Trop Anim Health Prod (2009) 41:1197–1202
DOI 10.1007/s11250-008-9300-9

ORIGINAL PAPER

Peste des petits ruminants virus (PPRV) infection; Its association with species, seasonal variations and geography

Muhammad Abubakar · Syed Muhammad Jamal · Muhammad Javed Arshed · Manzoor Hussain · Qurban Ali

Accepted: 9 December 2008 / Published online: 7 January 2009

Abstract The present investigation included a detailed description of the factors directly associated with PPRV infection in Pakistan. A total of 1,056 suspected serum samples were analyzed for the presence of antibodies to PPRV with no history of vaccination against PPR. The samples were collected from sixty two (62) suspected outbreaks from twenty five (25) major regions of the country. Samples were collected from the animals suffering from diarrhea and showing severe respiratory signs. Competitive enzyme linked immuno-sorbant assay (cELISA) was performed to detect the presence of antibodies in the serum against PPRV. Findings suggested that the overall PPR antibody sero-prevalence recorded in sheep was 54.09% as compared to 44.15% in goats. Geography, species, sex, age and season are the major factors associated with PPRV infection. Among various age groups, the animals showed the higher prevalence (67.48% and 52.28% in sheep and goats, respectively) at >2 years as compared with the other age groups. The area-wise highest sero-prevalence was in Sindh province which was 55.10%. It was highest in the plains of Punjab and Sindh province and also in hilly areas (AJK, northern areas and northern Punjab). These findings may be correlated with variations in the sheep and goats husbandry practices within different geographic regions and the topography of different areas. The study also indicated the scenario of antibodies circulation in the population and proving that PPR is now becoming endemic and is one of the major emerging TAD in Pakistan.

Keywords PPRV · Sero-prevalence · cELISA · Seasonal variations and geography

M. Abubakar (✉) · S. M. Jamal · M. J. Arshed · Q. Ali
National Veterinary Laboratory,
Park Road, Islamabad, Pakistan
e-mail: hayee42@yahoo.com

M. Hussain
Regional Epidemiologist,
FAO Project (GTFS/INT/907/ITA),
Islamabad, Pakistan

Introduction

Despite the large populations of sheep and goats in Pakistan, production performance is low. Among other limiting factors, infectious diseases such as contagious caprine pleuropneumonia, pasteurellosis, Peste des petits ruminants (PPR) and contagious ecthyma are a significant impediment to productivity. Peste des petits ruminants (PPR) is a highly contagious disease and it is responsible for high morbidity and mortality in sheep and goats. The huge number of small ruminants, which are reared in the endemic areas, makes PPR a serious disease threatening the livelihood of poor farmers (Diallo et al. 2007).

The PPR virus belongs to the genus Morbillivirus in the family Paramyxoviridae. Morbilliviruses form a small group of antigenically related viruses: measles virus, rinderpest virus, canine distemper virus, phocine distemper virus and dolphine and porpoise morbilliviruses (Barrett et al. 1993). Rapid detection of infected animals is very important for PPR controls to be effective. Severe cases in which animals show clinical signs in the field can easily be detected through clinical surveillance and the detection of antigen in clinical samples, while the diagnosis of PPRV infection in sub-clinically infected animals can be achieved by serological surveillance.

It is endemic in the Arabian Pennsylvania, the Middle East and in the Indian subcontinent (Shaila et al. 1996). The existence of PPR has been recognized in Pakistan (Athar et al. 1995; Hussain et al. 1998) but these reports were mainly based on clinical diagnosis and not on laboratory confirmation. Information on the prevalence of antibodies to PPRV in small ruminants is available from a number of countries in which the disease is reported, including the Sultanate of Oman (Taylor et al. 1990), Jordan (Lefèvre et al. 1991), Turkey (Ozkul et al. 2002) and various African countries (Anderson and Mckay 1994; Martrenchar et al. 1995). However, the pattern of PPRV infection and its association with seasonal variation and geography in small ruminants of Pakistan have not been systematically studied to date.

The test prescribed for the detection of PPRV antibody, virus neutralization test (VNT), is laborious and expensive and requires infectious virus. For these reasons, VNT is not ideal for large-scale routine testing. Due to their simplicity, high sensitivity, and economy, several competitive enzyme-linked immunosorbent assays (c-ELISAs) have been recognized as suitable systems for use for diagnosis and sero-epidemiological surveillance which we used in this study.

In the present study, efforts have been made to collect preliminary information on the factors affecting the prevalence of antibodies to PPRV in the sheep and goats population. The investigation included attending the PPR suspected outbreaks and data generated from the screening of 1056 sera samples throughout Pakistan. The other main objective of this sero-prevalence study of PPRV was to determine the seasonal variation, regional and spatial distribution of PPRV in the country.

Materials and methods

Study area and sample collection

The study was conducted on samples from twenty-five (25) major regions of Pakistan. A total of 1056 serum samples were collected and analyzed from 440 sheep and 616 goats. The information was gathered according to the questionnaire prepared for this purpose. The tissue samples from morbid animals in the outbreaks were also collected and analyzed for antigen detection. The areas were divided into regions known to be PPR affected. The spatial distribution was studied. Data and sera were collected and processed in a period of three years from 2005 to 2007.

Serum collection

Blood was collected by jugular-vein puncture in vacutest tubes. The blood was left to clot overnight at 4°C. Serum was decanted into sterile tubes and kept on ice for transportation to the laboratory. In the lab, where it was centrifuged, transferred to 1.8 ml screw capped serum tubes and stored at −20°C.

Frequency distribution of antibodies to PPRV in sheep and goat

The competitive ELISA was conducted to determine the prevalence and distribution of antibodies against PPRV and sera showing PI value greater than 50% were considered as positive. The competitive ELISA was used to clearly differentiate the exposed (infected) population from the unexposed (not infected) population. The PPR competitive ELISA kit (collectively produced by Biological Diagnostic Supplies Ltd, Flow Laboratories and Institute for Animal Health Pirbright, Surrey, England) was used for this purpose. The kit is based on a standard competitive enzyme linked immunosorbant assay (cELISA) principle to determine the presence of anti-PPR antibodies in serum. The test is based on the competition between the anti-H protein of PPR virus monoclonal antibodies and the serum samples binding the PPR antigen (Libeau et al. 1992). The presence of antibodies to PPRV in the serum samples block reactivity of the monoclonal antibodies which caused reduction in the expected colour following the addition of enzyme labeled anti-mouse conjugate and chromogen solution. The negative and positive cut-off values were used

from the controls of the test procedure. The ELISA micro-plates were read using an immuno-skan reader with an inference filter of 492 nm. The reader was connected to a computer loaded with ELISA data interchange (EDI) software, which was used to automate the reading and calculation of percent inhibit (PI) values. The OD values were converted to percent inhibition by using the following formula:

$$\mathbf{PI} = \mathbf{100} - \frac{(\mathbf{OD\ control/test\ serum})}{(\mathbf{OD\ monoclonal\ control})} \times \mathbf{100}$$

The samples with PI>50% were considered as positive.

Results

Based on information supplied in outbreak records, the most frequent clinical findings in diseased animals were a high body temperature (up to 106°F), severe muco-purulent nasal and ocular discharges, necrotic stomatitis and respiratory distress; diarrhea was also present but abortions were reported in a few cases. The most common post-mortem lesions reported were necrotic enteritis, pneumonia, spleenomegaly and enlargement of the lymph nodes. Interestingly, at least 19 of the 62 outbreak were associated with either the entry of newly purchased animals from a common market or the intermixing of migratory/nomadic animals with local animals. A complete history of the movement or the nomadic nature of the animals was not available for all of the outbreaks investigated. Amongst the 62 outbreaks of PPRV, 36 of the outbreaks involved goats alone, 21 of the outbreaks involved sheep alone, 5 of the outbreaks involved both sheep and goats (Table 1). Outbreaks were reported to be more severe in goats than sheep. Based on data of 427 samples for antigen detection by immuno-capture ELISA, the prevalence of PPRV in small ruminants of Pakistan was 40.98% (Abubakar et al. 2008). Season also affected the prevalence of PPRV infection, the greatest frequency of PPR outbreaks was noticed in the first and last quarter of the years (Fig. 1).

The species-wise PPR antibody sero-prevalence recorded in sheep was 54.9% as compared to goats 44.15% (Table 2). The area-wise highest sero-prevalence was 55.10% in sheep and goats of Sindh province (Table 3). The second highest prevalence (76%) was in Chakwal, followed by 75%, 64.28%, 64.71%, 61.29%, 60.66% and 60.31% in districts Bahawalpur, Haiderabad, Northern areas, Sahiwal, Azad Jammu & Kashmir (AJK) and Rawalpindi, respectively (Table 4). The distribution and prevalence of antibodies to PPRV among various age groups of animals was also studied (Table 5). Findings suggested that the majority of animals were exposed when older than 2 year (67.48% and 52.28% in sheep and goats, respectively). A higher proportion (42.58%) of sheep between the ages of 0–12 months were tested positive for PPRV as compared to goats (35.44%). Similarly the percentage positive of adult animals (1−2 years) for PPRV was greater in the sheep (54.94%) versus the goat (45.69%) population.

Table 1 Month-wise prevalence of PPR outbreaks (positive samples)

Sr. No.	Months	Year 2005 (28 outbreaks = 145 samples Positive)	Year 2006 (19 outbreaks = 204 samples Positive)	Year 2007 (15 outbreaks = 161 samples Positive)	Total	Percentage (%)
1)	January	16	28	14	**58**	6.8
2)	February	30	19	24	**73**	14.85
3)	March	17	36	19	**72**	32.57
4)	April	15	16	22	**53**	19.43
5)	May	12	21	15	**48**	5.14
6)	June	5	17	12	**34**	1.71
7)	July	0	0	6	**6**	-
8)	August	0	3	0	**3**	-
9)	September	6	12	12	**30**	2.85
10)	October	09	19	7	**35**	6.28
11)	November	13	13	11	**37**	5.71
12)	December	22	20	19	**61**	4.57
Total		**145**	**204**	**161**	**510**	

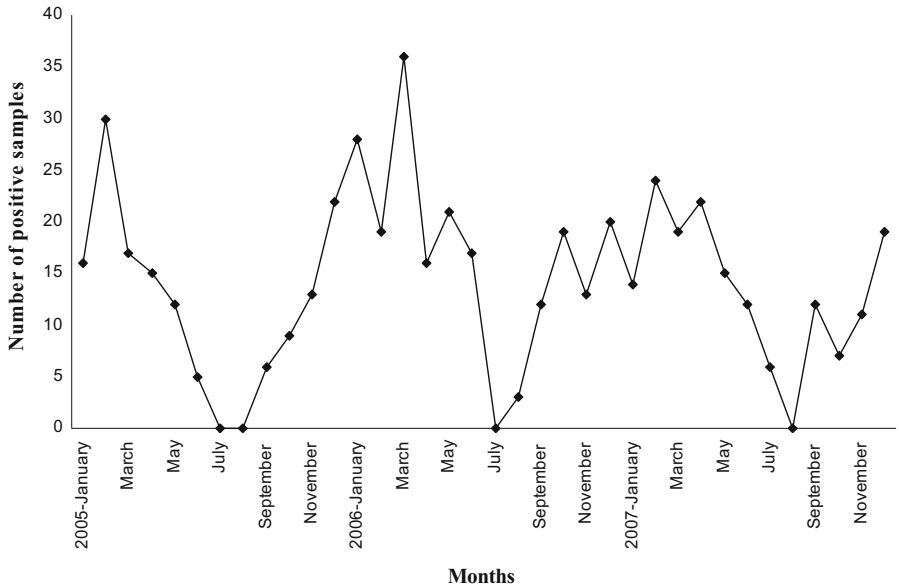

Fig. 1 Frequency of PPR outbreaks from January 2005 to December 2007

Discussion

Major factors, affecting the prevalence, host range, and distribution of PPRV were investigated in samples from twenty-five main regions of Pakistan. The presence of the disease was demonstrated by observing animals in the field and by virus confirmation from clinical specimens. This wide-ranging survey was the first to be carried out on this disease in Pakistan. The study also provided valuable data on the serologic status of the two domestic ruminant species (sheep and goats) with respect to PPRV.

The competitive ELISA used in the present investigation had high diagnostic specificity (99.8%) and sensitivity (90.5%) for the detection of PPRV antibody in convalescent sera when compared with the gold standard VNT (Anderson and McKay 1994; Singh et al. 2004; Libeau et al. 1992). The presence of antibodies to PPR virus in the serum samples blocked reactivity of the monoclonal antibodies which caused reduction in the expected colour following the addition of enzyme labeled anti-mouse conjugate and chromogen solution. The negative and positive cut-off values were used from the controls of the test procedure.

Table 2 Specie-wise prevalence of PPR antibodies

Specie	Total	Positive	Percent prevalence (%)
Sheep	440	238	54.09
Goat	616	272	44.15
Over-all prevalence	1056	510	48.30

The species and area were the main factors affecting the prevalence of the disease as overall prevalence of antibodies against PPRV was around 48.30% in sheep and goats. The disease was found in almost every major region across Pakistan but the incidence was highest in Sindh province. Occurrence of infection varied substantially by geographic locations of the animals tested. It was highest in the plains of Punjab and Sindh province and also in hilly areas (AJK, northern areas and northern Punjab). This might be due to the nomadic grazing in these parts of the country. It was found that climatic conditions and seasonal forage availability dictate grazing patterns in the areas of desert of Sindh and southern and northern Punjab.

The livestock utilize the alpine areas from June to October, when low temperatures retard plant growth, and then herders descend towards the plains or low valleys. During winter, livestock graze in Pothwar scrub ranges, abandoned cultivated lands, or browse in valleys along water channels, roads, and grazing

Table 3 Province-wise prevalence of PPR outbreaks (2005–07)

Sr. No.	Provinces	Total Samples	Positive (No./%)
1)	Punjab	668	**329** (49.25%)
2)	Sindh	98	**54** (55.10%)
3)	NWFP	121	**30** (22.90%)
4)	Balochistan	40	**16** (40%)
5)	AJK	61	**37** (60.66%)
6)	Northern areas	68	**44** (64.71%)
Total		1056	**510** (48.30%)

Table 4 Geographical distribution of PPR in the country (Prevalence of antibodies to PPRV in small ruminants population)

Sr. No.	Regions/area	Samples tested	Positive samples	Percent prevalence
1)	Islamabad	85	37	43.53
2)	Rawalpindi	194	117	60.31
3)	Murree	20	11	55
4)	Pakpaten	80	26	32.5
5)	Okara	58	34	58.62
6)	Lahore	20	06	30
7)	Sahiwal	31	19	61.29
8)	Mandi	21	10	47.62
9)	Chakwal	25	19	76
10)	Jand	22	03	13.64
11)	Gujranwala	22	05	22.73
12)	Layyah	20	07	35
13)	Faisalabad	25	06	24
14)	Bahwalnagar	24	18	75
15)	Attock	21	11	52.38
16)	Haripur	23	08	34.78
17)	Peshawar	44	08	18.18
18)	Tank	25	10	40
19)	Kohat	29	04	13.79
20)	Therparkar	26	11	42.31
21)	Mithi	30	16	53.33
22)	Haiderabad	42	27	64.28
23)	Quetta	40	16	40
24)	AJK	61	37	60.66
25)	Northern areas	68	44	64.71
Total		**1056**	**510**	**48.30**

grounds between agricultural fields. So the nutritional status of the animals improves during the rainy season due to increase availability of fodder that may lead to the increase resistance. Similar observations were recorded in humid zone of southern Nigeria by Wosu (1994). Climatic factors favorable for the survival and spread of the virus may also contribute to the seasonal distribution of PPR outbreaks. The highest frequency of PPRV outbreaks reported was in the first and last quarter of the years (Table 3), it was highest in the month of March followed by April.

The antibodies prevalence of the disease was as higher in sheep (54.09%) than goats (44.15%). PPR virus exhibits different levels of virulence between sheep and goats. Goats are severely affected while sheep generally undergo a mild form (Lefèvre and Diallo 1990). Although in few cases, the sheep showed a less clinical disease, but there was higher antibodies detection as compared to goats which is in congruent with Lefèvre and Diallo (1990). This increased antibodies response should not be misinterpreted as an increased susceptibility of sheep to infection with PPRV. Rather, this can be attributed to a higher recovery rate (lower case fatality rate) and/or a greater longevity of sheep versus goats. The presence of a large proportion of animals in the sheep population which have recovered from previous infections with PPRV and were maintained in the

Table 5 Peste des petits ruminants virus (PPRV) antibody prevalence in different age groups of sheep and goats

SHEEP+VE			GOAT+VE		
0–12M	1–2Y	>2Y	0–12M	1–2Y	>2Y
66/155	89/162	83/123	73/206	106/232	93/178
42.58%	54.94%	67.48%	35.44%	45.69%	52.28%

flock over many years for the purposes of wool production may indirectly account for the high prevalence of antibodies to PPRV detected in sheep.

Al-Majali et al. (2008) reported that the prevalence of PPRV in sheep and goats was 29 and 49%, respectively, in Jordan while Abraham et al. (2005) claimed in contrast, saying that the peste des petits ruminants (PPR) antibody seroprevalence was 3% in camels, 9% in cattle, 9% in goats and 13% in sheep which is in congruent with results of above study. In both sheep and goat flocks, large flock size, visiting live animals market and inadequate veterinary services were identified as risk factors for PPR seropositivity. Similarly, Szkuta et al. (2008) explored the spatial distribution and risk factors of PPR in Ethiopia and found that Sero-prevalence was very heterogeneous across regions and even more across *wereda*, with prevalence estimates ranging from 0% to 52.5%.

Thus the present study provided valuable data on the serologic status of PPRV in sheep and goats and its association with seasonal variations and geography in Pakistan which must be kept in mind while deciding the vaccination strategy for the control of disease.

Acknowledgments We are thankful to In-charges of district laboratories and disease diagnostic officers for their help in the sampling process. The financial assistance was provided by Strengthening of Livestock Services (EU) Project and FAO Regional Project (GTFS/INT/907/ITA).

References

Abraham, G., A. Sintayehu, G. Libeau, E. Albina, F. Roger, Y. Laekemariam, D. Abayneh and K. M. Awoke, 2005. Antibody sero-prevalences against peste des petits ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. *Preventive Veterinary Medicine*, **70** (1–2): 51–57. doi:10.1016/j.prevetmed.2005.02.011.

Abubakar, M., S. M. Jamal, M. Hussain and Q. Ali, 2008. Incidence of Peste des Petitis Ruminants (PPR) Virus in Sheep and Goat as Detected by Immuno-capture ELISA (Ic ELISA). *Small Ruminant Research*, **75** (2/3): 256–259. doi:10.1016/j.smallrumres.2007.12.001.

Al-Majali, A. M., N. O. Hussain, N. M. Amarin and A. A. Majok, 2008. Sero-prevalence of, and risk factors for, peste des petits ruminants in sheep and goats in Northern Jordan. *Preventive Veterinary Medicine*, **85** (1–2): 1–8. doi:10.1016/j.prevetmed.2008.01.002.

Anderson, J., and J. A. McKay, 1994. The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epidemiology Infection.*, **112** (1): 225–231.

Athar, H., Q.U. Islam, F. Azim, A. Shakoor, A. Maqbool and N.I. Chaudhary, 1995. An outbreak of peste des petits ruminants-like disease among goats in Punjab (Pakistan). *Pakistan Veterinary Journal*, **15**: 140–143.

Barrett, T.,Visser, I.K.G., Mamaev, L., Goatley, L., Bressem, M.F., and Van Osterhaus, A.D.M., 1993. Dolphine and porpoise morbilliviruses are genetically distinct from phocine distemper virus. *Virology*, **193**: 1010–1012. doi:10.1006/viro.1993.1217.

Diallo A., C. Minet, C. Le Goff, G. Berhe, E. Albina, G. Libeau and T. Barrett, 2007. The threat of peste des petits ruminants: progress in vaccine development for disease control. *Vaccine*. **25** (30): 5591–5597. doi:10.1016/j.vaccine.2007.02.013.

Hussain, M., M. Afzal, R. Muneer, M. Ashfaq and E. U. Haq, 1998. An outbreak of peste des petits ruminants in goats in Rawalpindi. *Pakistan Veterinary Journal*, **18**: 224–226.

Lefèvre, P. C. and A. Diallo, 1990. Peste des petits ruminants virus. *Review scientific technology Office International Epizootics*, **9** 4, 951–965.

Lefèvre, P. C., A. Diallo, F. Schenkel, S. Hussein and G. Staak, 1991. Serological evidence of peste des petits ruminants in Jordan. *Veterinary Record*, **128** (5): 110.

Libeau, G., A. Diallo, D. Calvez, and P. C. Lefever, 1992. A competitive ELISA using anti-N monoclonal antibodies for specific detection of RP antibodies in cattle and small ruminants. *Veterinary Microbiology*, **31**: 147–160. doi:10.1016/0378–1135(92)90073–3.

Martrenchar, A., N. Zoyem, A. Ngangnou, D. Bouchel, A. C. Ngo Tama and A. Njoya, 1995. Main infectious agents involved in the etiology of lung diseases of small ruminants in northern Cameroon (in French). *Rev. Elev. Méd. vét. Pays trop.*, **48** (2): 133–137.

Ozkul A., Y. Akca, F. Alkan, T. Barrett, T. Karaoglu, S. B. Dagalp, J. Anderson, K. Yesilbag, C. Cokcaliskan, A. Gencay and I. Burgu, 2002. Prevalence, distribution, andhost range of peste des petits ruminants virus, Turkey. *Emerging infectious Diseases*, **8** (7): 708–712.

Shaila, M.S., S. David, M. A. Foryth, A. Diallo, L. Goatley, R. P. Kitching, and T. Barret, 1996. Geographical distribution and epidemiology of PPR viruses. *Virus Research*, **43**: 149–153. doi:10.1016/0168–1702(96)01312–3.

Singh R. P., Saravanan, P., Sreenivasa, B. P., Singh, R. K., & Bandyopadhyay, S. K. 2004. Prevalence and distribution of peste des petits ruminants (PPR) virus infection in small ruminants. *Review scientific technology Office International Epizootics*, **23** (3): 807–819.

Szkuta, A. W., R. François, D. Chavernac, L. Yigezu, G. Libeau, D. U. Pfeiffer and J. Guitián, 2008. Peste des Petits Ruminants (PPR) in Ethiopia: Analysis of a national serological survey. *BMC Veterinary Research*, **4**: 34–43. doi:10.1186/1746–6148–4–34.

Taylor W. P., S. AlBusaidy and T. Barrett, 1990. The epidemiology of peste des petits ruminants in the Sultanate of Oman. *Veterinary Microbiology*, **22** (4): 341–352. doi:10.1016/0378–1135(90)90021-M.

Wosu, L.O., 1994. Current status of peste des petits ruminants (PPR) disease in small ruminants. A review article. *Stud. Research Veterinary Medicine*, **2**: 83–90.