Indian J Anim Health (2023), 62(2): 286-291
DOI: https://doi.org/10.36062/ijah.2023.10822

Research Article

Comparative efficacy of S-ELISA, N & F gene based reverse transcriptase PCR and cell culture methods for detection of PPR virus in clinical specimens

M. M. Tajpara[1*], N. M. Shah[1], D. R. Patel[2], B. B. Bhanderi[3] and I. H. Kalyani[2]

[1]College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh- 362 001, Gujarat, India; [2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari- 396 450, Gujarat, India; [3]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand- 388 001, Gujarat, India

Abstract

The present study was aimed to detect PPRV in clinical samples using S-ELISA, N & F gene based reverse transcriptase PCR (RT-PCR) and cell culture techniques. A total of 119 clinical samples comprised of nasal (62), oral (16) and conjunctival swabs (20) and tissues (21) were collected from goats (95) and sheep (24) from different districts of Saurashtra region of Gujarat for detection of PPRV. Thirty-one animals were found positive for PPRV by all four tests. Two samples were found negative by N gene-based RT-PCR but positive by S-ELISA. Relative to S-ELISA, sensitivity, specificity, and overall agreement for N gene-based RT-PCR was 94.59%, 100% and 98.32%, respectively, while for F gene-based RT-PCR, it was 86.49%, 100% and 95.80%, respectively. Representative clinical samples positive for PPRV by S-ELISA (37) were subjected to isolation and propagation of PPR virus in Vero cells. Out of 37 S-ELISA positive samples, 31 samples showed CPE on first passage, which was further confirmed by RT-PCR.

Keywords: Cell culture, Goat, PPR, RT-PCR, S-ELISA

Highlights:

- PPR virus was detected from clinical samples of sheep and goats from Saurashtra region of Gujarat by sandwich ELISA (31.09%), F gene based RT-PCR (29.41%) and N gene based RT-PCR (26.89%) assays.
- PPR virus was isolated in cell culture (83.78%) from S-ELISA positive clinical samples.
- Relative to S-ELISA, sensitivity of F & N gene based RT-PCR was 86.49% and 94.59%, respectively.

INTRODUCTION

In India, sheep and goats play an essential role in the socio-economic development of rural households and are generally referred as "Any Time Money" to rural landless, marginal, and small landholding farmers. The majority of small ruminant population of the country is migratory in nature and moves to different places for grazing and water requirements. Goats suffer various infectious and non-infectious diseases, of which peste des petits ruminants, contagious ecthyma, viral pneumonia and goat pox are most predominant (Harish *et al.*, 2009; Balamurugan *et al.*, 2014).

PPR is a highly contagious, acute, febrile viral disease in goats and sheep, characterized by fever, anorexia, depression, nasal discharge, ocular discharge, anorexia, abortion, erosion of nasal mucosa, stomatitis, coughing and depression. PPR is caused by peste des petits ruminants virus (PPRV) under the genus *Morbillivirus* of the family *Paramyxoviridae* (Gibbs *et al.*, 1979). In India, PPR emerged as a real threat, especially after eradication of rinderpest (Saliki *et al.*, 1994). The disease is reported from all parts of India including Gujarat (Patel *et al.*, 2017; Sakhare, 2019). The World Organization of Animal Health (WOAH) has identified PPR as a notifiable and economically important transboundary viral disease of sheep and goats (Balamurugan *et al.*, 2010).

Conventional serological tests often fail to differentiate PPRV and RPV infections. The diagnosis of PPR is based on sandwich ELISA (Haq *et al.*, 2017), RT-PCR (Chukwudi *et al.*, 2020) and haemagglutination using piglet or chicken red blood cells (Shaila *et al.*, 1996). Virus isolation remains the "gold standard" for detection of the virus from the clinical samples (Fakri *et al.*, 2016). But in some cases, it requires high technical expertise, labor and is also expensive, besides the requirement of sterile condition of the samples. Monoclonal antibody based immunocapture (sandwich) ELISA (Libeau *et al.*, 1994) was found to be more sensitive than virus isolation (Saliki *et al.*, 1994).

*Corresponding Author, E-mail: tajpara1978@rediffmail.com

Molecular techniques such as PCR have emerged as highly specific and sensitive tests, which are also useful in molecular characterization of the virus. Forsyth and Barrett (1995) developed a highly sensitive PCR using F-gene specific primer for detection of PPRV. It has become the most popular tool so far for diagnosis as well as molecular epidemiological studies (Shaila *et al*., 1996). The N gene was found to be more suitable for detection of PPR from field samples than the F gene by RT-PCR (Sakhare, 2019).

Considering the above facts and importance of disease on the economics of sheep and goat farming, the present research work was carried out to establish the comparative efficacy of sandwich-ELISA, cell culture, and N & F gene-based RT-PCR for the detection of PPRV in clinical specimens.

MATERIALS AND METHODS

Sample collection: The study was carried out from January 2019 to December 2020. A total of 119 samples from goats (n=95) and sheep (n=24) of different districts of Saurashtra region were collected. The samples, including swabs (nasal, oral and conjunctival swabs) and tissue (lungs, intestine, spleen, etc.), were collected aseptically from clinically ailing animals showing symptoms suggestive of PPR and also from dead carcasses. All the samples were transported to the laboratory under a cold chain and stored at -80°C till further use.

Detection of virus: All the nasal, oral and conjunctival swabs and tissues like lungs, intestine and spleen were processed for detection of PPRV using S-ELISA and RT-PCR, while those samples positive in S-ELISA were also processed for isolation and identification of PPRV. S-ELISA was performed using PPR S-ELISA kit supplied by the Division of Virology, ICAR-IVRI, Mukteswar as per the instructions of the manufacturer (Singh *et al*., 2004).

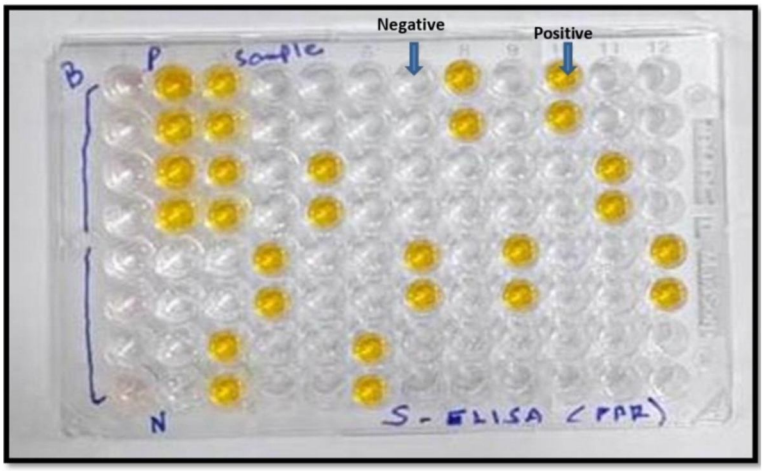
RT-PCR: PPR Sungri 95 strain was used as a positive control. Viral RNA was extracted using QIAamp Viral RNA Mini Kits (Qiagen, France) as per the manufacturer's instructions. RNA was quantified by spectrophotometric analysis using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). The reverse transcription and PCR were carried out sequentially in the same tube. The RNA obtained was converted to cDNA using a reverse transcriptase enzyme. The cDNA was amplified using PPRV specific N and F gene specific primers. The primers and PCR protocol for NP3 and NP4 (Couacy-Hymann *et al*., 2002) and F1 and F2 (Forsyth and Barett, 1995) were carried out for the detection of PPRV. RT-PCR was carried out in a final reaction volume of 50 µL using 200 µL capacity thin-walled PCR tubes comprising of 10 µL Qiagen one-step RT-PCR Buffer (5×), 2 µL of dNTP mix (10 mMol), 3.0 µL of each primer (10 pmol), 2 µL of Qiagen one-step RT-PCR enzyme mix, 10 µL of RNA template (30 ng/µL) and 20 µL of RNAse free water. The RT-PCR cycle conditions for N gene were reverse transcription at 50°C for 30 min, initial denaturation of 95°C for 15 min followed by 35 cycles of denaturation, annealing and extension at 94°C for 30s, 58°C for 30s and 72°C for 60s, respectively, and the final extension was carried out at 72°C for 5 min. The RT-PCR cycle conditions for F gene were reverse transcription at 50°C for 30 min, initial denaturation of 95°C for 15 min followed by 35 cycles of denaturation, annealing and extension at 94°C for 30s, 50°C for 60s and 72°C for 120s, respectively, and the final extension was carried out at 72°C for 10 min. Agarose gel electrophoresis was carried out to confirm the targeted amplification and documented by gel documentation system (Vilber-lourmat, France).

Virus isolation and identification: Isolation of PPR virus was carried out following the protocol given by Nanda *et al*. (1996). Thirty-seven clinical samples (12 tissues, 7 nasal swabs, 3 conjunctival swabs,7 oral swabs from goats and 2 tissues and 6 nasal swabs of sheep), ascertained positive for PPR virus by S-ELISA were further processed for isolation and identification of PPRV in Vero cell line. The Vero cell line was procured from National Centre for Cell Sciences, Pune (Maharashtra), India. The samples were filtered through 0.22 µm filters and 500 µL filtrate was inoculated in the tissue culture flasks (25 cm^2) with confluent Vero cell monolayer. DMEM (2.5 mL) supplemented with 2% FBS was added to each flask. The flasks were then incubated at 37°C for two hours with intermittent shaking for adsorption of the virus. At the end of incubation, flasks were filled with 7.5 mL of maintenance medium. Finally, the flasks were incubated in CO_2 incubator at 37°C with 5% CO_2 for 5 days with intermittent change of maintenance medium. The cells were observed for CPE under a microscope and harvested on the 5th day using three cycles of freeze-thaw.

RESULTS

S-ELISA: Out of 119 clinical samples, 37 samples were found positive by S-ELISA with an overall incidence rate of 31.09 percent. In case of goats, 30.52% (29/95) samples were detected positive, whereas in sheep, 33.33% (8/24) cases were confirmed as positive (Table 1 and Fig. 1).

Comparative diagnostic assays for PPR



[A1 to H1: - Antigen blank wells; A2 to D2: - Positive control; E2 to H2: - Negative control; A3 to D3, G3, H3, E4, F4, C5, D5, G6, H6, E7, F7, A8, B8, E9, F9, A10, B10, C11, D11, E12, F12: - Positive sample; Rest of wells are negative sample]

Fig. 1. S- ELISA for detection of PPR antigen in sheep and goats

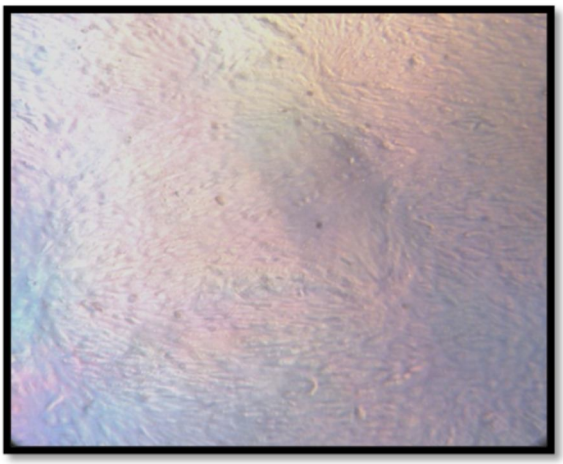


Fig. 4a. Uninfected Vero-cell

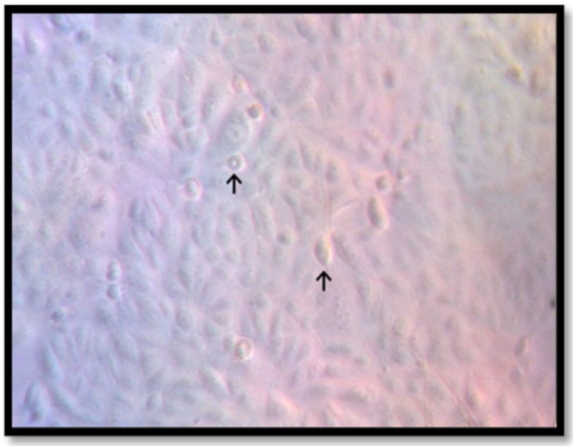


Fig. 4b. Ballooning of Vero-cell after 24-36 hours

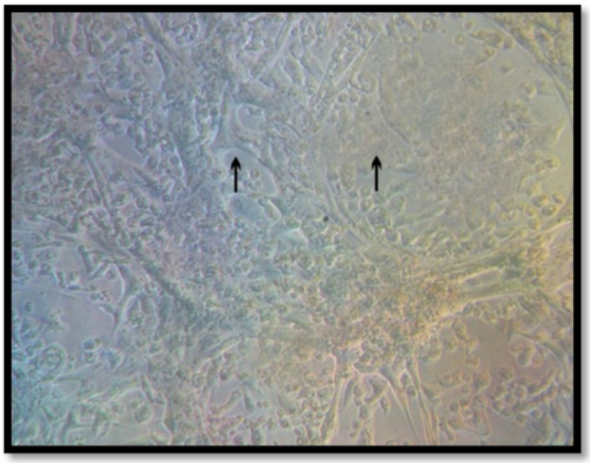


Fig. 4c. Aggregation of cells after 36-72 hours

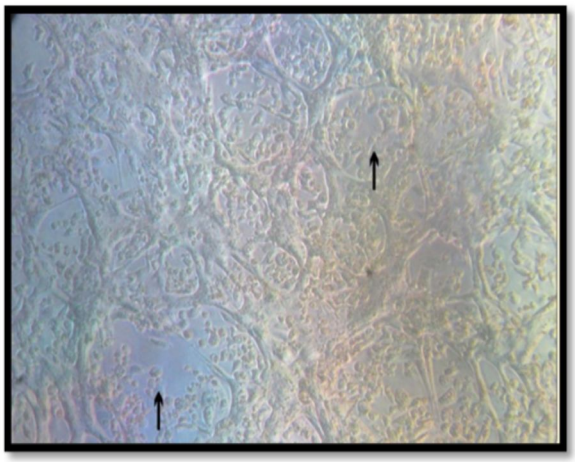
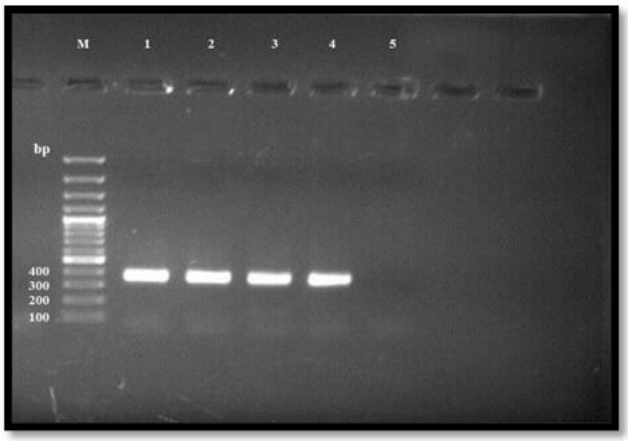


Fig. 4d. Fusion and syncytia formation after 72-96 hours

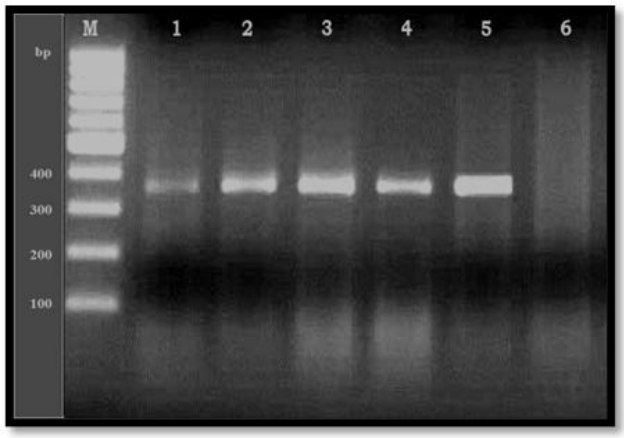
Table 1. Positive by S-ELISA, 'N' and 'F' gene RT-PCR and cell culture

Sl. no.	Types of samples	No of sample tested	Animals	S-ELISA positive	N-gene RT-PCR positive	F-gene RT-PCR positive	Cell culture positive
1	Nasal	40	Goat	7 (17.50)	7 (17.50)	6 (15.00)	6 (15.00)
2		22	Sheep	6 (27.27)	5 (22.72)	4 (18.18)	4 (18.18)
3	Conjunctival	20	Goat	3 (15.00)	3 (15.00)	2 (10.00)	2 (10.00)
4		00	Sheep	0	0	0	0
5	Oral	16	Goat	7 (43.75)	6 (37.50)	6 (37.50)	5 (31.25)
6		00	Sheep	0	0	0	0
7	Tissues	19	Goat	12 (63.16)	12 (63.16)	12 (63.16)	12 (63.16)
8		02	Sheep	2 (100)	2 (100)	2 (100)	2 (100)
Total (%)		119	Goat & Sheep	37/119 (31.09)	35/119 (29.41)	32/119 (26.89)	31/37 (83.78)



[1:- Positive control; 2, 3, 4:- Positive sample 5:- Negative control]

Fig. 2. Agar-gel electrophoresis showing F (372 bp) gene specific product for confirmation of PPR



[1 to 4: - Positive sample; 5: - Positive control; 6: - Negative control]

Fig. 3. Agar-gel electrophoresis showing N (351 bp) gene specific product for confirmation of PPR

RT-PCR: The result of N gene-based RT-PCR revealed 351bp product (Fig. 3) in 29.41% (35/119) samples, while F gene-based RT-PCR yielded 372bp product (Fig. 2) by 26.89% (32/119) samples (Table 1). The vaccine strain used in the study was also found to be positive for both genes.

Cell culture: Out of 37 S-ELISA positive clinical samples, 31 (83.78%) (10 nasal swabs, 2 conjunctival swabs, 5 oral swabs and 14 tissues) were found to be positive by cell culture on first passage. CPE characterized by ballooning of cells by 24-36 hr, and later on aggregation of the cells in 36-72 hr followed by formation of fusion mass and syncytia upto 72-96 hr post infection was observed. Cell lysis was also observed in some cases. The monolayer infected with sterile PBS (negative control) showed no such changes (Table 1 and Fig. 4a-4d).

DISCUSSION

The overall incidence of PPR was found to be 31.09% by S-ELISA. Incidence of PPR in goats in the present study remained lower than the observations reported by other workers in India so far, where the rate ranged from 54.54% to 66.7% (Tiwari, 2004; Malik *et al*., 2011; Mahajan *et al*., 2013; Muthuchelvan *et al*., 2014; Sakhare, 2019). Nagraj (2006) and Choudhary *et al*. (2009) also reported significantly high incidence of PPR in Gujarat. In the present study, the low incidence rate of PPR might be due to aggressive PPR control programs in Gujarat state through vaccination. Detection of PPRV antibodies can confirm the diagnosis of PPR; however, in areas where specific vaccination against PPR is practiced, detection of PPRV antibodies may yield a false picture of the prevalence of infection. The presence of maternal antibodies may further contribute to this

problem. Thus, in such cases, detection of PPR virus in clinical samples becomes essential.

Detection of N and F genes of PPRV using RT-PCR could be concluded that the disease outbreaks in the current study areas were caused by PPRV. N gene based RT-PCR was employed by Kerur *et al.* (2008), who amplified and characterized N gene and Kwiatek *et al.* (2010), who proposed that N gene is the most divergent and hence most appropriate for molecular characterization of closely related isolates. N gene codes for an internal structural protein, and also mRNAs of N gene are the most abundant transcripts of the virus, making it an attractive target for development of a highly sensitive and specific diagnostic assay for PPRV (George, 2002). Chowdhury *et al*. (2014) and Kgotlele *et al.* (2014) carried out N gene based PPRV detection. Luka *et al*. (2011) and Sundarpandian (2014) reported 51.52% and 9.7% positivity by F gene RT-PCR.

Isolation of the PPRV is the gold standard and confirmatory method of PPR disease in sheep and goats. Cell culture isolation of PPRV has been described by Ozkul *et al.* (2002) and Sakhare (2019) isolated PPR virus in Vero cells following first passage and also confirmed the isolates by RT-PCR. Biruk (2014) and Malik (2016) successfully isolated PPRV in Vero cells and detected CPE on day one after infection without any subsequent blind passage. Furley *et al.* (1987) isolated PPR virus in secondary calf kidney cells during second passage.

Comparative evaluation of Sandwich ELISA with N gene & F gene RT-PCR and cell culture: Out of the total 119 samples tested, PPRV could be detected in 37, 35 and 32 samples by S-ELISA, N gene based RT-PCR and F gene based RT-PCR, respectively. Out of 37 S-ELISA positive samples, PPRV could be detected in 31 samples by cell culture.

Thirty-one samples were positive to all four tests. Two samples negative by N gene RT-PCR were found positive by S-ELISA. Five samples negative by F gene RT-PCR were found positive by sandwich ELISA. Six samples negative by cell culture were found positive by sandwich ELISA. Compared to sandwich ELISA, the sensitivity and specificity of N gene based RT-PCR were 94.59 and 100 per cent, respectively as per the statistical formula (Samad *et al.*, 1994). Overall agreement between the two tests was 98.32 per cent (Table 2). Compared to sandwich ELISA, the sensitivity and specificity of F gene based RT-PCR was 86.49 and 100 per cent, respectively. Overall agreement between the two tests was 95.80 per cent (Table 3). Relative to sandwich ELISA, the sensitivity of cell culture was 83.78 per cent (Table 1).

Although RT-PCR was found to be better than virus isolation (Brindha *et al.*, 2001), very little information was available on the comparative efficiency of PPRV detection in the same field samples by RT-PCR and with any OIE/FAO approved field test such as Ic-ELISA. Thus, in the present study, the detection of PPRV was done in the same samples with both the tests, i.e., S-ELISA and gene based RT-PCR. A total of 119 samples were tested by both S-ELISA and RT-PCR. S-ELISA proved to be the most sensitive in detecting PPRV than N gene based RT-PCR, as it detected 37 samples by S-ELISA and 35 samples N gene based RT-PCR, respectively. Considering S-ELISA as the reference, the relative sensitivity of N gene RT-PCR was 94.59%. Significantly lower sensitivity of RT-PCR to sandwich-ELISA, i.e., 12.50 and 53.33 per cent was observed by George (2002) and Tiwari (2004), respectively; however, specificity was 100 per cent in their studies, using the F1-F2 primer pair

Table 2. Comparative evaluation of Sandwich ELISA and N- gene based RT-PCR in detection of PPRV

Test		S-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RT-PCR	Positive	35	00	35	(35/37) x100 = 94.59	(82/82) x100 = 100	(117/119) x100 = 98.32
	Negative	02	82	84			
Total		37	82	119			

Table 3. Comparative evaluation of Sandwich ELISA and F- gene based RT-PCR in detection of PPRV

Test		S-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RT-PCR	Positive	32	00	32	(32/37) x100 = 86.49	(82/82) x100 = 100	(114/119) x100 = 95.80
	Negative	05	82	87			
Total		37	82	119			

for PPRV detection.

Saliki *et al*. (1994) concluded that sandwich-ELISA was significantly more sensitive in comparison to single passage virus isolation. Comparatively, the higher significance of Sandwich ELISA than the RT-PCR in our study corroborates the results of Nagraj (2006) and Vartika (2006), who observed 73.33% and 65.22% specificity using F and N genes, respectively. Sakhare (2019) reported 89.18% sensitivity and 100% specificity using N gene based RT-PCR with S-ELISA. In contrast to our findings, Biruk (2014) found that those samples negative in S-ELISA were found to be positive by RT-PCR and vice versa with one sample.

PPRV could be detected in 31.09, 29.41 and 26.89 per cent by S-ELISA, N & F gene based RT-PCR, respectively. Those samples were positive in S-ELISA, in which 83.78% were positive in virus isolation. Relative to S-ELISA, the sensitivity and specificity of N gene based RT-PCR was 94.59 and 100 per cent, respectively. Overall agreement between the two tests was 98.32 per cent. Relative to S-ELISA sensitivity and specificity of F gene based RT-PCR were 86.49 and 100 per cent, respectively. Overall agreement between the two tests was 95.80 per cent. Relative to S-ELISA, sensitivity of cell culture was 83.78 per cent. The finding revealed that the comparison of S-ELISA, N & F gene based RT-PCR and cell culture methods were found to be comparatively sensitive and specific for the detection of PPR virus from clinical specimens.

Conflict of interest: The present research work has no conflict of interest.

Author's contribution: MMT: Performed sandwich ELISA, RT-PCR; DRP, IHK: Performed cell culture; MMT, NMS: Involved in statistical analysis, methodology and editing; BBB: Involved interpretation of result and final editing. All the authors contributed equally to the research work and writing of the research manuscript.

ACKNOWLEDGEMENT

Authors are thankful to the Animal Disease Investigation Office, Ahmedabad for providing sandwich ELISA kit.

REFERENCES

Balamurugan V, Hemadri D, Gajendragad MR, Singh RK and Rahman H, 2014. Diagnosis and control of peste des petits ruminants: A comprehensive review. Virusdisease, 25(1): 39-56, doi: 10.1007/s13337-013-0188-2

Balamurugan V, Sen A, Venkatesan G, Yadav V, Bhanuprakash V *et al*., 2010. Isolation and identification of virulent peste des petits ruminants viruses from PPR outbreaks in India. Trop Anim Health Prod, 42(6): 1043-1046, doi: 10.1007/s11250-010-9527-0

Biruk A, 2014. Epidemiology and identification of peste des petits ruminants (PPR) virus circulating in small ruminants of Eastern Amhara region bordering Afar, Ethiopia. MSc Thesis submitted to College of Veterinary Medicine and Agriculture, Bishoftu, Addis Ababa University. Available At: http://etd.aau.edu.et/handle/123456789/4319

Brindha K, Raj GD, Ganesan PI, Thiagarajan V, Nainar AM *et al*., 2001. Comparison of virus isolation and polymerase chain reaction for diagnosis of peste des petits ruminants. Acta Virol, 45(3): 169-172

Choudhary P, Jhala MK and Kanani AN, 2009. Incidence of PPR virus in Gujarat by S-ELISA and molecular detection by F, N and H gene based RT-PCR. R Vet J India, 5(1/2): 1-4

Chowdhury EH, Bhuiyan AR, Rahaman MM, Siddique MSA and Islam MR, 2014. Natural peste des petits ruminants virus infection in Black Bengal goats: virological, pathological and immunohisto-chemical investigation. BMC Vet Res, 10: 263-272, doi: 10.1186/s12917-014-0263-y

Chukwudi IC, Obgu KI, Luka PD, Malesa RP, Heath LE *et al*., 2020. Comparison of colorimetric loop mediated isothermal amplification kit and reverse transcription polymerase chain reaction in the diagnosis of peste des petitis ruminants in sheep and goats in southeast Nigeria. Vet World, 13(11): 2358-2363, doi: 10.14202/vetworld.2020.2358-2363

Couacy-Hymann E, Roger F, Hurard C, Guillou JP, Libeau G *et al*., 2002. Rapid and sensitive detection of peste des petits ruminants virus by a polymerase chain reaction assay. J Virol Methods, 100(1-2): 17-25, doi: 10.1016/s0166-0934(01)00386-x

Fakri F, Elarkam A, Daouam S, Tadlaoui K, Fassi-Fihri O *et al*., 2016. VeroNectin-4 is a highly sensitive cell line that can be used for the isolation and titration of peste des petits ruminants virus. J Virol Methods, 228: 135-139, doi: 10.1016/j.jviromet.2015.11.017

Forsyth MA and Barrett T, 1995. Evaluation of polymerase chain reaction for the detection and characterization of rinderpest and peste des petits ruminants viruses for epidemiological studies. Virus Res, 39(2-3): 151-163, doi: 10.1016/0168-1702(95)00076-3

Furley CW, Taylor WP and Obi TU, 1987. An outbreak of peste des petits ruminants in a zoological collection. Vet Rec, 121(19): 443-447, doi: 10.1136/vr.121.19.443

George AA, 2002. Comparative evaluation of different gene targets for PCR diagnosis of PPR. M.V.Sc thesis submitted to Deemed University IVRI, Izatnagar, India

Gibbs EP, Taylor WP, Lawman MJ and Bryant J, 1979. Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. Intervirology, 11(5): 268-274, doi: 10.1159/000149044

Haq AA, Ramasamy S, Chakravarti S, Yadav AK, Rajak KK *et al*., 2017. Investigation on peste des petits ruminants outbreak in goats of Bareilly district of Uttar Pradesh, India.

Vet Immunol Immunopathol, 19(1): 47-54, doi: 10.5958/0973-9149.2017.00007.7

Harish BR, Chandranaik BM, Shivaraj RH, Venkatesha MD and Renukaprasad C, 2009. Epidemiology and diagnosis of Peste des petits ruminants in Karnataka. Indian Vet J, 86(8): 773-775

Kerur N, Jhala MK and Joshi CG, 2008. Genetic characterization of Indian peste des petits ruminants virus (PPRV) by sequencing and phylogenetic analysis of fusion protein and nucleoprotein gene segments. Res Vet Sci, 85(1): 176-183, doi: 10.1016/j.rvsc.2007.07.007

Kgotlele T, Macha1 ES, Kasanga CJ, Kusiluka LJM, Karimuribo ED *et al*., 2014. Partial genetic characterization of peste des petits ruminants virus from goats in northern and eastern Tanzania. Transbound Emerg Dis, 61(Suppli1): 56-62, doi: 10.1111/tbed.12229

Kwiatek O, Keita D, Gil P, Fernández-Pinero J, Jimenez Clavero MAJ *et al*., 2010. Quantitative one-step real-time RT-PCR for the fast detection of the four genotypes of PPRV. J Virol Methods, 165(2): 168-177, doi: 10.1016/j.jviromet.2010.01.014

Libeau G, Diallo A, Colas F and Guerre L, 1994. Rapid differential diagnosis of rinderpest and peste des petits ruminants using an immunocapture ELISA. Vet Rec, 134(12): 300-304, doi: 10.1136/vr.134.12.300

Luka PD, Erume J, Mwiine FN, Ayebazibwe C and Shamaki D, 2011. Molecular characterization and phylogenetic study of peste des petits ruminants viruses from North Central States of Nigeria. BMC Vet Res, 7: 32, doi: 10.1186/1746-6148-7-32

Mahajan S, Agrawal R, Kumar M, Mohan A and Pande N, 2013. Incidence of peste des petits ruminants in nomadic sheep and goat of Jammu region. Vet World, 6(7): 384-387, doi: 10.5455/vetworld.2013.384-387

Malik M, 2016. Molecular Pathomorphology and Immunodiagnosis of PPR in Goats in Assam. Ph.D thesis, Assam Agricultural University, India

Malik YS, Singh D, Chandrashekar KM, Shukla S, Sharma K *et al*., 2011. Occurrence of dual infection of peste-des-petits-ruminants and goatpox in indigenous goats of central India. Transbound Emerg Dis, 58(3): 268-273, doi: 10.1111/j.1865-1682.2011.01201.x

Muthuchelvan D, De A, Debnath B, Choudhary D, Venkatesan G *et al*., 2014. Molecular characterization of peste-des-petits ruminants virus (PPRV) isolated from an outbreak in the Indo-Bangladesh border of Tripura state of North-East India. Vet Microbiol, 174(3-4): 591-595, doi: 10.1016/j.vetmic.2014.10.027

Nagraj, 2006. Assessment of different gene target for detection of peste des petits ruminants by RT-PCR and sequence analysis of F and N gene segments. M.V.Sc thesis submitted to Anand Agricultural University, Anand, India

Nanda YP, Chatterjee A, Purohit AK, Diallo A, Innui IK *et al*., 1996. The isolation of peste des petits ruminants virus from northern India. Vet Microbiol, 51(3-4): 207-216, doi: 10.1016/0378-1135(96)00025-9

Ozkul A, Akca Y, Alkan F, Barrett T, Karaoglu T *et al*., 2002. Prevalence, distribution, and host range of peste des petits ruminants virus, Turkey. Emerg Infect Dis, 8(7): 708-712, doi: 10.3201/eid0807.010471

Patel YR, Patel JM, Vihol PD, Kalyani IH, Sakare PS *et al*., 2017. Seroprevalence of peste des petits ruminants (PPR) in Navsari and Valsad districts of South Gujarat. Int J Curr Microbiol App Sci, 6(8): 221-228, doi: 10.20546/ijcmas.2017.608.030

Sakhare PS, 2019. Detection, isolation and molecular characterization of peste des petitis ruminants virus in small ruminants of south Gujarat. Ph.D Thesis, Vanbandhu College of Veterinary Science & Animal Husbandry, Navsari Agricultural University, Navsari. India

Saliki JT, Brown CC, House JA and Dubovi EJ, 1994. Differential immunohistochemical staining of peste des petits ruminants and rinderpest antigens in formalin fixed, paraffin-embedded tissues using monoclonal and polyclonal antibodies. J Vet Diagn Invest, 6(1): 96-98, doi: 10.1177/104063879400600117

Samad A, Awaz KB and Sarkate LB, 1994. Diagnosis of bovine traumatic reticulo peritonitis I: strength of clinical signs in predicting correct diagnosis. J Appl Anim Res, 6(1): 13-18, doi: 10.1080/09712119.1994.9706019

Shaila MS, Shamaki D, Forsyth MA, Diallo A, Goatley L *et al*., 1996. Geographic distribution and epidemiology of peste des petits ruminant viruses. Virus Res, 43(2): 149-153, doi: 10.1016/0168-1702(96)01312-3

Singh RP, Sreenivasa BP, Dhar P and Bandyopadhyay SK, 2004. A sandwich-ELISA for the diagnosis of pestedes petits ruminants (PPR) infection in small ruminants using anti-nucleocapsid protein monoclonal antibody. Arch Virol, 149(11): 2155-2170, doi: 10.1007/s00705-004-0366-z

Sundarpandian G, 2014. Development of DNA vaccine for PPR. Ph.D Thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai- 600 051, India

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.V.Sc Thesis, Anand Agricultural University, Anand, India

Vartika C, 2006. Assessment of novel N-gene target for detection of peste des petites ruminants (PPR) virus by RT-PCR and characterization by RFLP profile. M.V.Sc thesis submitted to Anand Agricultural University, Anand, India

Received- 18.11.2022, Accepted- 13.07.2023, published- 12.09.2023 (Online), 01.12.2023 (Print)

Section Editor: Prof. S. N. Joardar, Associate Editor