Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Occurrence of Peste Des Petitis Ruminants in Five Districts of Punjab, Pakistan

Muhammad Moazam Jalees, Iftikhar Hussain*, Muhammad Arshad, Ghulam Muhammad[1], Qaiser Mehmood Khan[2] and Muhammad Shahid Mahmood

Institute of Microbiology; [1]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad; [2]National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan
*Corresponding author: driftikharuaf@gmail.com

ARTICLE HISTORY

Received: July 28, 2011
Revised: September 06, 2012
Accepted: November 09, 2012

Key words:
c-ELISA
Pakistan
Peste des petits ruminants
Punjab
RT-PCR

ABSTRACT

The present study was carried out to know the seroprevalence, molecular characterization and molecular epidemiology of peste des petitis ruminants (PPR) virus. The serum (n=440) and tissue (n=242) samples were collected from sheep and goat population reared in the central and peripheral districts of the Punjab province. The specimens were subjected to RT-PCR for the specific detection of fusion (F) protein genes of PPR virus. The cELISA test was applied to serum samples to know seroprevalence. Overall 51.5 and 46.5% seropositivity was found in sheep and goat, respectively. Seropositivity under different ages 1, 2 and >2years was recorded as 46.5, 60.5 and 37.8%, respectively. Species and sex variation of seropositivity was recorded at 60.3 and 32.8% in buck and doe, respectively and 64.8 and 50.7% in ewes and ram, respectively. Sixty percent goat and 55.6% sheep population in Faisalabad district was seropositive for PPR virus, followed by the Bhakkar district where PPR virus seropositivity was 55.1% in sheep and 53.5% in goat. Specimen samples recovered from outbreak cases showed 372bp RT-PCR product indicating the presence of F specific protein region of PPR virus. Maximum predilection for PPR virus was lymph nodes (87.5%) in sheep as well as in goats followed by spleen (62.5%), nasal swabs (59.1%) and blood (51.4%). The phylogenetic analysis showed similar percentages and relationship with already reported data of this region. The present study reports that PPR virus is affecting the sheep and goat of less than two years of age with high frequency. The prevalent PPR virus is homologous with most of the reported PPR viruses from the Asian outbreaks.

To Cite This Article: Jalees MM, I Hussain, M Arshad, G Muhammad, QM Khan and MS Mahmood, 2013. Occurrence of peste des petitis ruminants in five districts of Punjab, Pakistan. Pak Vet J, 33(2): 165-169.

INTRODUCTION

Peste des petitis ruminants (PPR) is an acute and highly contagious viral disease of small ruminants caused by a morbillivirus (Roeder *et al*., 1994), which is antigenically related to other morbilliviruses such as Canine distemper virus, Measles virus and Rinderpest virus. PPR is endemic in many parts of the world, including Africa, Asia, Middle East and sub Saharan Africa, India, Pakistan, Iran, Iraq, Bhutan and Bangladesh (Shaila *et al*., 1996; Kwiatek *et al*., 2007; Sande *et al*., 2011; Abubakar *et al*., 2011a). PPR was first recognized in Pakistan during 1991 by Amjad *et al*. (1996) and in Punjab province by Athar *et al*. (1995). Since then, the disease is reported in many parts of country (Hussain *et al*., 2003). The disease has serious economic and trade implications because of its high mortality (Asim *et al*., 2009). Naturally, the disease affects both sheep and goat but more severity with high case fatality recorded in goats than sheep (Atta-ur-Rehman *et al*., 2004).

PPR is characterized by high fever, mucopurulent discharge, ulceration of mucus membrane and inflammation of gastrointestinal tract with morbidity between 80-90% and mortality between 50-80% (Khan *et al*., 2008b). Abubakar *et al*. (2011b) reported the morbidity and mortality as high as 100 and 90%, respectively, and in certain cases these rates were recorded as low as 20%. The mortality rate varies between 70-80% in acute out break cases with death of affected animals in 10-12 days (Diallo *et al*., 2007). Due to its high mortality and morbidity rates, PPR has been rated as a notifiable viral disease by OIE.

PPR virus has been reported continuously from Pakistan with different frequencies using a variety of techniques i.e., virus isolation, ELISA, and PCR (Anderson and Meckay, 1994; Singh *et al*., 2004; Khan *et al.,* 2008b). PPR outbreaks were reported annually and in various areas and seasons in Pakistan (Tahir *et al*., 1998; Hussain *et al*., 1998; Khan *et al*., 2008a; Khan *et al.,* 2009). The previous reports were based upon small number of samples per district encompassing many districts and secondly RT-PCR was not the main techniques to conduct epidemiology of PPR on large scale. Moreover detailed analysis regarding age, sex and species were not reported solely from the area selected in the present study. The present study hypothesized to bridge the gap regarding seroprevalence of PPRV using cELISA and PCR to compile molecular epidemiology of PPRV with reference to location, age sex in sheep and goat samples collected from different districts of Punjab.

MATERIALS AND METHODS

Sample collection: Blood and specimen samples were collected from sheep n=134 and goats n=108 at different outbreaks in and around five districts (Faisalabad, Attock, D.G Khan, Bhakkar, Kasur) of Punjab, Pakistan (January-December 2010). The districts heavily populated with sheep and goats (Anonymous, 2006) were selected for the present study. A total of 440 serum samples were collected from the randomly selected apparently healthy sheep and goat for specific antibody screening, while 242 specimen samples were collected from convalescent cases for antigen detection (Table 1). The samples preservation was done according to FAO Manual for Trans-boundary diseases. Ice pads were used for the transportation of lymph node, spleen and serum samples to the laboratory.

Detection of antibodies by cELISA: Sera were titrated for antibodies by cELISA kit manufactured by Institute for Animal Health, Pirbright laboratory UK following instruction of the manufacturer. The negative and positive cut off values was used for the control of test procedure. The ELISA plates were read using (Humma Reader, USA) inference filter of 492 nm. EDI (ELISA data interchange) software was used to read and calculate the percentage of inhibition (PI). The optical density (OD) was converted to PI by using following formula (Libeau *et al.,* 1992).

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

Samples having P1 >50% were considered as positive.

Detection of PPRV using RT-PCR: The samples were subjected to RNA extraction using the protocol described by Nanda *et al*. (1996). Extracted RNA was subjected to cDNA synthesis by using iScript protocol (Bio-Rad Laboratories CA). The iScript reaction mix (4µl) was added into 1µl reverse transcriptase, nuclease free water (10µl) and RNA template (5µl) to make final volume of reaction mixture 20µl. This reaction mixture was incubated at 25°C for 5 minutes and 42°C for 30 min and 85°C for 5 minutes with final holding at 4°C.

PCR amplification: A pair of primers *i.e.*, F1 (Forward) 5′ATCACAGTGTTAAAGCCTGTAGAGG3 and primer F2 (Reverse) 5′GAGACTGAGTTTGTGACCTACAAG C3 were added to 5µl cDNA, 5 µl of 10x PCR buffer, 1.5 µl of 50 mM $Mgcl_2$, Taq polymerase (5 µl) and 1µl dNTP (10mM each) and sterile distilled water to make final volume 50 µl. The cocktail was finally subjected to denaturation, annealing, synthesis and extension as reported by Nanda *et al*. (1996). PCR product was subjected to 1.5% agarose gel electrophoresis and was visualized by UV trans-illuminator Dolphin Doc, Wealtech USA. PCR product was sent to Eurofins MWG Operon U.S.A and phylogenetic analysis was performed using clustal W2 program of multiple sequence alignment. Chi-square analysis was performed to statistically analyze the differences found in sero-prevalence of PPR in sheep and goat.

RESULTS

Detection of PPR antibodies: A total of 440 serum samples from goat (n=131) and sheep (n=309) were randomly collected from five districts of Punjab. Of these 51.5% sheep and 46.5% Goat samples were positive. The percent positivity of the sheep samples is significantly different (P=0.042) and in goat samples highly significant (P=0.000) among different districts under study (Table 1). Age and gender based interaction is described in Table 2. It was found that the seroprevalence of PPR in ewes upto 1 year with buck 2 years and doe more than 2 years of age were significantly higher than other groups. While gender wise the values showed that the seroprevalence in ewe and buck, and ram and doe are significantly different.

Virus detection and F gene sequencing: Samples of sheep (n=134) and goat (n=108) were collected from various outbreak cases at five different districts of Punjab (Table 3). RT-PCR was applied on these samples targeting protein sequence of 372bp size (Fig. 1). Maximum PPRV was detected from lymph node (87.5%) followed by spleen, nasal swabs and blood. Whereas significant differences were observed in nasal swab and blood samples (P=0.000) followed by spleen samples from sheep, which were significantly different (P=0.001). The analysis of F gene sequence revealed that homology between previously reported and characterized Pakistani and surrounding areas isolates ranges between 98-99%. A phylogenetic tree was constructed for F gene based on sequence recovered from gene bank representing lineages. This tree revealed that the sample in this study describing the F gene sequence cluster together in the region of South East Asia and Middle East with the identification of new indigenous isolate of PPR virus found in the lineage 4 (Fig. 2) of isolate was obtained after submission of sequencing results to NCBI, USA.

DISCUSSION

PPR is an acute viral disease caused by a morbillivirus that mainly effect sheep and goats. The disease is having variable degree of morbidity and mortality. So far field cases in Pakistan are diagnosed on

Table 1: Statistics of PPR Virus detected through RT-PCR in specimen samples of sheep and goat in five districts of Punjab

Sampling Area	Total population	Total sample collected				Positive samples			
		NS	Blood	Spleen	LN	NS	Blood	Spleen	LN
Sheep									
Central Punjab (Faisalabad district)	54753	12	7	2	2	8	5	2	2
Northern Punjab (Attock district)	180947	11	8	3	2	7	4	2	2
Southern Punjab (D.G Khan district)	645035	24	8	-	2	14	4	-	2
Western Punjab (Bhskksr district)	459540	12	6	4	4	7	3	1	4
Eastern Punjab (Kasur ditrcit)	98730	7	8	6	6	3	3	3	4
Total	1439005	66	37	15	16	39	19	8	14
Prevalence percentage						59.100	51.400	53.300	87.500
Chi square values						33.636	24.667	17.579	1.714
P values						0.000	0.000	0.001	0.788
Goats									
Central Punjab (Faisalabad district)	904918	7	6	2	2	2	3	2	2
Northern Punjab (Attock district)	628642	12	5	1	1	7	4	1	1
Southern Punjab (D.G Khan district)	858437	27	4	3	3	18	2	1	2
Western Punjab (Bhskksr district)	655774	12	6	2	2	6	2	1	2
Eastern Punjab (Kasur ditrcit)	365792	9	4	-	-	3	2	-	-
Total	3413564	67	25	8	8	36	13	5	7
Prevalence percentage						53.700	52	62.50	87.500
Chi square values						91.134	18.440	2.00	0.750
P values						0.000	0.002	0.736	0.945

NS= Nasal swab; LN = Lymph node.

Table 2: Seroprevalence of PPR virus in sheep and goat using c-ELISA

Age group	+ve Sheep (Positive/Total)		+ve Goat (Positive/Total)	
	Ram	Ewe	Buck	Doe
Upto 1 year	48/82	28/42	12/25	8/18
Upto 2 years	34/7	4/38	19/24	7/19
More than 2 years	20/44	18/28	13/24	4/21
Total	102/201	70/108	44/73	19/58
	(50.7%)	(64.8%)	(60.3%)	(32.8%)
Chi square values	70.861	7.852	10.014	55.793
P values	0.000	0.049	0.018	0.000

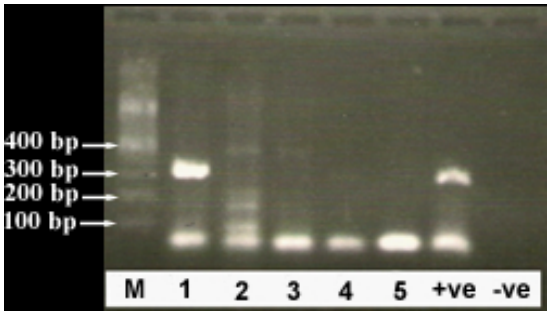

Fig. 1: Ethidium bromide stained agarose gel electrophrosis of the product amplified with PCR using specific primers for PPR virus. M; 100bp DNA ladder Lane 1 showing 372 bp size lanes 2-5 showing –ve samples; +ve lane showing vaccine positive band.

three prong strategy i.e. recording, detection of antibodies and detection of virus. Case recording helps to find out the location where this disease is epidemic. Conventional serological tests often gave confusing results due to cross reaction between PPR and RPV that is why specific technique is employed simultaneously for attaining more specificity and sensitivity in diagnosis (Abubakar *et al*., 2011a).

In the present study, the overall seroprevalence in sheep and goat population in Punjab was 51.5 and 46.5%, respectively, which was higher than the already reported 43.33% by Khan *et al*. (2007; 2008a). This difference may be due to wider spread of PPR virus particularly in the sheep and goat population reared probably without proper vaccination. The seroprevalence of PPR was significantly higher (P=0.000) in goats of central and western Punjab with 60 and 53.5%, respectively as compared to eastern and northern Punjab with 33.3 and 37.5%, respectively. The seroprevalence in sheep population was significantly higher (P=0.042) than the seroprevalence of the sheep samples collected from central Punjab.

Significantly higher seroprevalence of PPR in sheep and goat population in western Punjab has been reported by Khan *et al.* (2007), but in the present study goat samples showed significantly high prevalence. It may be possible that Khan *et al*. (2007) could have conducted a study having more emphasis on goat only. The seroprevalence was significantly higher in doeling upto 1 year of age followed by ewe with more than 2 years. Sheep showed higher seropositivity in ewe than in ram. These findings are in congruent with Khan *et al*. (2007), who reported that male are usually slaughtered at early ages and female sheep and goats are retained. Abubakar *et al.* (2009) also described concrete variation existing with species, age, sex, season, and geographical location as considerable factors associated with PPRV infection.

Molecular techniques such as PCR have emerged as highly specific and sensitive, which are also useful tools for molecular epidemiology and in molecular characterization of the virus. Nanda *et al*. (1996) developed highly sensitive RT-PCR using F-gene primers for detection of PPRV. In the present study, the prevalence of PPR virus detected by RT-PCR was 62.8 and 63.9% in sheep and goat, respectively. The goats were found more susceptible to PPRV infection than the sheep. Durrani *et al*. (2010), Dhand *et al*. (2002) and Kumar *et al*. (2002) conducted the prevalence studies of PPRV in different locations at different time and reported that goats are more susceptible than sheep. However, in present study difference between sheep and goat prevalence is non-significant. Shankar *et al*. (1998) on the other hand reported significantly higher incidence rate of PPRV in sheep (39.1%) than goats (23%). These results were higher than 50% as reported by Bahadar *et al.* (2009) that may be attributed to the difference in nature of infectious agent (Abraham and Berhan, 2001).

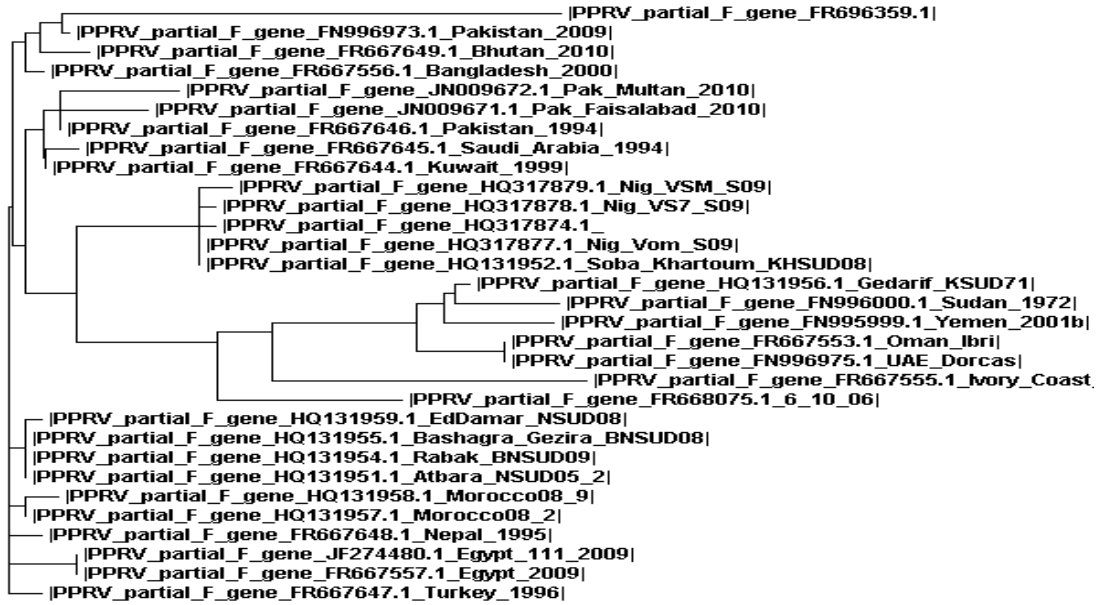

Fig. 2: Phylogenetic relationship among Pakistani and different isolates following alignment of sequences using clustal w 2 program of multiple sequence alignment.

Table 3: Region wise distribution of serum samples showing seropositivity of sheep and goat using c-ELISA test

	Goat Sampling				Sheep Sampling				Total	
Sampling Area	Total Tested	+ve Samples	-ve Samples	% +ve	Total Tested	+ve Samples	-ve Samples	% +ve	Total +ve	% +ve
Central Punjab (Faisalabad district)	5	3	2	60.0	82	42	40	51.2	45	55.6
Northern Punjab(Attock district)	16	6	10	37.5	57	31	26	54.4	37	45.9
Southern Punjab(D.G khan district)	58	28	30	48.3	78	45	33	57.7	73	52.9
Western Punjab(Bhakkar district)	43	23	20	53.9	60	34	26	56.7	57	55.1
Eastern Punjab(kasur ditrcit)	9	3	6	33.3	32	20	12	62.5	23	47.91
TOTAL	131/440	63	68	46.5	309/440	172	137	56.3	235	51.5
Chi square		43.806	37.882			9.919	15.737			
P values		0.000	0.000			0.042	0.003			

In F gene phylogenetic relationship the sequence in study clustered with sequence pattern from Pak 09 with accession no. (FN996973.1), Bhutan 10 (FR667649.1) and Bangladesh 2000 (FR667556.1), branching pattern following neighbor joining method of corresponding sequences indicating high mutation rate, which need to be investigated further (Fig. 3). Variation in F gene based on nucleotide variation for analysis of phylogenetic relationship. Similarity percentages in already reported and the sequence of F gene from the present study is in agreement with Munir *et al*. (2012).

Conclusion: It was concluded that young sheep and goat population is getting infection of PPR virus with higher frequency than the adults and the predilection site of PPR virus remained the lymph nodes.

REFERENCES

Abraham G and A Berhan, 2001. The use of antigen-capture enzyme-linked immunosorbent assay (ELISA) for the diagnosis of rinderpest and peste des petits ruminants in Ethiopia. Trop Anim Health Prod, 33: 423-430.

Abubakar M, S Ashiq, AB Zahoor, MJ Arshed and AC Banyard, 2011a. Diagnosis and control strategies for peste des petits ruminants virus: global and Pakistan perspectives. Pak Vet J, 31: 267-274.

Abubakar M, S Ashiq, Z Hussain, M Hussain, Sh Saleha, MJ Arshed and AB Zahoor, 2011b. Comparison of antigen detection methods of peste des petits ruminants virus in clinical samples of small ruminants. Bulg J Vet Med, 14:103-108.

Abubakar M, SM Jamal, MJ Arshed, M Hussain and Q Ali, 2009. Peste des petits ruminants virus (PPRV) infection; its association with specie, seasonal variation and geography. Trop Anim Health Prod, 41: 1197-1202.

Amjad H, QU Islam, M Forsyth, T Barrett and PB Rossiter, 1996. Peste des petits ruminants in goats in Pakistan. Vet Record, 139: 118-119.

Anderson K and JA Mckay, 1994. The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goat and the possible implications to rinderpest control programmes. Epidemiol Infect, 112: 225-231.

Anonymous, 2006. Agriculture Census Organization, Statistics Division, Government of Pakistan.

Asim M, A Rashid, AH Chaudhary and MS Noor, 2009. Production of homologous live attenuatedcell culture vaccine for the control of Peste des petits ruminants in small ruminants. Pak Vet J, 29: 72-74.

Athar H, QU Islam, F Azim, A Shakoor, A Maqbool, NI Chaudhary, 1995. An outbreak of peste des petits ruminants-like disease among goats in Punjab Pakistan. Pak Vet J, 15: 140-143.

Atta-ur-Rahman, M Ashfaque, SU Rahman, M Akhtar and S Ullah, 2004. Peste des petits ruminants antigen in mesenteric lymph nodes of goats slaughtered at DI Khan. Pak Vet J, 24: 159-160.

Bahadar S, AA Anjum, MD Ahmad and A Hanif, 2009 Isolation and identification of peste des petits ruminants Virus by Cell Culture And Immunocapture Enzyme Linked Immunosorbent Assay. J Anim Plant Sci, 19: 119-121.

Dhand NK, Sharma CS, Sandhu KS, Sharma DR and Singh J, 2002. Outbreaks of peste des petits ruminants (PPR) in Punjab. Indian J Anim Sci, 72: 853-854.

Diallo A, C Minet, CL Goff, G Berhe, E Albina, G Libeau, T Barrett, 2007. The threat of peste des petits ruminants: progress in vaccine development for disease control. Vaccine, 25: 5591-5597.

Durrani AZ, N Kamal, N Mehmood and AR Shakoori, 2010. Prevalence of peste des petits ruminants (KATA) in sheep and goats of Punjab. Pak J Zool, 42: 211-216.

Hussain M, M Afzal, R Muneer, M Ashfaq and EU Haq, 1998. An outbreak of peste des petits ruminants in goats in Rawalpindi. Pak Vet J, 18: 224-226.

Hussain M, R Muneer, M Jahangir, AH Awan, MA Khokhar, AB Zahur, M Zulfiqar and A Hussain, 2003. Chromatographic strip technology: A pen side test for the rapid diagnosis of peste des petitis ruminants in sheep and goat. J Biol Sci, 3: 1-7.

Khan HA, M Siddique, M Arshad, M Abubakar, M. Akhtar, MJ Arshad and M Ashraf, 2009. Post-vaccination antibodies profile against Peste des petits ruminants (PPR) virus in sheep and goats of Punjab, Pakistan. Trop Anim Health Prod, 41: 427-430.

Khan HA, M Siddique, MJ Arshad, QM Khan and SU Rahman, 2007. Seroprevalance of Peste des petitis ruminants (PPR) virus in sheep and goat in Punjab province of Pakistan. Pak Vet J, 27: 109-112.

Khan HA, M Siddique, SU Rahman, M Abubakar and M Ashraf, 2008a. The detection of antibody against peste des petits ruminants virus in Sheep, Goats, Cattle and Buffaloes. Trop Anim Health Prod, 40: 521-527.

Khan MA, NS Hussain, S Bahadar, A Ali and Al Shah, 2008b. An outbreak of Peste des petitis ruminants (PPR) in goats in district chitral, N.W.F.P., Pakistan. ARPN J Agri Biol Sci, 2: 19-22.

Kumar P, Kumar R, Sharma A and Tripathi BN, 2002. Pathology of peste des petits ruminants (PPR) in goats and sheep: spontaneous study. Indian J Vet Pathol, 26: 15-18.

Kwiatek O, C Minet, C Grillet, C Hurard, E Carlsson, B Karimov, E Albina, A Diallo and G Libeau, 2007. Peste des petits ruminants (PPR) outbreak in Tajikistan. J Comp Pathol, 136: 111-119.

Libeau G, A Diallo, D Calvez and PC Lefever, 1992. A competitive ELISA using anti-N monoclonal antibodies for specific detection of RP antibodies in cattle and small ruminants. Vet Microbiol, 31: 147-160.

Munir M, S Zohari, A Saeed, QM Khan, M Abubakar, NL Blanc and M Berg, 2012. Detection and phylogenetic analysis of Peste des Petits Ruminants Virus isolated from recent outbreaks in Punjab, Pakistan. Transbound Emerg Dis, 59: 85-93.

Nanda YP, A Chatterjee, AK Purohit, A Diallo, K Innui, RN Sharma, G Libeau, JA Thevasagayam, A Bruning, RP Kitching, J Anderson, T Barrett and WP Taylor, 1996. The isolation of peste des petits ruminants virus from northern India, Vet Microbiol, 51: 207-216.

Roeder PL, G Abraham, G Kenfe and T Barrett, 1994. Peste des petits ruminants in Ethopian goats. Trop Anim Health Prod, 26: 69-73.

Sande R, C Ayebazibwe, C Waiswa, F Ejobi, FN Mwiine, W Olaho-Mukani, 2011. Evidence of peste des petits ruminants virus antibodies in small ruminants in Amuru and Gulu districts, Uganda. Pak Vet J, 31: 363-365.

Shaila MS, S David, MA Foryth, A Diallo, L Goatley, RP Kitching and T Barret, 1996. Geographical distribution and epidemiology of PPR viruses. Virus Res, 43: 149-153.

Shankar H, Gupta VK and Singh N, 1998. Occurrence of pest des petitis ruminants like disease in small ruminants in Uttar Pradesh. Indian J Anim Sci, 68: 38-40.

Singh RP, BP Sreenivasa. P Dhar and SK Bandyopadhyay, 2004. A Sandwich-ELISA forthe diagnosis of peste des petits ruminants (PPR) infection in small ruminants using anti-nucleoprotein monoclonal antibody. Arch Virol, 149: 2155-2170.

Tahir MT, R Ahmad, I Hussain and M Hussain, 1998. Counter immunoelectrophoresis -A rapid technique for the diagnosis of peste-des-petitis ruminants. Pak Vet J, 18: 55-56.