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PREVALENCE OF PESTE DES PETITS RUMINANTS VIRUS ANTIBODIES IN SMALL RUMINANTSIN SINDH, PAKISTAN

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ABSTRACT

Peste des petits ruminants (PPR) is an acute viral disease of small ruminants which is clinically characterized by occulo-nasal discharges, oral erosive lesions, diarrhea, pneumonia and leucopenia.It leads to high morbidity and moderate mortality rates.The present study was aimed at determining the seroprevalence of PPR in small ruminants of Sindh province by competitive-ELISA. A total of 7096bloodsamples were collected from sheep (1309) and goats (5787) fromeleven districts of the Sindh. The overall seroprevalence was found to be35.23 percent in the small ruminant population.Prevalence was found higher in sheep (37.2%) than in goats (34.78%). Seroprevalence varied in various age groups and was found to be 33.41%, 33.34% and 39.15% in three age groups, i.e.<1 year, 1 to 2 years and > 2 years, respectively.Prevalence rates were higher in females (35.94%) than in males (31.23%). Prevalence of PPRvaried in various agro-ecological zones of the province. It was found highest in Dadu district (69.3%) and lowest in Badin district (15.7%). Southern coastal districts of Badin (15.7%) and Thatta (30.9%) showed lower and eastern districts of Tando Allah Yar (60.3%) along with Tharparkar (50.3%) a higher seroprevalence of PPR. PPR is endemic in Sindh, therefore there is an urgent needfor preventive vaccination disease control.

Key words: PPR, Sindh, c-ELISA, Seroprevalence, Goat, Sheep.

INTRODUCTION

Peste des petits ruminants (PPR) is a highly contagious viral disease of sheep and goats. The infectionis characterized by fever, anorexia, ulcerative stomatitis, diarrhea, oculo-nasal discharges, cough and pneumonia (Zahur *et al.*,2007). In non-endemic areas, mortality and morbidity rates may vary depending upon susceptible population and in severe cases can reach up to 90 and 100%, respectively (Hussain *et al.*, 2003). Concurrent bacterial, viral or parasitic infections may aggravate condition and increase mortality up to 100% (Kitching, 1988).

In sub-continent, PPR was first reportedin southern India in 1987 (Shaila *et al*., 1989).The disease may have existed earlier as diagnostic tools needed for differentiation from rinderpest were lacking. Confirmatory diagnostic tests for PPR such as cDNA clones (Diallo *et al*., 1989), monoclonal antibody based ELISA (Libeau, 1994) and PCR (Forsyth and Barrett, 1995) made the confirmatory differential diagnosis possible. PPRwas first reported in Pakistan in early 1990s on the bases of clinical and epidemiological observations (Pervez *et al*., 1993 and Athar *et al*., 1995). However it was confirmed through laboratory tests by Amjad *et al*. in1996. PPR has been serologically confirmed in various parts of Pakistan and an overall prevalence rate of about 50% in small ruminant population hasbeen reported by various studies (Khan *et al*., 2007, Abubakar *et al*., 2009, Aslam *et al*., 2009). Pestedes petits ruminants virus (PPRV) from various outbreaks in Pakistan has also been genetically characterized and found to belong to lineage IV (Zahur *et al*., 2014 and Munir *et al*., 2015).

After eradication of Rinderpest, importance of PPR has come to limelight, and the need of eradication of PPR is being increasingly felt (Abubakar *et al*., 2011a, Anderson *et al*., 2011, Baron *et al*., 2011 and Albina *et al*., 2013) as an effective vaccine and diagnostic tools are already available. Food and Agriculture Organization (FAO) and OIE have now plans for global eradication of PPR by 2030 (OIE, 2015).Nationaleradication strategy can only be based on latest data on prevalence of PPR in various parts of Pakistan. The present study was carried out to determine currentsero-prevalence of the disease in small ruminants invarious districts of Sindh province.

MATERIALS AND METHODS

Study area: The area covered in current study included 11 districts (45 Tehsils) of Sindh province. The area includes various agro-ecological zones of Sindh including western hilly areas, eastern Thar and Nara deserts, southern coastal belt and Northern/Central irrigated plains.

Sample collection: A total of 7096bloodsamples from small ruminants including 5787from goats and 1309from

sheep were collected from 11 districts (45 Tehsils) of Sindh Province of Pakistan between June 2012 and December 2013. Random samples were collected by field veterinarians and information regarding age and gender was recorded for each sample. The sera were separated andstored at –20ºC till use.

Competitive Enzyme-LinkedImmunosorbentAssay(c-ELISA): Theanti-PPRV antibodiesin serum samples were determined by a commercial competitive enzyme linked immunosorbant assay (c-ELISA) kit according to instructions of the manufacturer (collectively produced by CIRAD. EMVT, Montpellier, France, distributed by BDS, United Kingdom). The negative and positive cut-off values were used for the control of test procedure. Optical density (OD) values were read at 492nm with ELISA plate reader (Immunoskan BDSL, Thermo Lab. Systems, Finland). The absorbance wasconverted to percentage inhibition (PI) by ELISA Data Interchange (EDI) software which uses following formula:

PI = 100 - (OD control/test serum) X 100
(OD monoclonal control)

The serum samples showing PI value of 50 or above were taken as positive for PPRV antibodies, while those below value of 50 were considered negative.

Data Analysis: The prevalence of PPRVantibodies along with 95%confidence intervals (CI) and odd ratioswerecalculated using standard statistical methodsusing the computer package Student Edition of Statistics (SXW), version. 8.1 (copyright 2005, Analytical Software, USA).

RESULTS AND DISCUSSION

The frequency distribution of the PI values of the animals tested for PPR antibodies using c-ELISA are given as in Figure 1. Among the samples considered negative for PPRV antibodies (PI<50%), most of the samples had a PI values between 10 to 20 percent. Whereas, peak frequency distribution was observed between 80 to 90%. Similar observations on peak PI values ranges for anti-PPRV antibodies in negative and positive samples have been reported by Khan *et al*. (2008).2500 out of 7096 serum samples were found positive for PPRshowing a prevalence rate of 35.23% in small ruminant population of the province (Table 1). These results are similar to the findings of Singh *et al*. (2004)and Aslam*et al*. (2009) who found a seroprevalence of 33% and 31.25% in India and Khyber-Pakhtunkhwa, Pakistan, respectively. However, seroprevalence of PPR in Sindh province in this is study is lower than what was reported by Khan *et al*. (2007) for Punjab Province of Pakistan which was 43.33%. The prevalence of PPR in present study is also lower than reported by Abubakar *et al*. (2009 and 2011) who reported an overall prevalence of about 53 % in Sindh. The difference of prevalence rates between two studies may be due to difference in number of serum samples used and sampling strategy. Prevalence rates determined from samples submitted to laboratories from suspected outbreaks of PPR, as has been done in the study of Abubakar *et al*. (2009) are likely to be higher than would be case in random sampling. In our study random samples have been taken from sheep and goat population in various districts of Sindh.

In the current study, 2013 (34.78%) serum samples from goats and 487 (37.20%) serum samples from sheep were found positive forPPR. A higher seroprevalence in sheep as compared to goats has been reported in numerous other studies as well (Singh *et al*., 2004, Raghavendra *et al*., 2008, Munir *et al*., 2008,Khan *et al*., 2007, Khan *et al*., 2008 and Abubakar *et al*., 2011b).Although sheep and goats may not vary greatly in terms of disease incidence but there tends to be higher mortality in PPRV infected goats than in sheep (Soundararajan *et al*., 2006). Higher recovery rate in sheep leads to longevity as a result of which a higher proportion of sheep shows positive for anti-PPRV antibodies than goats (Singh *et al*., 2004). Moreover, due to higher fecundity rate of goats, herd is quickly replaced by young ones which are more susceptible to PPRV (Singh *et al*., 2004). However, findings in present study are not in agreement with Balamurugan *et al*.(2011)and Rashid *et al*.(2008)who found a lower seroprevalence in sheep than goats.

Prevalence of PPR in our study was found higher in females (35.95%) than males (31.24%) (Table 2). Seroprevalence in males of goats was 29.74% and in females it was35.68%. Whereas in sheep, prevalence was 37.44% in males and 37.16% in females. Some studies have shown a higher prevalence in female population of small ruminants (Khan *et al*., 2007 and Khan *et al*., 2008) while in others no significant difference have been found (Munir *et al*., 2008 and Mahajan *et al*., 2012). In most of the studies measuring seroprevalence of PPR, including ours, sample size of two sexes is usually variable with fewer samples of male animals which renders comparisons difficult. The physiological demand on females in the form of lactation, pregnancy and estrus can increase susceptibility to infections (Susan and Asamays, 1998) and it may also be responsible for higher prevalence of PPR in female population of small ruminants.

Seroprevalence of PPRV antibodies varied among three age-groups under study. The age groups<1 year, 1 to 2 years and >2 years showed overall seroprevalence rates as 33.41%, 33.34% and 39.15% respectively (Table 2). Prevalence of PPR remained higher within age group >2 years in sheep (40.59%) and goats (38.81%) than other two age-groups. This is in accordance with results of several previous studies

Table 1. Seroprevalence of PPR in small ruminant population of Sindh province

	Goat			Sheep			Overall		
District	**No. of samples**	**Positive**	**+ve %**	**No. of samples**	**Positive**	**+ve %**	**No. of samples**	**Positive**	**+ve %**
Badin	700	121	17.3	250	28	11.2	950	149	15.7
NaushahroFeroze	883	215	24.3	108	53	49.1	991	268	27.0
Thatta	709	225	31.7	87	21	24.1	796	246	30.9
Shaheed Benazirabad	750	258	34.4	200	66	33.0	950	324	34.1
Umerkot	740	242	32.7	159	93	58.5	899	335	37.3
Khairpur	815	332	40.7	200	58	29.0	1015	390	38.4
Shikarpur	82	39	47.6	7	2	28.6	89	41	46.1
Hyderabad	70	33	47.1	16	9	56.3	86	42	48.8
Tharparkar	800	399	49.9	200	104	52.0	1000	503	50.3
Tando Allah Yar	149	89	59.7	70	43	61.4	219	132	60.3
Dadu	89	60	67.4	12	10	83.3	101	70	69.3
Overall / total	5787	2013	34.8 (CI: 33.5 to 36.0)	1309	487	37.2 (CI: 34.6 to 39.8)	7096	2500	35.2 (CI: 34.1 to 36.3)

Table 2. Sex and age-wise prevalence PPR in Sindh

Species/Sex/Age groups	**No. of samples**	**Positive samples**		**95% CI**	**Odd Ratio**
		No	**%**		
Goats					
Male	871	259	29.74	26.7 to 32.9	0.294
Female	4916	1754	35.68	34.3 to 37.0	0.356
Age groups (years)					
<1	1791	598	33.39	31.2 to 35.6	0.333
1-2	2164	704	32.53	30.5 to 34.5	0.325
>2	1832	711	38.81	36.5 to 41.0	0.388
Sheep					
Male	211	79	37.44	30.9 to 44.3	0.374
Female	1098	408	37.16	34.3 to 40.1	0.371
Age groups (years)					
<1	365	123	33.7	28.9 to 38.8	0.368
1-2	508	187	36.81	32.6 to 41.2	0.368
>2	436	177	40.6	35.9 to 45.4	0.406

reporting a higher seroprevalence of PPRV antibodies in the >2 years age group in both sheep and goats (Khan *et al*., 2008, Abubakar *et al*., 2009 and Abubakar *et al*., 2011b). The presence of PPRV antibodies in both sheep and goats increases with age. The >2 years age-group includes a proportion of small ruminants which were exposed to PPR during young age but survived and sero-converted.

Prevalence rates of PPR varied in various districts of Sindh province. Southern coastal districts of Badin (15.7%) and Thatta (30.9%) had considerably lower prevalence while in eastern districts of Tando Allah Yar(60.3%) and Tharparkar (50.3%) seroprevalence of PPR was found higher (Table 1). The highest prevalence was found in Dadu district (69.3%). Prevalence was found higher in districts which are in the desert zone (Tharparkar) and in irrigated plains (Tando Allah Yar, Hyderabad and Khairpur) near Thar/Nara deserts and dry hilly terrain of Kohistan (Dadu). These results are in accordance with findings with Abubakar *et al*. (2011b) who found higher seroprevalence of PPR antibodies in south-eastern parts of Sindh. Similarly, Singh *et al*. (2004) found higher prevalence of PPRV antibodies in South and South-western parts of India. Variable seroprevalence have been hypothesized to be due to multiple factors including animal migrations and nutritional deficiencies (Singh *et al*., 2004). Poor nutrition in arid and semi-arid zones may increase disease incidence due decreased immune resistance. Under drought conditions, small ruminants are moved from barren hilly areas of Sindh along with Thar and Nara deserts to river irrigated parts of Sindh where they are in

close contact with susceptible local small ruminant population. Similar findings have been reported for Punjab province where prevalence of PPRV was found to be highest in Southernand Western parts including Cholistan desert (Khan *et al*., 2007 and Khan *et al*., 2008).

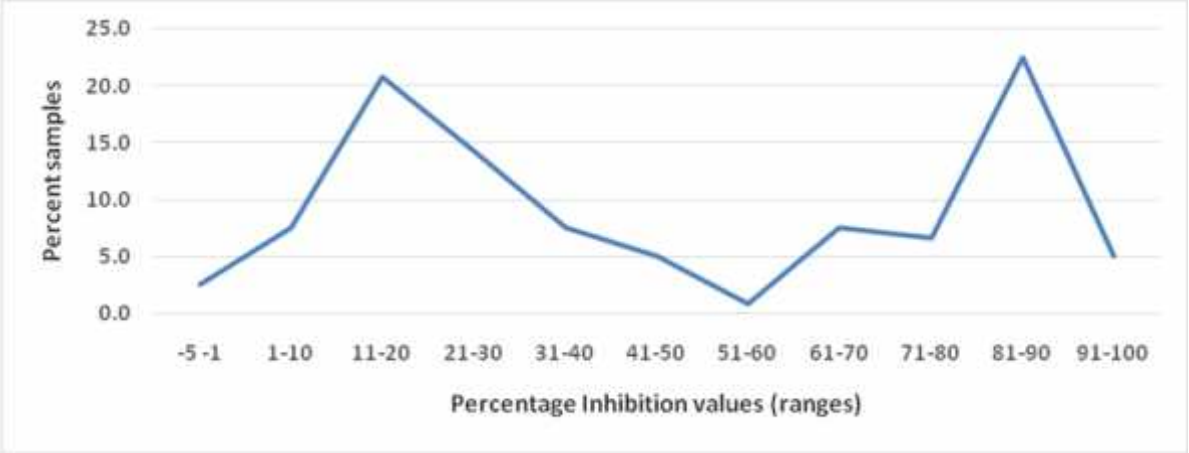

Fig. 1. Percent Inhibition (PI) values trend in seroprevalence of PPR

Conclusion: PPR is endemic throughout Sindh but prevalence ishighest in female goats >2 years of age in the eastern Thar and Nara desert zone and adjacent irrigated areas. There is an urgent need for local PPRV vaccine production for control and eradication efforts.

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