)	
1-2 Year	31	9 (29.03)	7	2 (28.57)	38	11 (28.95)	
>2 Year	20	3 (15.00)	5	1 (20.00)	25	4 (16.00)	
Total	95	29 (30.53)	24	8 (33.33)	119	37 (31.09)	
		$\chi^2 = 3.670 (P>0.05)$			$\chi^2 = 0.847 (P>0.05)$		$\chi^2 = 4.494 (P>0.05)$
3. Sex							
Male	39	12 (30.77)	7	2 (28.57)	46	14 (30.43)	
Female	56	17 (30.36)	17	6 (35.29)	73	23 (31.51)	
Total	95	29 (30.53)	24	8 (33.33)	119	37 (31.09)	
		$\chi^2 = 0.0018 (P>0.05)$			$\chi^2 = 0.100 (P>0.05)$		$\chi^2 = 0.015 (P>0.05)$

Note: Figures in parentheses indicate percentage.

Table.3 Breed wise incidence of PPRV antigen

Breed (Goat)	No. of tested	No. of positive	Breed (Sheep)	No. of tested	No. of positive	Total No. of tested	Total No. of positive	
Zalawadi	10	2	Marwari	3	0	13	2	
Gohilwadi	31	8	Patanwadi	4	1	35	9	
TD	41	10(24.39)	TD	7	1(14.29)	48	11(22.92)	
ND	54	19(35.19)	ND	17	7(41.18)	71	26(36.62)	
G. Total	95	29	Total	24	8	119	37	
		$\chi^2 = 1.281 (P>0.05)$			$\chi^2 = 1.613 (P>0.05)$			$\chi^2 = 2.51 (P>0.05)$

Note: Figures in parentheses indicate percentage. TD: Total Descript, ND: Non-Descript

Table.4 Sample wise PPR antigen detection by S-ELISA

Sr. No.	Type of samples	Species of the animals				Total	
		Goat		Sheep		Tested	Positive
		Tested	Positive	Tested	Positive		
01	Nasal swabs	40	7 (17.5)	22	6 (27.27)	62	13 (20.97)
02	Conjunctival swabs	20	3 (15.00)	0	0	20	3 (15.00)
03	Oral swab	16	7 (43.75)	0	0	16	7 (43.75)
04	*Tissue Table 4-A	19	12 (63.16)	2	2 (100)	21	14 (66.67)
Total		95	29 (30.53)	24	8 (33.33)	119	37 (31.09)
		$\chi^2=16.333^* (P<0.05)$				$\chi^2= 18.984^* (P<0.05)$	

Note: Figures in parentheses indicate percentage.

Table.4A Tissue wise PPR antigen detection by S-ELISA

Sr. No.	Type of tissue	Species of the animals				Total	
		Goat		Sheep		Tested	Positive
		Tested	Positive	Tested	Positive		
01	Lung	9	8 (88.89)	1	1 (100)	10	9 (90.00)
02	Trachea	4	2 (50.00)	0	0	4	2 (50.00)
03	Spleen	3	1 (33.33)	0	0	3	1 (33.33)
04	Intestine	3	1 (33.33)	1	1 (100)	4	2 (50.00)
Total		19	12 (63.16)	2	2 (100)	21	14 (66.67)
		$\chi^2=5.15 (P>0.05)$				$\chi^2=4.95 (P>0.05)$	

Note: Figures in parentheses indicate percentage.

Fig.1 Nasal Discharge from Sheep



Fig.2 Mucopurulent Nasal Discharge from Goat

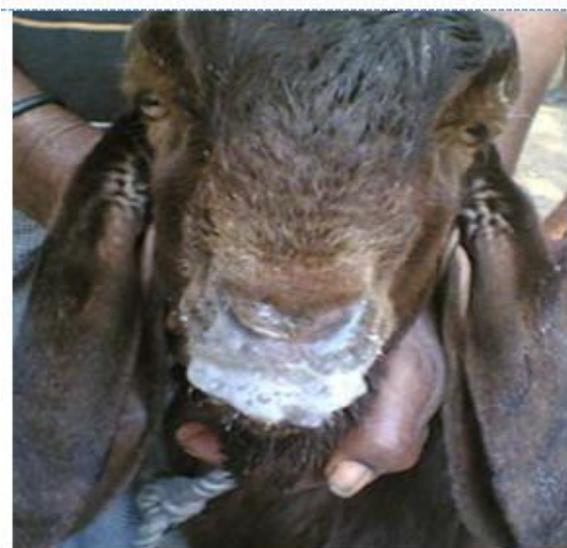


Fig.3 Sandwich ELISA for detection of PPR Antigen in sheep & Goats
(A1 to H1:- Antigen blank wells, A2 to D2:- Positive control, E2 to H2:- Negative control, A3 to D3,G3,H3,E4,F4,C5,D5,G6,H6,E7,F7,A8,B8,E9,F9,A10,B10,C11,D11,E12,F12:-Positive sample, Rest of the wells are negative sample)



Breed wise incidence of PPRV antigen

In the present study incidence of PPRV viral antigen was statistically non-significant between Descript & Non-Descript breed of small ruminant. Out of 119 clinical samples, 37 (31.09%) samples including 29 goats and 8 sheep were found positive. Out of 29 clinical positive samples of goat, 19 Non-descript breed, 8 Gohilwadi, 2 Zalawadi breed of goat was found positive. Out of 8 clinical positive samples of sheep, 7 Non-descript breed, 1 patanwadi breed of sheep was found positive (Table 3).

The present study finding corroborate with Chauhan *et al.*, (2011) reported that seroprevalence was highest in Non-descript goats (46.18%) than Mehsani breed (40.77%).

Contrary to our finding Abhijit *et al.*, (2005) reported that the prevalence of the PPR infection in goats was found to be highest in Jamunapari breed (52%) as compared to Black Bengal (30%) and non-descript breeds (28%). Rahman *et al.*, (2015) reported that the prevalence of PPR was higher in Black Bengal

goats than in Jamunapari breed. In contrast to our finding, Chauhan *et al.*, (2011) reported that seroprevalence was highest in chokla (64.44%) & Marwari (60.16%) breed of sheep than crossbred sheep (40.00%). Jalees *et al.*, (2016) observed that Cholistani breed of sheep showed higher prevalence than rest of the breeds of sheep.

Sample wise positivity by sandwich ELISA

Out of 119 clinical samples, 37 (31.09%) samples including 13 Nasal swabs, 3 conjunctival swabs, 7 oral swabs and 14 tissues were found positive. The overall positivity for PPRV was significant ($P < 0.05$) among various clinical samples.

In the present study, we found presence of viral antigen in goat 63.16% (12/19), 43.75% (7/16), 17.5% (7/40) and 15.00% (3/20) in tissue, oral swab, nasal swabs and conjunctival swabs, respectively. However presence of viral antigen in sheep was 27.27% (6/22) and 100% (2/2) innasal swab and tissue, respectively (Table 4).

In this study found more number of tissue samples 66.67% (14/21) positive by S- ELISA in small ruminant. Similar observations have also been made earlier from studies of Gujarat, (95.83%) by Ranaware *et al.*, (2013) and 87.17% by Sakhare (2019).

Among tissue samples, Rajeshwari *et al.*, (2000) concluded that spleen (63.68% positivity) is the choice of material for antigen detection compared to lymph node and lung, which revealed 51.95% and 48% positivity, respectively (Table 4-A). In present study, we found positivity of PPRV in 43.75% (7/16) in oral swabs and 20.97% (13/62) in nasal swabs by S-ELISA. These positivity corroborates with the finding of Mahajan *et al.*, (2012) who reported that, the nasal swab samples recorded maximum number of positive samples (57.96%) followed by ocular swabs (53.38%), oral swabs (46.15%) and blood samples (42.30%), whereas least positivity was reported in rectal swab (19.23%) samples. The results are also in line with Singh *et al.*, (2004) who reported that maximum quantity of PPRV antigen is excreted in ocular and nasal secretions which are the best ante-mortem material for diagnosis of PPR by sandwich ELISA. In present study we found presence of PPR viral antigen in sheep 27.27% (6/22) and 100% (2/2) in nasal swab and tissue respectively. The positivity corroborate with finding of Albayrak and Alkan (2009) who observed that the nasal and conjunctival swab samples are more valuable as diagnostic material from sheep with clinical symptoms. However, Haq *et al.*, (2017) revealed only 18.75% (18/96) samples were positive for PPRV-specific antigen in anti-N monoclonal antibody-based sandwich-ELISA.

Comparatively higher positivity rate in tissue samples than other swabs as observed in the present study can also be explained by the fact, that it is possible that few of the animals might not be in viremic stage at the time of

sample collection. As tissues are collected on post- mortem, the virus after causing sufficient damage to the level of death obviously remains in active growth phase and hence may turn out to be more positive (Chaudhary, 2009).

A total of 119 different clinical samples (nasal swabs, conjunctival swabs, oral swabs and lung, trachea, spleen, intestinal tissue) from goats and sheep were collected from Rajkot, Amreli and Bhavnagar districts of Saurashtra region of Gujarat for detection of PPR antigen by Sandwich ELISA. Out of 119 clinical samples, 37 samples were found positive in small ruminants by S-ELISA, giving an overall incidence rate of 31.09 percent. In Goat and sheep, 30.52% (29/95) and 33.33% (8/24) samples were detected positive, respectively.

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