**DETECTION AND ISOLATION OF *PESTE-DES-PETITS* RUMINANTS VIRUS (PPRV) INFECTION IN SHEEP AND GOATS**

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**ABSTRACT**

 The present study was conducted for isolation and molecular detection of PPR virus in sheep and goat in Punjab region. Samples of ocular (25), nasal (25) and rectal swabs (25) separately from sheep and goats were collected thus making it to total of 150 samples. These samples were subjected to Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for molecular detection of PPR virus. Out of 75 samples from 25 goats, three samples (4%) were found positive by F gene specific RT-PCR and all the samples from sheep were negative for PPR virus. From these samples, one PPRV isolate was isolated in Vero cell line.

**Keywords:** PPR, Sheep, Goat, RT-PCR, Virus isolation, Vero cell line

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 **INTRODUCTION**

 *Peste des petits ruminants (*PPR) are known by numbers of name viz goat plague (Dhar *et al* 2002). Peste des petits ruminants (PPR) is an acute, viral and highly contagious disease of sheep and goats characterized by fever, catarrhal inflammation of ocular and nasal mucous membrane ,diarrhea, leucopenia, erosive stomatitis, gastroenteritis and pneumonia (Barrett *et al* 2005). The causative agent, PPR virus (PPRV) is an enveloped RNA virus belonging to the genus Morbillivirus of the family *Paramyxoviridae* (sub family Paramyxovirinae) under the order Mononegavirales (Gibbs *et al* 1979).The virus is pleomorphic (Haffar *et al* 1999) and the genome is a negative sense single stranded-RNA, approximately 16 Kilo bases (kb) long with negative polarity (Chard *et al* 2008). Based on sequence analysis of the F gene, phylogenetically, there are four different lineages (I–IV) of PPRV. Whereas lineage IV is believed to be prevalent in India, other lineages are prevalent in African countries (Sen *et al* 2010). PPR was first described in 1942 by Gargadennec and Lalanne in the Ivory Coast, West Africa (Gargadennec *et al* 1942.) PPRV was first isolated in sheep cell culture in 1962 (Gilbert and Monnier 1962) and it was first time observed under electron microscope in 1967 (Bourdin and Laurent-Vautier 1967). In India, its presence was first reported in 1987. The first confirmed outbreak of PPR in sheep with 25 % mortality was reported in Arasur village, Villipuram district of Tamil Nadu during 1987 (Shaila *et al* 1989). Thus to study the status of PPR in region under study; the present study was undertaken to isolate PPR virus from sheep and goats and also to detect the virus using molecular techniques.

**MATERIAL AND METHODS**

**Sample collection**

The samples were collected during the period between August 2017 to April 2018. A total of 150 samples viz.ocular(25), nasal (25) and rectal swabs (25) each from sheep as well as goat were collected in phosphate buffer saline (PBS); pH-7.4 from the various regions of Punjab, India. The samples were collected irrespective of the breed, age, sex, vaccination status and health status of the animal.

**Processing of samples**

The ocular, nasal and rectal swabs were collected individually in 4 ml of PBS. The samples were divided equally into two different microcentrifuge tubes (2 ml each). Then the samples were centrifuged at 3000 rpm for 2 minute and supernatant was collected. One part of the sample was kept at -20°C for RNA extraction. To another part of the sample, 10µl of antibiotic- antifungal solution (Sigma, USA) was added and it was incubated at 37°C for 30 min. and later stored at -80°C for virus isolation.

**RNA isolation**

RNA isolation from ocular, nasal and rectal swab samples was performed by using the commercially available kit according to the manufacture’s guidelines (Takara RNAiso Plus, Japan).The extracted RNA pellet was eluted in 25µl of nuclease-free water and stored at -80°C.The purify of the RNA was checked by measuring the optical density at 280-nm wavelength by Nanodrop (Thermo Fisher Scientific.USA).

**First Strand cDNA Synthesis**

The extracted RNA was converted into cDNA by using the commercially available kit (Takara-Prime ScriptTM First Strand Synthesis cDNA was done by 8µl of RNA template,1µl of random hexamers and 1µl of dNTPs (2.5mM).It was incubated at 65°C for 5min and then cooled immediately on ice. To this mixture a master mixture was added which was prepared by adding 4.0µl of Prime Script Buffer (5x), 0.5µl of RNA inhibitor (40 U/µl), 1.0µL of Prime Script RTase (200U/µL) and 4.5µL RNase-free water. The PCR tubes were placed in the thermocycler with the following thermal conditions, initially at 30°C for 10min, followed by at 42°C for 60 min and finally 95°C for 5 min for inactivation of the enzyme. The cDNA synthesized was stored at -20°C.

**Amplification of F Gene of PPR Virus**

PCR amplification was carried out by set of F-gene-specific primers (Table 1).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S.No** | **Gene** | **Primer name** | **Primer Sequence** | **Product size (bp)** | **Reference** |
| **1.** | F-gene | **‘F’** | 5´GAGACTGAGTTTGTGACCTACAAGC-3´ | **372** | **Forsyth and Barret****(1995)** |
| **‘R’** | 5´ATCACAGTGTTAAAGCCTGTAGAGG-´3 |

 The PPR vaccine was used as positive control and nuclease-free water was used as negative control. The master mix was prepared by adding 5.0µL of cDNA, 2.5µL of 10x PCR buffer (with MgCl2), 1.0µL of dNTPs (2.5Mm each),0.5µL of Taq polymerase (5U/µL), 1.0µL each of forward (20 pmol/µL) and reverse primer (20 pmol/µL). The final volume was made 25µL with nuclease-free water. The reaction mixture was placed in thermocycler with following thermal cyclic conditions 1 cycle of 95°C for 5 min, 30 cycles of 94°C for 1 min, 50°C for 1min, 70°C for 7 min. PCR products (10µL) were visualized in 1.5% and run at 90V electrophorised gel for 30-35 min and visualized by gel documentation system.

 **Isolation of PPR Virus**

Vero cell line available at PPR Laboratory of IVRI, Mukteshwar (India) was used for isolation of the virus

**Virus isolation in vero cells**

Vero (African green monkey kidney) cells were propagated in Eagle’s minimum essential medium (EMEM, Sigma, ST.Louis, MO, USA) containing 10% bovine calf serum (BCS) and antibiotics viz., Penicillin G and streptomycin 100 unit and 100µg per ml respectively. Incubated at 37°C with 5%CO2 for 25-36hr, 25cc flask with 80-85% confluency were selected for virus infection. Then Discarded the EMEM and rinsed with fresh serum free EMEM 1ml of PCR positive PPRV inoculum were added to the flask for adsorption of virus. Incubated at 37°C for 1 hr with intermittent shaking of flask at 15min interval. After 1hr, fresh EMEM with 2-3% FBS was added and incubated at 37°C with 5% CO2. Observe the flask daily for CPE and after 10th day of post-infection cells showed 80% CPE. CPE was observed after 3 passages. Cells were harvested and kept at 4°C for further use. The cell culture supernatant of sample showing CPE was subjected to RT-PCR for further confirmation.

**RESULTS AND DISCUSSION**

A total of 150 samples (ocular, nasal and rectal swabs) from sheep as well as goat were collected and subjected to Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for molecular detection of PPR virus. Out of 75 samples from 25 goats, three samples (4%) were found positive by F gene specific RT-PCR showing an amplicon size of 372bp (Fig. 1) and all the samples from sheep were found negative for PPR virus. From the field samples, one PPRV isolate was isolated in Vero cell line. The presence of virus was further confirmed by RT-PCR.

 Luka *et al* (2012b) conducted F-gene based molecular studies on PPR suspected oculo-nasal and blood samples. They found that 67 samples out of 383 were positives**.**

Chowdhury *et al* (2014) detected viral RNA by RT-PCR in 69 out of 84 nasal swab 59 out of 84 blood and 21 out of 21 lymph node samples in Bangladesh.

Kiran *et al* (2017) also conducted F-gene based molecular studies on PPR suspected oculo-nasal swab samples and found 28 positive samples out of 100 samples.

 Ullah *et al* (2016) also observe isolation of PPR virus on Vero cells of faecal samples found positive in RT-PCR.

Kiran *et al* (2017) also subjected virus isolation of PPRV on B95a cell line and observed one sample showed the cytopathic effects (rounding of cells, syncytia formation and aggregation of cells) from four samples.

 Fallahi (2017) studied on diagnosis of PPR virus from oral and nasal samples of sheep and goats and observed two isolates and were confirmed by using RT-PCR. Similar findings were reported by Bahadar *et al* (2009) in nasal and ocular samples

**Fig.1. PCR for detection of PPR virus.**



372bp

500bp

 M 1 2 3 4 5 6 7 8 3 4 5

Lane M- Marker; Lane 1-Positive control; Lane 3,5,6 Positive samples; Lane 2, 4, 7 negative samples, Lane 8-Negative control

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