Pakistan J. Zool., vol. 42(3), pp. 211-216, 2010.

Prevalence of Peste des Petits Ruminants (KATA) in Sheep and Goats of Punjab

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Abstract.- The present study aims at determining the prevalence of an acute viral disease of small ruminants Peste des petits ruminants (PPR) caused by a *Morbillivirus* and is characterized by fever, oculonasal discharges, stomatitis, diarrhoea and pneumonia. Samples of nasal swabs and blood samples, 504 each, collected from sheep and goats showing signs of pneumoentritis in three different geographical regions viz. North, South and Central Punjab were screened for PPR virus by RT-PCR test. Out of 504 blood samples tested 79 were positive for PPR showing prevalence of 15.7 per cent. Of 504 nasal swabs collected from animals already sampled for blood, 85 samples were positive for PPR showing prevalence of 16.9 per cent in the small ruminant population of the Punjab province. Among the three regions prevalence was 24.7, 38.8 and 36.5 percent in North, South and Central regions of Punjab province respectively from nasal swabs. Thirty nine nasal swab samples from sheep and 46 from goat were found positive by RT-PCR giving a specie wise prevalence of 15.5 and 18.3 per cent, respectively in both species. The hematological observations of infected animals positive for PPR by RT-PCR test showed anemia in sheep and goats.

Keywords: Peste des petits ruminants, Pakistan, RT-PCR, Nasal swabs, Anemia.

INTRODUCTION

P*este des Petits Ruminants* (PPR) is an an acute,febrile, highly contagious disease of goats and less commonly in sheep, caused by a morbillivirus of paramyxoviridae and first described by Gargadennec and Lalane (1942) from Ivory Coast in West Africa. Like all members of the family, the PPR virus is an enveloped pleomorphic particle of size between 150 and 390 nm (Durojaiye *et al*., 1985) containing nonsegmented single stranded RNA genome of negative polarity. The disease resembles rinderpest and characterized by pneomoentritis.The disease is characterized by high fever, ocular and nasal discharge, pneumonia, necrosis and ulceration of the mucous membrane and inflammation of gastro-intestinal tract leading to severe diarrhoea (Gibbs *et al.*, 1979). Morbidity and mortality rates can be as high as 100 and 90 per cent, respectively (Abu-Elzien *et al.*, 1990). PPR outbreaks are now regular feature in different parts of Pakistan. Although serological tests were simple and cheap, they were not sensitive enough to detect low quantities of virus, especially if the quantities of viral antigen in pathological samples were dependent on the severity of the disease as suggested by some authors (Scott and Brown, 1961). In late 1980s, molecular technique based on nucleic acid hybridization using rinderpest and PPR specific cDNA probes was developed (Diallo *et al.*, 1989; Shaila *et al.*, 1989; Pandey *et al.*, 1992), later a polymerase chain reaction (PCR) technique using F-gene primers has been developed (Forsyth and Barett, 1995) which was found highly sensitive tool for diagnosis of PPR (Regasamy *et al*., 2007). In the present study an attempt has been made to standardize and apply RT-PCR for detection of PPR virus from field samples collected from three different geographical regions of Punjab province to find out the prevalence of disease in small ruminants.

MATERIALS AND METHODS

Sample collection

Nasal swabs and blood samples from unvaccinated sheep and goats in Lahore, Multan, Rawalpindi, Jhelum, Sheikhupura and Bahawalpur

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0030-9923/2010/0003-0211 $ 8.00/0

districts located in north, south and central regions of Punjab province were collected randomly during 2006-2007.The reference population of sheep and goats was screened on the basis of signs of pneumonia with entritis. A total of 504 samples consisting of 252 each from sheep and goats respectively were collected. Of the 252 sheep samples, 42 were from six districts each. Similarly, of the 252 goat samples, 42 samples were collected from each of six districts. Prevalence of PPR location wise and specie wise was recorded on the basis of RT-PCR. The samples were also processed for hematological observations.

Extraction of RNA from blood

Whole blood (200 µl) was taken in a centrifuge tube and to it, 20 µl of 5N acetic acid was mixed. The mixture was further processed for RNA extraction with TRIReagent® Guanidinium thiocynate-Phenol–monophasic solution: TRI Reagent® (Sigma Aldrich,USA, Cat No. T-9424 as described by Tiwari (2004). RNA was quantified by a spectrophotometer at 260 nm and its purity was judged on the basis of optical density ratio at 260:280 nm. The samples with acceptable purity (*i.e.* ratio 1.7-2.0) were qualified.

Extraction of RNA from Nasal swab suspension

Virus suspensions from swab samples and freeze dried PPR vaccine were prepared. Swab samples were suspended in one ml of 0.5X TE buffer, vortexed and squeezed along the wall of tube to drain out all the contents of the swabs. The virus suspension (250 µl) was taken in a 1.5 ml centrifuge tube, to which 750µl of TRI Reagent® was added and mixed by passing the suspension several times through a pipette. RNA pellet was dissolved in 25 µl of DEPC treated/ RNase free water and stored at -20°C. The methodology adopted for RNA extraction from nasal swabs was described by Tiwari (2004).

Reverse transcription

Omniscript™ Reverse Transcriptase Kit (Cat no. 205110, QIAGEN, Germany) for 50 reactions was supplemented with 100 units of Ribonuclease inhibitor (Cat no. R 2520, Sigma Aldrich, USA) before use. The method of reverse transcription adopted by Tiwari (2004) was used in the present study. For enzyme inactivation, the cDNA samples were heated up to 94^{o}C for 5 min**.** The cDNA formed was stored at - 40^{o}C until used for PCR. To confirm the synthesis of cDNA and integrity of reverse transcription step, alkaline gel (2%) electrophoresis of an aliquot of the cDNA was performed as per the method described by Sambrook and Russel (2001). cDNA (5 µl) was mixed with one µl of 6X alkaline gel-loading buffer, and electrophoresed at 3.5 V/cm. When the bromocresol green had migrated into the gel approx. 0.5-1 cm, the power supply was turned off, and a glass plate was placed on top of the gel. Electrophoresis was then continued until the bromocresol green migrated up to approximately two third part of the gel. The gel was soaked in neutralizing solution for 45 min at room temperature, followed by staining with 0.5 µg/ml ethidium bromide in 1X TAE for 30 min at room temperature. The gel was then visualized under UV light and documented by gel documentation system.

Polymerase chain reaction

Two sets of primers (synthesized by Sigma Genosys) spanning the lineage specific sequences of F-gene of PPR virus were used. Details of the primers are given in Table I. PCR Master Mix (Cat no. K0171, MBI Fermentas) containing 0.05U/µl *Taq*DNA polymerase (recombinant) in reaction buffer, $MgCl_2$ (4mM) and dNTPS (0.4 mM of each) was used in PCR. A reaction mixture was prepared as per details given in Table II. PCR tubes containing the mixture were tapped gently and spun briefly at 10,000 rpm. The steps and conditions of thermalcycler for PCR are shown in Table II. To confirm the targeted PCR amplification, five µl of PCR product from each tube was mixed with one µl of 6X gel loading buffer from each tube and electrophoresed along with 50bp DNA molecular weight marker (GeneRuler, MBI Fermentas) on 2.0 % agarose gel containing ethidium bromide (one per cent solution at the rate of 5 µl/100 ml) at constant 80 V for 30 min in 0.5X TBE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system.

Table I.- Details of primer used for RT- PCR.

Sr. No.	Primer	Primer sequence position	Position of primers on F-genes	Reference
1.	F1 (F)	5'ATC ACA GTG TTA AAG CCT GTA GAG G 3'	777-801	Forsyth and Barett
2.	F2 (R)	5' GAG ACT GAG TTT GTG ACC TAC AAG C 3'	1124-1148	(1995)
3.	F1b (F)	5'AGT ACA AAA GAT TGC TGA TCA CAG T- 3'	760-784	Dhar *et al.* (2002)
	F2d (R)	5'GGG TCT CGA AGG CTA GGC CCG AAT A-3'	1183-1207	

Table II.- Overall and Location-wise prevalence of PPR.

	Districts	No. of positive samples	Location wise +ive (%)	Overall +ive (%)
South	Bahawalpur	18	21.2	3.6
Region	Multan	15	17.6	2.98
	Total	33/85		38.8
Central	Lahore	18	21.2	3.6
Region	Sheikhupura	13	15.3	2.6
	Total	31/85		36.5
North	Jhelum	11	12.9	2.2
Region	Rawalpindi	10	11.8	1.98
	Total	21/85		24.7

RESULTS

Overall prevalence

A total of 504 nasal swabs and blood samples from sheep and goats collected from six districts of Punjab were screened for PPR using RT-PCR test. The primer pair F1 and F2 generated 372 bp amplicon, while F1b and F2d primers amplified approximately 448 bp amplicon (Fig. 1). Out of 504 blood samples tested, 79 were positive for PPR yielding an overall prevalence of 15.7 per cent. Of 504 nasal swabs collected from animals already sampled for blood 85 samples were positive for PPR yielding an overall prevalence of 16.9 per cent in the small ruminant population of the Punjab province included in the study.

Location-wise prevalence

Among the three regions prevalence was 24.7, 38.8 and 36.5 percent in North, South and Central regions of Punjab province respectively from nasal swabs. The location wise prevalence was highest in district Jhelum and district bahawalpur (21.2%) while lowest prevalence was recorded in district Rawalpindi (11.8%).

Species-wise prevalence

Of the 504 blood samples tested, 252 blood samples each were collected from sheep and goat. Thirty nine blood samples from sheep and 46 from goat were found positive by RT-PCR giving a species wise prevalence of 15.5 and 18.3 per cent, respectively in both species (Fig. 2.).

Hematological observations

The hematological observations post infection in animals positive for PPR by RT-PCR test shows anemia in sheep and goats. The comparative hematological observations showed that values of WBC, RBG and HgG were lower in sheep as compared with goats (Table III).

Table III.- Comparison of normal and post infection hematological values in goat and sheep.

Parameter	Goat		Sheep	
	Normal range	Post infection	Normal range	Post infection
WBC ($Lx10^3/\mu l$)	4-13	20.7	4-12	18.2
RBC($Lx10^6/\mu l$)	8-18	4.49	9-15	1.95
HgG (*g/dL)	8-16	11.7	9-15	6.8
PCV (%)	22-38	16.9	27-45	6.2
MCV(fL)	16-25	35.3	28-40	31.9
MCH (pg)	5.2-8	24.5	8-12	34.8
MCHC (g/dL)	30-36	69.3	31-34	78.6

DISCUSSION

PPR is an acute viral disease caused by a Morbillivirus that mainly affects sheep and goats (Singh *et al.*, 2004). The disease causes varying degree of morbidity and mortality in susceptible

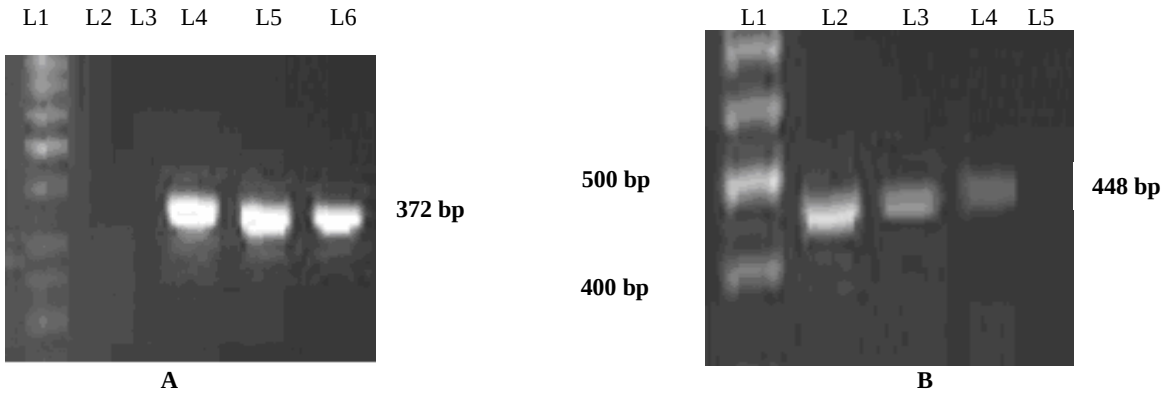

Fig.1: PPRV F-gene specific PCR product amplified with primer pair F1-F2 (A) and F1b-F2d. (B).
Lanes in A: L1, DNA marker; L2, negative control; L3, negative samples; L4-L6, positive control.
Lanes in B: L1, DNA marker; L2, positive control; L3, positive sample; L4, positive control; L5, negative control.

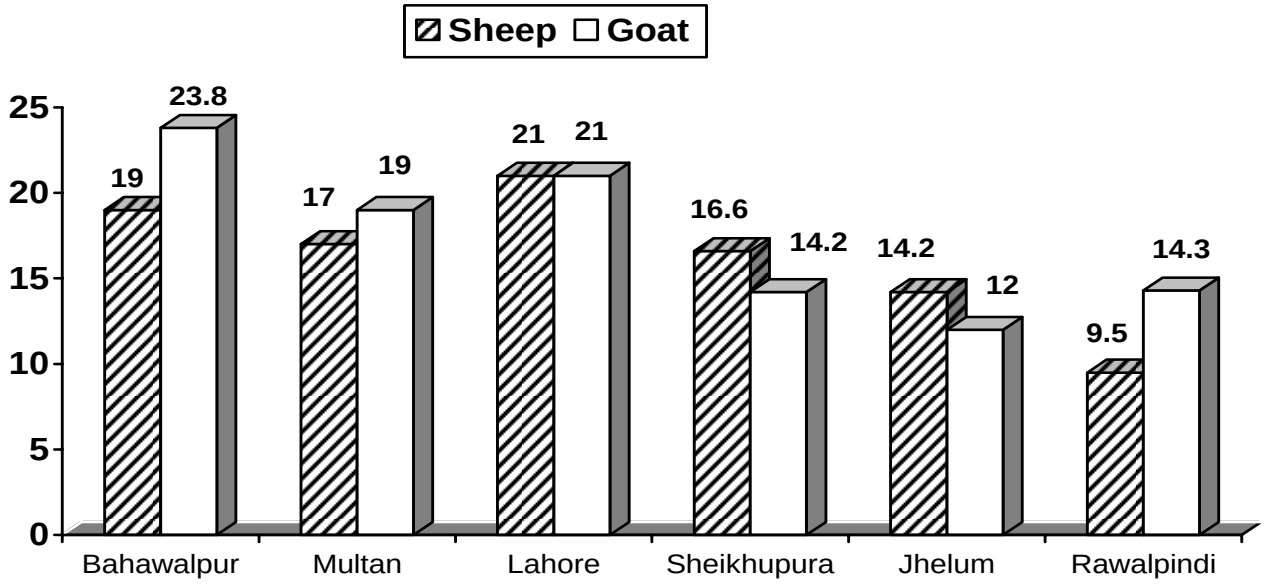

Fig. 2: Species wise prevalence of PPR in six districts of Punjab province.

population (Radostits *et al.,* 2000). Three methods may be used to diagnose and monitor the distribution and prevalence of PPR *i.e.,* Case recording of PPR outbreaks, serological detection of PPR specific antibodies and detection of the virus. Although, case recording of PPR outbreaks could give some clues in the areas where the disease is endemic, laboratory diagnosis is essential for confirmation. Conventional serological tests often fail in specific diagnosis of PPR due to cross reaction between PPR virus and RPV (Tiwari, 2004). Molecular techniques such as PCR have emerged as highly specific and sensitive tests, which are also useful in molecular haracterization of the virus. Forsyth and Barrett (1995) developed a highly sensitive PCR using F-gene primers for the detection of PPRV. In sheep and goats prevalence recorded in present study was 15.5 and 18.3 per cent respectively. Thus, it appears that goats were more susceptible to PPRV infection than sheep. This finding was in accordance with observations made by Dhand *et al.* (2002) and Kumar *et al.* (2002) in India. Obi *et al.* (1983) also reported five field outbreaks of PPR in Southern Nigeria between 1976

and 1980 where, only goats were affected. Similarly, Amjad *et al.* (1994) also observed outbreak of PPR among goats in Pakistan, where sheep kept on the same farm remained unaffected clinically. Taylor (1984) opined that sheep had possible innate resistance to PPR virus. On the contrary, Shankar *et al.* (1998) observed significantly higher incidence rate of PPRV in sheep (39.1%) than goats (23.0%).

Conventionally, detection of PPRV in field samples is carried out serologically or by virus isolation, which, besides less sensitive and cumbersome, are often difficult to interpret. The nucleic acid based detection assay like RT-PCR, overcome these limitations and have been successfully used for detecting the virus as described by Tiwari (2004). Among the various techniques developed for the detection of PPR virus, RT-PCR test developed by using F-gene primers (F1-F2), which is known to amplify a 372 bp fragment of F-gene (Forsyth and Barrett, 1995; Shaila *et al.*, 1996). However, Dhar *et al.* (2002) described another F-gene primer pair (F1b-F2d), which amplifies a 448 bp fragment of the F-gene encompassing the sequence amplified by primer pair F1-F2. Thus, RT-PCR test using the above two sets of F-gene primers, was used in present study. Results indicated that both the primer pairs performed equally in the present study .It was also evident that RT-PCR performed equally well with all the nasal swab samples 16.9 while, for blood samples 15.7% samples were found positive by RT-PCR, showing that RT-PCR using swab samples appears more efficacious than the blood samples. The pathological evidence of anemia was recorded during the present study in sheep and goats declared positive for PPR. The hematological values were lower in sheep as compared to goats.

REFERENCES

ABU-ELZEIN, E.M.E., HASSANIEN, M.M., AL-AFALEQ, A. I., ABD-ELHADI M.A. AND HOUSAWI, F.M.I., 1990. Isolation of peste des petits ruminants from goats in Saudi Arabia. *Vet. Rec.*, **127**: 309-310.

AMJAD, H., QAMAR, I., FORSYTH, M., BARRETT, T. AND ROSSITER, P.B., 1996. Peste des petits ruminants in goats in Pakistan. *Vet. Rec.*, **139**: 118-119.

DHAND, N.K., SHARMA, C.S., SANDHU, K.S., SHARMA, D.R. AND SINGH, J., 2002. Outbreaks of peste des petits ruminants (PPR) in Punjab. *Indian J. Anim. Sci*., **72:** 853-854

DHAR, P., SREENIVASA, B.P., BARRETT, T., SINGH, R.P. AND BANDYOPADHYAY, S.K., 2002. Recent epidemiology of peste des petits ruminants virus (PPRV). *Vet. Microbiol*., **8:** 153-159.

DUROJAIYE, O.A., TAYLOR, W.P. AND SMALE, C., 1985. The ultrastructure of peste des petits ruminants virus. *Zbl. Vet. Med. B*., **32:**460-465.

FORSYTH, M. AND BARRETT, T., 1995. Detection and differentiation of rinderpest and peste des petits ruminants viruses in diagnostic and experimental samples by polymerase chain reaction using P and F gene-specific primers. *J. Virol. Res*., **39**: 151-163.

GARGADENNEC, L. AND LALANE, A., 1942. La peste des petits ruminants. *Bull. Serv. Zootech. Epizoot. Afr. Occid. Francaise*, **5**: 16-21.

GIBBS, E.P.J., TAYLOR, W.P., LAWMAN, M.P.J. AND BRYANT, J., 1979. Classification of the pestedes-petits-ruminants virus as the fourth member of the genus *Morbillivirus*. *Intervirology*, **11:** 268-274.

KUMAR, P., KUMAR, R., SHARMA, A. AND TRIPATHI, B.N., 2002. Pathology of peste des petits ruminants (PPR) in goats and sheep: spontaneous study. *Indian J. Vet. Pathol*., **26**: 15-18.

OBI, T.U., OJO, M.O., TAYLOR, W.P. AND ROWE, L.W., 1983. Studies on the epidemiology of peste des petits ruminants in Southern Nigeria. *Trop. Veterinarian*, **1**: 209-217.

PANDEY, K.D., BARON, M.D. AND BARRETT, T., 1992. Differential diagnosis of rinderpest and PPR using biotinylated cDNA probes. *Vet. Rec*., **131**: 199-200.

RADOSTITS, O.M., GAY, C.C., BLOOD, D.C. AND HINCHCLIFF, K.W., 2000. Peste des petits ruminants. In: *Veterinary medicine*, 9th edition .W.B. Saunders, London. pp. 1077-1079.

RENGASAMY, R., MANICAVASA, K.A.D., KARUNAKARAN, K., BALASUBRAMANIAM, G.A., SELVARAJU, G. AND BALAKRISHNAN, S., 2007. Seroprevalence of peste des petits ruminants (PPR) in sheep and goats. *Tamilnadu J. Vet. Anim. Sci*., **3**: 135-139.

SAMBROOK, J. AND RUSSEL, D.W., 2001. *Molecular cloning: A laboratory manual*, 3rd Ed., Cold Spring Harbor Laborartory, Cold Spring Harbor, NY.

SCOTT, G.R. AND BROWN, R.D., 1961. Rinderpest diagnosis with reference to the agar gel double diffusion test. *Bull. Epizoot. Dis. Afr.*, **9**: 83-120.

SHAILA, M.S., PURASHOTHAMAN, V., BHAVSAR, D., VENUGOPAL, K. AND VENKATESAN, R.A., 1989. Peste des petits ruminants of sheep in India. *J. Indian Vet. Rec.*, **125**: 602.

SHAILA, M.S., SHAMAKI, D., FORSYTH, M.A., DIALLO, A., GOATLEY, L., KITCHING, R.P. AND BARRETT,

T., 1996.Geographic distribution and epidemiology of peste des petits ruminants virus. *J. Virol. Res*., **43:**149-153.

SHANKAR, H., GUPTA, V.K. 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, R.P., SREENIVASA, B.P., DHAR, P., SHAH, L.C. AND BANDYOPADHYAY, S.K., 2004. Development of a monoclonal antibody based competitive-ELISA for detection and titration of antibodies to peste des petits ruminants (PPR) virus. *Vet. Microbiol.*, **98**: 3-15.

TAYLOR, W.P., 1984. The distribution and epidemiology of 'peste des petits ruminants'. *Prev. Vet. Med*., **2**: 157-166.

TIWARI, A., 2004. *Prevalence of peste des petits ruminants (PPR) virus in Small ruminants of gujarat and its characterization by RT PCR/RFLP and SSCP profiles*. M.Sc. thesis. Department of Veterinary Microbiology college of veterinary science and animal husbandry Anand Agricultural University, Anand.

(Received 25 May 2009, revised 8 June 2009)