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Isolation of PPRV in vero cell line from Saurashtra region of Gujarat

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Abstract
The present study was designed to study isolation of PPR virus from various clinical samples and to observe cytopathic effects of PPR. For this, total 37 representative clinical samples positive for PPRV by S-ELISA were subjected for isolation and propagation of PPR virus in vero cells. Out of 37 S-ELISA positive samples, 31 samples showed CPE in first passage. The characteristic cytopathic effects includes ballooning of cells, aggregation of cells and syncytia formation. The conformation of virus was checked by N gene based reverse transcriptase PCR (RT-PCR).

Keywords: Cell culture, CPE, S-ELISA, vero cell line

Introduction
Despite the explosion of new techniques for 'same day diagnosis' of viral disease, virus isolation (VI) remains the "gold standard" against which the newer methods must be compared (Brindha *et al*., 2001) [1]. Also, VI is a prerequisite for studying the biological characterization of PPR virus. Vero cells have been the most commonly used host for isolation and cultivation of morbilli viruses including PPRV (Diallo *et al*., 1989) [2]. The pattern of PPR infection is largely determined by the property of tropism of PPRV to host cells. Cellular receptors are one of the major determinants of the host range restriction and tissue tropism. All the viruses initiate infection of susceptible cells by first binding to cell surface receptors. Although, PPR virus enters the host through respiratory tract, like other Morbilliviruses, the major site for viral replication is lymphoid tissues (Tatsuo *et al.*, 2001) [3]. After entering the upper respiratory tract, the virus exhibits pronounced tropism for monocytic and lymphocytic cells, and soon viral replication is detected in draining lymph nodes. Subsequent viraemia results in dissemination of the virus to visceral lymph nodes, spleen, bone marrow and mucosae of the gastrointestinal and respiratory systems. The PPR virus has got higher affinity for epithelial cells of gastrointestinal tract and lymphoid tissue. Like other Morbilli viruses, PPRV remains highly cell-associated during the course of its spread and can be isolated from blood leukocytes early during the infection.
The "gold standard" for any diagnostic test is the isolation and identification of causal agent of the specific disease. Gilbert and Monnier (1962) [4] were first to isolate PPR virus in cell culture. Nigerian strains of PPRV could be isolated in primary calf kidney (BK) cells but the cytopathic effects (CPE) were poorly defined and far harder to detect than those in lamb kidney cells. Sarkar (2006) [5] developed a stable Vero cell line constitutively expressing Caprine SLAM (Signalling lymphocyte activating molecule) and adapted PPR vaccine virus in both Vero and Vero/SLAM cells.

Material & Methods
Cell line
Vero cell line was procured from National Centre for Cell Sciences, Pune (Maharashtra), India. Vero cells (African green monkey kidney cells) were propagated in growth medium (GM) containing Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Sigma). The cells were maintained in DMEM supplemented with 2% FBS.

Sub culturing of cells
Vero cells were grown as per protocol described by (Ammerman *et al*., 2008) [6].

The Vero cell line was procured from National cell culture centre, Pune which had been transported in maintenance medium to our lab as monolayer in T-10 flask. Upon receipt of cell line, quality parameters were checked and cells were subcultured. The medium over the monolayer was decanted and cells were washed with sterile DPBS twice, then 1 ml warm TVGS (temperature-37 ℃) was poured in to flask with the help of 2 ml sterile plastic pipette with rubber bulb and removed after 30 seconds only. Again, 2 ml of TVGS was put in to flask and kept for 1 min, then medium was removed and a film of TVGS was left in the flask. Flask was kept at 37 ℃ for 5 min. Detachment of cell monolayer could be visualized against the tube light. As next step, 10 ml growth medium (DMEM added with 10% FBS with 1X antibiotic solution) was infused into flask and thereby serum in the medium blocked activity of trypsin. Medium was aspirated and infused repeatedly for 15-20 time with the help of 5 ml sterile pipette fitted with rubber bulb so that clumps of cells could be broken down. Two T- 25 cell culture flasks (Nunc, Denmark) were seeded from this cell suspension where each flask received 5 ml growth media. The flasks were incubated in CO_2 incubator (Labtech, Korea) at 37 ℃ and under 5% moist CO_2 concentrations. The cells were allowed to form a continuous monolayer sheet which usually took 48 hours. Otherwise medium was changed after every 2 days upon change of colour of medium to orange upon drop of pH drop towards acidic side. The same cycle of steps were repeated during the subculture of flasks keeping split ratio at 1:3. Sometimes growth surface was increased from T-25 to T-75 flask where subculture was done at split ratio of 1:1. Fifteen ml of growth medium was used instead of 5 ml for T-75 flask.

Preparation of inoculum

Preparation of swabs/clinical sample

Swabs were agitated in the transport medium to elute virus and left at room temperature for 30 min. The swabs were removed and supernatant was filtered through 0.22 µm filters and the filtrate was used as inoculums for virus isolation.

Preparation of tissue sample

The pooled tissue sample was cut into small pieces, ground in mortar and pestle using sterile sand. PBS (0.01M, pH7.4) was added to make 10% w/v suspension and then it was centrifuged at 2000 rpm for 15 minutes. The supernatant was collected and filtered through membrane filter (0.22 µm) and the filtrate was used as inoculum for virus isolation.

Virus isolation

Virus isolation was performed in Vero cell line by adsorption method using thirty seven samples (12 tissues, 7 nasal swabs, 3 conjunctival swabs,7 oral swabs from goats along with 2 tissues, 6 nasal swabs of sheep), which had given highest O.D. in s-ELISA. The isolation of PPR virus was carried out using a protocol given by Nanda *et al.* (1996) [7] with little modification. Tissue culture flasks (25 cm^2) with confluent Vero cell monolayer were taken and the media was decanted from the flasks. Tissue homogenates / swabs filtrate (500 µl) were inoculated on to confluent Vero cell monolayer. DMEM (2.5ml) supplemented with 2% FBS was added to each flask. The flasks were then incubated at 37 ℃ 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 ℃ with 5% CO_2 tension for further observation. Media were replaced the following day with maintenance media. Then, media were replaced each alternate day till 5th day. The inoculated monolayer was observed under inverted light microscope daily for evidence of cytopathic effect (CPE). Inoculated cultures were harvested after 5th day using three cycles of freeze (-80 ℃) thaw.

Confirmation virus from cell culture fluid BY RT-PCR

The cell culture fluid of the samples showed CPE were screened for the confirmation of PPRV by N gene-based RT-PCR. For this purpose, the cell culture fluids were subjected to RNA extraction using commercial RNA extraction kit QIAMP (Qiagen, France) as per manufacturer's instruction and then analyzed by RT-PCR.

Result and Discussion

In present study an S-ELISA positive 37 representative clinical samples i.e. tissues (N=14) and swabs (N=23) consisting (7 Nasal swabs, 3 conjunctival swabs, 7 oral swabs and 12 tissue from goats along with 6 nasal swabs and 2 tissue from sheep) were subjected to inoculation, isolation and propagation of PPR virus in Vero cells following standard protocol (OIE, 2013). Isolation of PPRV in Vero cells were successful from the first passage of the samples with the characteristic CPE which is in concordance with that described by the World Organisation for Animal Health (Adombi *et al.*, 2011; OIE, 2013) [8,9].

The presence of the virus in the Vero cells medium was confirmed by collecting and testing of the cell culture supernatant employing RT-PCR as per Singh *et al.* (2009) [10]. Thirty one samples could be grown on Vero cells in first passage. CPE characterized by ballooning of cells by 24-36 hrs (Plate 2), and later on aggregation of the cells in 36-72 hrs (Plate 3) followed by formation of fusion mass and syncytia upto 72-96 hrs (Plate 4) post infection were observed. Cell lysis was also observed in some cases. The monolayer infected with sterile PBS (negative control) did not show such changes (Plate 1). Out of S-ELISA positive 37 clinical samples, 31 samples including 10 Nasal swabs, 2 conjunctival swabs, 5 oral swabs and 14 tissues were found positive by Cell culture on first passage.

Table 1: Detection of viral genome in S-ELISA positive clinical samples by cell culture.

Sr. No.	S-ELISA positive clinical sample	Species of animals				Total	
		Goat		Sheep			
		Tested	Positive	Tested	Positive	Tested	Positive
01	Nasal Swab	7	6 (85.71)	6	4 (66.67)	13	10 (76.92)
02	Conjunctival Swab	3	2 (66.67)	0	0	3	2 (66.67)
03	Oral Swab	7	5 (71.43)	0	0	7	5 (71.43)
04	Tissue* Table (A)	12	12 (100.00)	2	2 (100.00)	14	14 (100.00)
Total		29	25 (86.21) ((86.21)	8	6 (75.00)	37	31 (83.78)

Note: Figures in parentheses indicate percentage.

Similar observations to the present study, have been described by Dhinakar Raj *et al*. (1992) [11] who have worked on isolation of PPRV from nasal swabs of ailing goat, Ozkul *et al.* (2002) [12] and Sakhare (2019) [13] isolated PPR virus in Vero cells following first passage and also confirmed the isolates by RT- PCR, Balamurugan *et al*. (2006) [14] isolated the PPRV successfully in Vero cells at passage level one after 8–10 days of infection. Biruk (2014) [15] and Malik (2016) [16] successfully isolated PPRV in vero cells and detected CPE on day 1 after infection without any subsequent blind passage. Furley *et al.* (1987) [17] isolated PPR virus in secondary calf kidney cells during second passage.

On the contrary, Nanda *et al.* (1996) [7] isolated PPR virus from sheep lung and intestine after two blind passages in Vero cells and observed characterized CPE as cell rounding, increased refractivity and slow detachment from supporting surface. Similarly, PPRV after three blind passages in Vero cells could be isolated by Brindha *et al.* (2001) [1] and Chaudhary (2009) [18]. Sarkar (2006) [5] developed a stable Vero cell line and studied the susceptibility of PPR vaccine virus where intensive CPE was found.

Contrary to our time period, Adombi *et al*. (2011) [8] detected syncytia after one day of infection, in CHS-20 cells. By day 2 the syncytia had enlarged so much that many of them had fused. By day 3 many cells had infection by PPRV resulted in the development of syncytia in a similar manner to that demonstrated by Vero cells expressing human, canine or bovine SLAM protein and infected by MV, CDV of RPV.

PPRV remains highly cell-associated during the course of its spread. Till date, the concept has been that entry of PPR virus in host cells is mediated by attachment of viral haemagglutinin (H) protein to cellular sialoglycoproteins (Murphy *et al*., 1999) [19], which might result in syncitia formation. In the present study, isolation of the virus indicated quick adaptation of the virus in the Vero cells. Quick adaptation of virus might be attributed to the quality of samples, proper collection, proper storage till inoculation and the quantity of inoculums, which greatly affect multiplicity of infection (Choudhary *et al*., 2009) [18].

Isolation of virus from fresh samples was more efficient than when deriving the virus from frozen samples. This difference could be explained by a decrease in virus infectivity during the freeze-thaw process. We found that isolation of wild-type PPRV was more efficient from post-mortem tissues than from swabs of infected animals. Thirty one cell culture passage lysates and reference vaccine virus were processed for RNA extraction using QIAmp mini viral kits. Reference vaccine virus as well as 31 cell culture passage aliquots of samples was confirmed by single compact band of 351 bp with NP3 and NP4 (Plate 5).

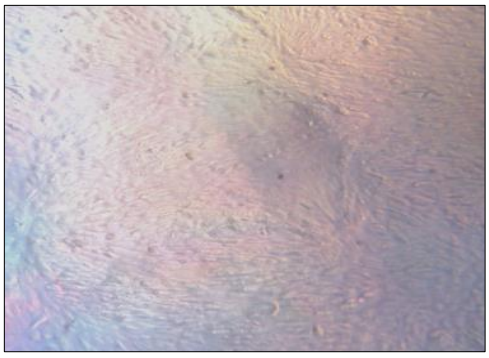

Plate 1: Uninfected vero-cell

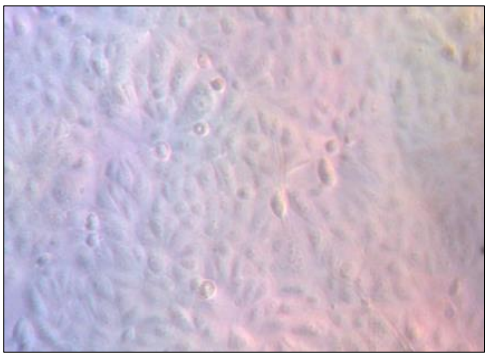

Plate 2: Balloning of vero-cell after 24-36 hours

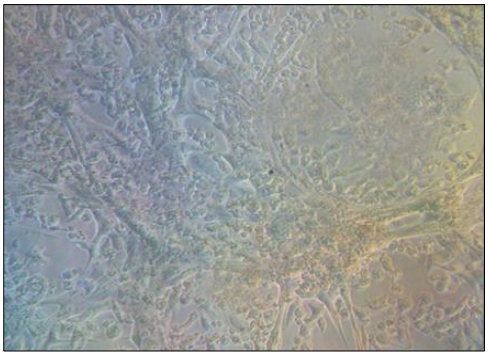

Plate 3: Aggregation of cells after 36-72 hours

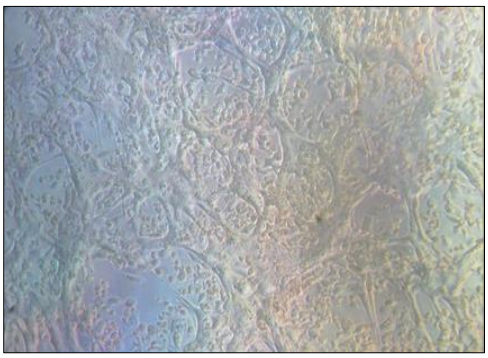

Plate 4: Fusion and syncytia formation after 72-96 hours

References

1. Brindha K, Raj GD, Ganesan PI, Thiagarajan V, Nainar AM, Nachimuthu K. Comparison of virus isolation and polymerase chain reaction for diagnosis of peste des petits ruminants. Acta Virologica. 2001;45(3):169-172.
2. Diallo A, Barrett T, Barbron M, Subbarao SM, Taylor WP. Differentiation of rinderpest and peste des petits ruminants viruses using specific cDNA clones. Journal of Virological Methods. 1989;23(2):127-136.
3. Tatsuo H, Ono N, Yanagi Y. Morbilliviruses use signaling lymphocyte activation molecules (CD150) as cellular receptors. Journal of Virology. 2001;75(13):5842-5850.
4. Gilbert Y, Monnier J. Adaptation des virus da le peste des petits ruminants aux cultures cellularies. Revue d'elevage et de medecine veterinaire des pays tropicaux, 1962;15:32-34.
5. Sarkar J. Molecular analysis of putative receptor (SLAM) for modulation of Peste des petits ruminants virus infectivity in Vero cells. Ph.D. Thesis. Submitted to Deemed University, IVRI, Izatnagar, India; c2006.
6. Ammerman NC, Beier-Sexton M, Azad AF. Growth and

maintenance of Vero cell lines. Curr. Protoc. Microbiol. 2008;11(1):A-4E.
7. Nanda YP, Chatterjee A, Purohit AK, Diallo A, Innui IK, Sharma RN, *et al*. The isolation of peste des petits ruminants virus from northern India. Veterinary Microbiology. 1996;51(3-4):207-216.
8. Adombi CM, Lelenta M, Lamien CE, Shamaki D, Koffi YM, Traore A, *et al*. Monkey CV1 cell line expressing the sheep-goat SLAM protein: a highly sensitive cell line for the isolation of peste des petits ruminants virus from pathological specimens. Journal of Virological Methods. 2011;173(2):306-313.
9. OIE. Peste des petits ruminants. Chapter 2.7.11. In Manual of diagnostic tests and vaccines for terrestrial animal health. World Organization for Animal Health (OIE), Paris (I and II); c2013.
10. Singh D, Malik YPS, Chandrasekhar KM. Design and evaluation of N gene primers for detection and characterization of peste des petits ruminants (PPR) virus from central India. Indian Journal of Virology. 2009;20:47.
11. Dhinakar Raj G, Thangavelu A, Nachimuthu K, Venugopalan TA. Cytopathic effect of peste des petits ruminants virus in Vero cells. Indian Journal of Animal Sciences. 1992;62(12):1135-1136.
12. Ozkul A, Akca Y, Alkan F, Barrett T, Karaoglu T, Dagalp SB, *et al*. Prevalence, distribution, and host range of Peste des petits ruminants virus, Turkey. Emerging Infectious Diseases. 2002;8(7):708-712.
13. Sakhare PS. Detection, isolation and molecular characterization of Peste des petitis ruminants virus in small ruminants of south Gujarat. Ph.D. Thesis, Vanbandhu College of Vet. Sci. &A.H., Navsari Agricultural University, Navsari. India; c2019.
14. Balamurugan V, Sen A, Sarvanan P, Singh RP, Singh RK, Rasool TJ, *et al*. One Step multiplex RT-PCR for detection of PPR virus in clinical samples. *Veterinary Research Communications*, 2006;30(6):655-666.
15. Biruk Alemu. 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; c2014.
16. Malik M. Molecular Pathomorphology and Immunodiagnosis of PPR in Goats in Assam. Ph.D. thesis, Assam Agricultural University. India; c2016.
17. Furley CW, Taylor WP, Obi TU. An outbreak of peste des petits ruminants in a zoological collection. Veterinary Record. 1987;121(9):443-447.
18. Choudhary P, Jhala MK, Kanani AN. Incidence of PPR virus in Gujarat by s-ELISA and molecular detection by F, N and H gene based RT-PCR. Royal Veterinary Journal of India. 2009;5(1/2):1-4.
19. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ. Veterinary Virology. 3rd Edn., Academic Press, San Diego, CA., USA.; c1999. p. 423-425. ISBN-13: 9780080552033.