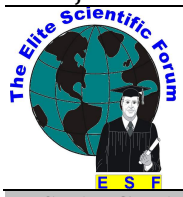
Pak. j. life soc. Sci. (2016), 14(1): 12-17 E-ISSN: 2221-7630;P-ISSN: 1727-4915



Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH ARTICLE

Seroprevalence and Molecular Detection of Peste des Petits Ruminants Virus (PPRV) in Different Breeds of Sheep and Goat of Punjab (Pakistan) and Its Status in Gravid Animals

Muhammad Moazam Jalees[1,*], Iftikhar Hussain[1], Muhammad Arshad[1], Ghulam Mohammad[2] and Qaiser Mahmood Khan[3]
[1]Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan
[2]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan
[3]National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

ARTICLE INFO
Received: Jan 10, 2016
Accepted: Mar 01, 2016
Online: Mar 04, 2016

Keywords
Sheep and goats
Peste des petits ruminants
RT-PCR
Punjab, Pakistan

***Corresponding Author:**
mmjalees@gmail.com

ABSTRACT

Present study was aimed to investigate the seroprevalence of peste des petits ruminants (PPR) in different breeds of sheep and goat population of Punjab, Pakistan. A total of 800 sera samples collected from apparently healthy/aborted animals were subjected to c-ELISA test. Further, the nasal swabs, lymph nodes, blood and spleen specimens were collected during various outbreaks from five districts of Punjab and molecular detection of PPR virus was performed by RT-PCR targeting fusion (f) protein genes. The overall breed wise seroprevalence was found maximum in Cholistani sheep (67.85%) as compared to other sheep breeds ($P>0.05$; $\chi^2=2.119$). Among the goats the Beetal breed showed significantly higher prevalence (61.58%) ($P<0.05$; $\chi^2=11.514$) than rest of the goat breeds under study. The seroprevalence of PPR was found apparently higher in pregnant goats (48.97%) and sheep (45.31%) as compared to non-pregnant animals. The abortion rate in sheep was found to be 19% while in goats it was 23%. The PCR results revealed a product of 372bp. In conclusion, all the sheep breeds under study showed a similar pattern of PPR susceptibility; whereas, the goat breeds such as Beetal was most susceptible, followed by Nachi, Hairy and teddy breeds. The persistent seroprevalence of PPRV in the pregnant sheep and goat population indicated a possible carrier state from which infection may be transmitted to the young ones vertically.

INTRODUCTION

Peste des petits ruminants (PPR) is an endemic disease of small ruminants affecting various regions of the world from Africa to Asia (Balamurugan et al., 2012, 2014; Dhar et al., 2002). PPR Virus contains an enveloped, pleomorphic negative-stranded RNA genome. Its genomic length is 15,948 nucleotides having six genes that encode eight proteins (Kumar et al., 2014). The clinical manifestations of PPR are sub acute to acute onset of symptoms, characterized by high grade fever (up to 106 °F), profuse diarrhea, stomatitis, oculonasal discharge and respiratory discomfort; pregnant animals may even abort (Khan et al., 2007). This infection may lead to 80-90% morbidity in affected flocks. Generally goats appear more severely affected than sheep (Abraham et al., 2005). The lesions are often complicated by secondary bacterial infections by Pseudomonas species and *E. coli*; enteric parasites (helminth and protozoa) if present and blood protozoans (Purushothaman et al., 2006). Based on these differences in prevalence between sheep and goat, it is considered to be more related to breed and local environmental differences (Abubakar et al., 2015).

According to a recent survey, a total of 98 million ovine and caprine heads were recorded. Among these, around 30 million were sheep while around 68 million were goats (Anonymous, 2014-15). More than 37 breeds of goats have been recognized while around 25 sheep breeds were found in the country. Heavy losses inculcated from PPR due to high rates of mortality and

morbidity in the affected flocks threatens livestock industry every year (Zahur et al., 2009). Annually, it steers a total loss of around Rs 20.5 billion in Pakistan (Abubakar et al., 2015). In addition, the incidence and final outcome of PPR in gravid animals is not well known in the region. There is strong evidence suggesting the association between PPRV infections and abortions in gravid animals (Abu Elzein et al., 2004; Abubakar et al., 2007; Kul et al., 2008). Keeping in view, the current study was designed to rule out the status of the disease in various breeds of sheep and goat population; its prevalence in pregnant/non pregnant sheep and goat and its possible association with abortions/stillbirths.

MATERIALS AND METHODS

Study area and period

The study was carried out from January 2010 to March 2011 in five different districts of Punjab, Pakistan. To make study more significant and diversified based on topography and population density of small ruminants, districts selected from various locations. Geographical coordinates of Punjab Province are as follows: Attock (North) 33°25'0" North (N) longitude and 72°19'60" East (E) latitude; Dera Ghazi Khan (South) 30° 3' 22" N, 70° 38' 4" E; Bhakkar (West) 31° 10′ N 72° E; Kasur (East) 31° 0′ 0″ N and 74° 10′ 0″ E and Faisalabad (Central) 30°31.5 N and 73°74 E (Fig 1).

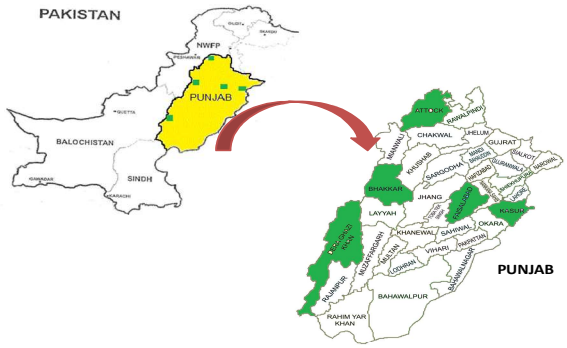


Fig. 1: Map of Pakistan (Punjab) showing the study location with highlighted districts.

Source of outbreak data

Sampling was done on the basis of number of reports of the different outbreaks of PPR disease in a particular area and from the apparently healthy flock/aborted animals of the same area. The data regarding the outbreaks was obtained from respective District Livestock Offices and National Veterinary Laboratory, (NVL) Islamabad Pakistan.

Sample collection

All the animals were randomly selected and bled for blood and serum samples.

Sample size calculation was made by the given formula (Thrusfield, 2013).

n= 1.962 Pexp (1- Pexp) / d2

Where;

n= required sample size

Pexp= expected prevalence

d=desired absolute precision

The number of samples calculated by this formula were subjected to following formula for the estimation of required sample size i.e.

n(adj)= (N x n) / (N + n)

Where:

N= Size of the study population

A total of 800 samples (582 females and 218 males) was collected from depending upon the population size of sheep and goat in the selected areas. Detailed information regarding age, sex, gravid status, vaccination history, previous exposure to the virus was taken about the collected samples. Their distribution with reference to various districts, tehsils (sub district) and species is presented in Table 1. The sample distribution was as follows: 279 from D.G khan district (179 sheep and 100 goats); 122 samples from Faisalabad (16 sheep and 106 goats); 205 from the Bhakkar district (128 sheep and 77 goats); 70 from Kasur (27 sheep and 43 goats); and 124 from the Attock district (50 sheep and 74 goats). Out of these 800 samples, female animals in the current study were 582. Out of these female animals, 64 sheep (n=280) were pregnant. In goats (n=302) 98 animals were pregnant.

Antibodies detection

The serological investigation was made by using the c-ELISA (competitive enzyme-linked immunosorbent assay) kit developed (ID.vet, ID Screen® PPR Competition CIRAD, Grabels, France). Results were interpreted according to the guidelines provided by the manufacturer.

Molecular identification

A set of primers F 5/ATCACAGTGTTAAAGCCTG TAGAGG3 and primer R 5/GAGACTGAGTTTGTGA CCTACAAG C3 was used to amplify the target F gene of PPRV. With the addition of molecular grade Depsi water, a total volume of 50 µl was set as a master mix for the PCR, which contains 5 µl cDNA, 5 µl PCR buffer, 1µl dNTP (10mM each), 1.5 µl of 50 mM Mgcl2, (iScript™ Bio Rad U.S.A). Downstream PCR (Polymerase chain reaction) was performed, the PCR reaction mixture was subjected to denaturation, annealing and extension as reported by Nanda et al. (1996). Subsequently 1.5 % agarose gel electrophoresis was performed for the estimation of PCR product and was visualized by UV trans-illuminator.

Statistical analysis

Data was subjected to the Statistical Package for Social Sciences (SPSS) V.17.0 for statistical analysis using Chi-square test. The results were considered significant at $P<0.05$.

RESULTS

Overall seroprevalalence was found highest in Cholistani breed (67.85%) followed by Khali breed (67.41%) and Latti breed (66.66%), whereas the difference was statisitcically non significant (P>0.05 with χ^2=2.119) Table 1. In goats highest seroprevalence was recorded in beetal breed (61.58%) and it was found to be 31 % to 35% in rest of three breeds under study (Nachi, Hairy and Teddy goat), whereas the difference was statistically significant (P<0.05 with χ 2 =11.514) Table 1.

District wise seroprevalence of PPR in sheep and goat population detected through c-ELISA test

Overall seroprevalence of PPR in sheep and goat was found at 61% and 44% respectively. Highest seroprevalence was found in sheep in the area of central Punjab (Faisalabad District) with 68.75%, followed by northern Punjab (Attock District) with 68% and its low percent positivity was found in Bhakkar District 58.6%, (χ^2 = 0.087, p=0.767) (Table 1). This percentage was lower when trial was performed on goats as it was found to be 53.48% in eastern Punjab (Kasur District) followed by central Punjab (Faisalabad District) 50% and northern Punjab (Attock districts) 48.64% ,(χ^2=2.197, P=0.138) (Table 1).

Seroprevalence of PPR on the basis of pregnancy status of animals

Overall seroprevalence of the PPR in pregnant animals was found to be apparently higher 48.97% in goats (χ^2=0.914, P= 0.339) and 45.31% in sheep (χ^2=0.014, P= 0.907) than in non- pregnant animals. By evaluating seroprevalence status district wise, it was found that maximum seroprevalence was detected (55.55%) in pregnant goats from southern Punjab district (Bhakkar) (Table 2). Furthermore, abortion percentage in sheep and goats were recorded 19% and 23% respectively (Fig 2).

Molecular Identification of PPRV

RT-PCR was employed to target the F gene and a PCR product of 372bp confirmed the presence of virus. Phylogenetic analysis revealed that the virus of the current study clustered in South East Asia and Middle East region (Fig 3). The National Center for Biotechnology Information (NCBI) accession number FR696359 has been given to the isolate of the present study. While exploring F gene Phylogenetic liaison, the sequence of study bunched with sequence pattern results obtained from Iran R22/10 with accession number (FN 995204), Nepal 09 (FN 996974) and Izatnagar 94 (KF 752444). This branching pattern was obtained by the help of the neighbor joining method of the corresponding sequences demonstrating high mutation rate, which require further investigation. Variation in F gene is based on nucleotide variation for analysis of Phylogenetic relationship. Similarity percentages have been already reported and the sequence of the F gene from the present study is in agreement with Munir et al. (2012) and Ashraf et al. (2015).

Table 1: District wise and breed wise seroprevalence of PPR in sheep and goat population

Areas	Sheep breeds						Goat breeds				
	Bahalwalpuri	Cholistani	Kahli	Lohi	Latti	Total (%)	Beetal	Nachi	Hairy	Teddy	Total (%)
Faisalabad	-------	------	-----	7/10	4/6	11/16(68.75)	34/38	10/36	4/17	5/15	53/106(50)
Bhakkar	--------	-----	40/56	35/72	------	75/128(58.6)	17/30	------	6/26	8/21	31/77(40.25)
Kasur	--------	-----	16/27	----	------	16/27(59.25)	16/22	4/12	------	3/9	23/43(53.48)
D.G.Khan	46/93	38/56	------	24/30	------	108/179(60.33)	12/28	11/23	4/29	6/20	33/100(33)
Attock	--------	-----	4/6	6/8	24/36	34/50(68)	14/33	------	14/17	8/24	36/74(48.64)
Total	46/93 (49.46%)	38/56 (67.85%)	(60/89) (67.41%)	72/120 (60%)	28/42 (66.66%)	244/400(61)	93/151 (61.58%)	25/71 (35.21%	28/89 (31.46%)	30/89 (33.70%)	176/400(44)

Goat breeds= χ^2 =11.514, p=0.009* Significant (P<0.05); Sheep breeds=χ^2=2.119, p=0.714 Non significant (P>0.05); District wise (goat) =χ^2=2.197, p=0.138 Non significant (P>0.05); District wise (sheep) = χ^2 = 0.087, p=0.767 Non significant (P>0.05).

Table 2: Seroprevalence of PPR virus distributed among pregnant sheep and goat population in five districts of Punjab

Sample Locale	Sheep sero-positive # (%)		Goat sero-positive # (%)	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
Faisalabad	2/4(50%)	2/6(33.33%)	16/30(53.33%)	18/46(39.13%)
Bhakkar	6/14(42.85%)	37/74(78.72%)	10/18(55.55%)	18/42(42.85%)
Kasur	4/10(40%)	3/8(37.5%)	3/8(37.5%)	10/22(45.45%)
D.G.Khan	11/23(47.82%)	43/106(40.56%)	11/25(44%)	19/58(32.75%)
Attock	6/13(46.15%)	10/22(45.45%)	8/17(47.05%)	16/36(44.44%)
Total	29/64(45.31%)	95/216(43.98%)	48/98(48.97%)	81/204(39.7%)

Sheep=χ^2=0.014, P-value=0.907 Non Significant (P>0.05); Goat=χ^2 0.914, P-Value=0.339 Non Significant (P>0.05).

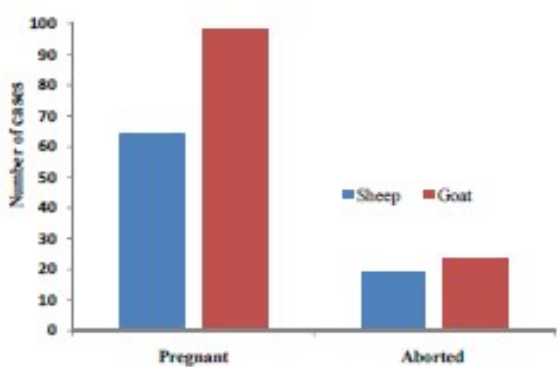


Fig. 2: Abortion percentage in sheep and goat infected with PPR Virus

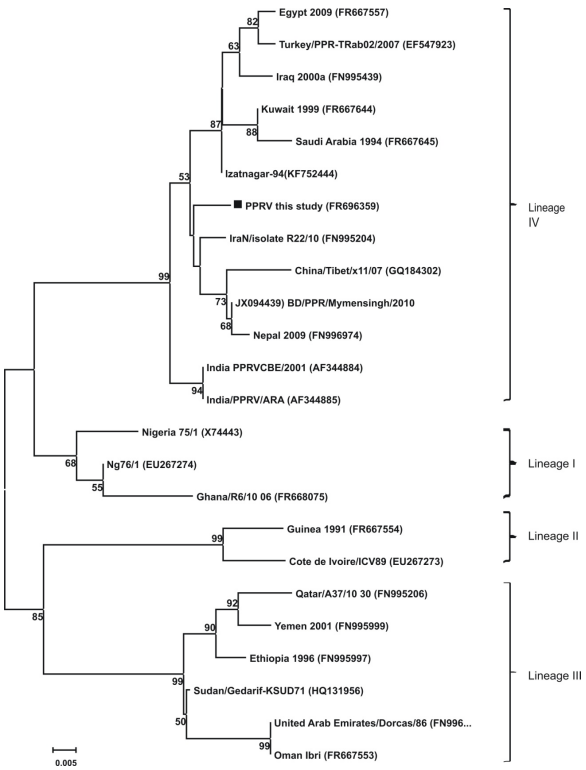


Fig. 3: Phylogenetic analysis using Mega4 (1000 bootstrap) neighbor joining method (with kimura-2 parameter model).

DISCUSSION

PPR is an acute viral infection of ovine animals and pose colossal economic importance (Hegde et al., 2009). The etiology of this threatening infection belongs to the genus Morbillivirus and family Paramyxoviridea (Ishag et al., 2015). The frequency distribution of the disease is variable and related to the region (Shaila et al., 1996).

The purpose of the current study was to estimate the seroprevalence of PPRV in different breeds of sheep and goat population and its incidence in gravid animals in Punjab. Overall seroprevalence of PPR in Pakistan was recorded at 48.5% by Zahur et al. (2011), however, in goats it was 52.9%, while in sheep it was recorded as 37.7%. Abubakar et al. (2009) calculated it in sheep as 54.9 % and in goats as 44.15 %. Khan et al., (2007) estimated the seroprevalence of PPRV in Punjab as 43.33%. Area wise, highest seroprevalence was calculated to be 55.10 % in sheep and goats in Sindh province. The other reported highest prevalence 76% was in Chakwal, followed by 75%, 64.71%, 64.28%, 61.29%, 60.66% and 60.31% in various districts i.e. Bahawalpur, Northern areas, Haiderabad, Sahiwal, Azad Jammu & Kashmir (AJK) and Rawalpindi, respectively (Abubakar et al., 2009). In the present study, overall seroprevalence of PPR in sheep and goat was found at 61% and 44%, respectively. Highest seroprevalence was found in sheep in the area of central Punjab (Faisalabad District) with 68.75%, followed by northern Punjab (Attock District) with 68%. This percentage was lower when trial was performed on goats as it was found to be 53.48% in eastern Punjab (Kasur District) followed by central Punjab (Faisalabad District) 50% and northern Punjab (Attock District) 48.64% (χ^2= 2.197; P=0.138). Sheep breed under study showed similar pattern of PPR susceptibility; whereas, the goat breeds such as Beetal was most susceptible, followed by Nachi, Hairy and Teddy breeds. There are some obvious causes which steer such elevation of the disease rate. These include the continuous spread of PPR virus in non-vaccinated population, (Jalees et al., 2013) unrestrained transportation of the animals and the imperfect quarantine measures.

Overall seroprevalence of PPRV in gravid sheep and goats was recorded as 45.31% and 48.97%, respectively. Highest seroprevalence of PPR in pregnant sheep was in Faisalabad (50%), whereas, in goats it was recorded up to 55% in District Bhakkar. Taken together, different districts showed variable seroprevalence of PPR in sheep and goat, but overall increased seropositivity was found in gravid animals. This increased seroprevalence in gravid animals may be related to the altered activity of immune system during pregnancy. There is an immunosuppressive environment within uterus to prevent severe immunological response against conceptus. In addition, under the influence of estrogen and progesterone, T-cell profile shifts towards helper T cells (Th-2) cell type in midgestation. The activation of uterine natural killer (uNK) cells and CD^{8+}/T is also inhibited on trophoblastic tissue (Schumacher et al., 2014). Overall, this immunosuppressive environment may favor the transmission of the virus to the fetus. Although the main transmission route of virus is horizontal and this virus is not known as abortifacient virus as yet, but a congenital form of PPRV has also been observed during

outbreaks (Kul et al., 2008). Our results completely endorse the above statement about the abortion, as in the findings of the present study about 19% and 23% PPRV prevalence was recorded among pregnant sheep and goats respectively. The same is evident from the study of Abu Elzein et al. (2004); Abubakar et al. (2007) and Kul et al. (2008) which is suggesting the association between PPRV infections and abortions in gravid animals. In another study, the same results were found, as a total of 105 abortions were reported among nine different goat flocks affected by PPRV (Kulkarni et al., 1996). Similarly 58 abortions were observed among 140 PPRV-infected gravid goats by Abubakar et al. (2007). These observations are reinforced by ozkul et al. (2008) who observed trans-placental transmission of the virus in sheep and goat off springs by immunolocalizing the virus in liver, lymphoid tissue and lungs. In the present study, we found the high prevalence of virus in pregnant animals. The higher prevalence of PPRV in precious breeds of sheep and goat and particularly in pregnant population is alarming which indicates the persistence of PPR virus and hence suggest stringent immunoprophylaxis measures in future.

Conclusion

In conclusion, PPRV is prevalent in gravid sheep and goats, higher prevalence of PPR was found in sheep than in the goat population. The F gene based PPRV detection and phylogenetic analysis showed that PPRV is homologous to virus prevalent in South East Asia and Middle East regions.

Acknowledgements

The author is thankful to Waqas Ashraf, Ameena Mobeen National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan and Dr Abubakar, Dr Javed Arshad National Veterinary Laboratory (NVL), Islamabad District Livestock Officer Faisalabad, Haider Ali Khan for laboratory work and provision of necessary facilities and being a source of information regarding outbreaks.

Authors' contribution

MMJ designed, executed the research work, data analysis and write up of article. IH supervised the research, and write up of the article. MA performed data analysis and facilitated lab work. GM wrote up and read the article. QMK provided molecular lab facilities and performed proof reading of article.

REFERENCES

Abu Elzein EME, FMT Housawi, Y Bashareek, AA Gameel, AA Afaleq and E Anderson, 2004. Severe PPR infection in gazelles kept under semi-free range conditions. Journal of Veterinary Medicine Series B, 51: 68–71.

Abubakar M, A Qurban and HA Khan, 2007. Prevalence and mortality rate of Peste des petits ruminants: possible association with abortion in goat. Tropical Animal Health and Production 40: 317-321.

Abubakar M, SM Jamal, MJ Arshed, M Hussain and Q Ali, 2009. Peste des petits ruminants virus (PPRV) infection; its association with species, seasonal variations and geography. Tropical Animal Health and Production, 41: 1197–202.

Abubakar M, M Irfan and S Manzoor, 2015. Peste des petits ruminants in Pakistan; past, present and future perspectives. Journal of Animal Science and Technology 57:32 DOI 10.1186/s40781-015-0066-0.

Abraham G, A Sintayehu, G Libeau, E Albina, F Roger, Y Laekemariam, D Abayneh and KM Awoke, 2005. Antibody seroprevalences against Peste des petits ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. Preventive Veterinary Medicine, 159: 51-57.

Ashraf W, QM Khan, A Mobeen, H Kamal, MM Jalees, M Farooq, A Diallo and H Unger, 2015. Molecular detection and genotyping characterization of PPRV in various areas of Pakistan, 2007 to 2012. Empress-animal health 360 | No. 45/2015 (http://www.fao.org/3/a-i4484e/i4484e05.pdf).

Anonymous, 2014-15. Economic survey of Pakistan, 2013-14. Ministry of Finance, Government of Pakistan, Islamabad, Pakistan.

Balamurugan V, P Krishnamoorthy, DSN Raju, KK Rajak, V Bhanuprakash, AB Pandey, MR Gajendragad, K Prabhudas and H Rahman, 2014. Prevalence of Peste des petits ruminant virus antibodies in cattle, buffaloes, sheep and goats in India. Virus Diseases, 1: 85–90.

Dhar P, BP Sreenivasa, T Barrett, M Corteyn, RP Singh, RK Singh and SK Bandyopadhyay, 2002. Recent epidemiology of peste des petits ruminants virus (PPRV). Veterinary Microbiology, 88: 153–159.

Hegde R, RG Amitha, HK Muniyellappa, SM Byregowda P Giridhar, and C Renukaprasad, 2009. Study on outbreaks of sheep and goat pox in Karnataka state of India. Indian Journal of Field Veterinarians, 4: 7-11.

Ishag OM, IK Saeed and YH Ali, 2015. Peste des petits ruminants outbreaks in White Nile State, Sudan. Onderstepoort Journal of Veterinary Research 82: 1-4.

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. Pakistan Veterinary Journal, 33: 165-169.

Khan HA, M Siddique, MJ Arshad, QM Khan and SU Rehman, 2007. Sero-prevalence of Peste des petits ruminants (PPR) virus in sheep and goats in Punjab province of Pakistan. Pakistan Veterinary Journal, 27: 109–112.

Kulkarni DD, MS Bhikane , MS Shaila, PV aralakshmi, MP Apte and BW Narladkar, 1996. Peste despetits ruminants in goats India. Veterinary Record, 138: 187–188.

Kul O, N Kabakci, A Özkul, KKalender and H TAtmaca, 2008. Concurrent Peste des petits ruminants virus and pestivirus infection in stillborn twin lambs. Veterinary Pathology, 45: 191–196.

Kumar N, S Maherchandani, SK Kashyap, SV Singh, S Sharma, KK Chaubey and H Ly, 2014. Peste Des Petits Ruminants Virus Infection of Small Ruminants: A Comprehensive Review. Viruses, 6, 2287-2327.

Munir M, S Zohari, A Saeed, QM Khan, M Abubakar and N LeBlanc 2012. Detection and Phylogenetic Analysis of Peste des Petits Ruminants Virus Isolated from Outbreaks in Punjab, Pakistan. Transboundary and Emerging Diseases, 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. Veterinary Microbiology, 51: 207-216.

Purushothaman V, S Hemalatha, G Ravikumar, R Govindarajan, N Sundar and A Kotteeswaran, 2006. An outbreak of Peste Des Petits Ruminants in small ruminants. Indian Veterinary Journal, 83: 133-136.

Shaila MS, D Shamaki, MA Forsyth, A Diallo, L Goatley, RP Kitching and T Barrett, 1996. Geographic distribution and epidemiology of peste des petits ruminants viruses. Virus Research, 43: 149-153.

Schumacher A, SD Costa and AC Zenclussen, 2014. Endocrine Factors Modulating Immune Responses in Pregnancy. Frontier in Immunology, 5: 196.

Thursfield M, 2013. Veterinary Epidemiology. Wiley Blackwell, USA.

Zahur AB, A Ullah, H Irshad, MS Farooq, M Hussain and M Jahangir, 2009. Epidemiological investigations of a Peste des petits ruminants (PPR) outbreak in Afghan sheep in Pakistan. Pakistan Veterinary Journal, 29: 174-178.

Zahur AB, A Ullah, M Hussain, H Irshad, A Hameed and M Jahangir, 2011. Sero-epidemiology of peste des petits ruminants (PPR) in Pakistan. Preventive Veterinary Medicine; 102: 87-92.