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Detection and Isolation of *Peste-des-petits* Ruminants Virus (PPRV) Infection in Sheep and Goats

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ABSTRACT

PPR also known as goat plague is an acute highly contagious disease of sheep and goats characterized by fever, catarrhal inflammation of ocular and nasal mucous membrane and diarrhea. The present study was conducted for molecular detection and isolation of *Peste des petits ruminants* (PPR) virus in sheep and goats in Punjab region. A total of 150 samples; ocular (25), nasal (25) and rectal swabs (25) separately from sheep and goats were collected. These samples were subjected to Reverse Transcriptase-PCR for molecular detection of PPR virus. Out of 75 samples from 25 goats, three samples (4%) were found positive for F gene by RT-PCR and all the samples from sheep were negative for PPR virus. PPR virus was isolated from one sample using vero cell line.

Key words: Goat, PPR, RT-PCR, Sheep, Virus isolation.

Peste des petits ruminants (PPR) also known as goat plague (Dhar *et al*., 2002) is an acute 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). This is an important disease affecting small ruminants and play an important role in the economy of the country as small ruminants are reared for milk, meat and wool and have a pivotal role in agriculture and employment generation of marginal farmers. The disease threatens food security and livelihoods of smallholders and affects the growth of animal husbandry sector (Gitao *et al*. , 2016). The morbidity of the infection is high approximately 80 to 90% and mortality between 50 to 80% (Lefevre and Diallo, 1990). The susceptibility of the disease is higher in goats as compared to sheep (Singh *et al*., 2009). 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 long (Chard *et al*., 2008). Among all the proteins of the virus, the fusion (F) and hemagglutinin (H) proteins constituting the majority of the viral envelope are responsible for induction of protective host immune response (Diallo *et al*., 2007). Based on sequence analysis of the F gene there are four lineages (I–IV) of the virus. The lineage IV is believed to be prevalent in India, other lineages are prevalent in African countries (Sen *et al*., 2010). In India, PPR was first reported in 1987 in an outbreak in sheep with 25% mortality in Arasur village, Villipuram district of Tamil Nadu (Shaila *et al*., 1989). Nowadays the importance of the disease has increased due to the government's initiative to control it under PPR control programme. Therefore, the present study was conducted to know the status of PPR in region under study.

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Sample collection and processing

The samples were collected during 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 goats were collected from the various regions of Punjab, India. The samples were collected irrespective of the breed, age, sex and vaccination status of the animal. The ocular, nasal and rectal swabs were collected individually in 4mlof PBS, pH 7.4. The samples were centrifuged at 3000 rpm for 2 minutes and supernatant was collected. One part of the supernatant was kept at -20°C for RNA extraction and to the other part was added 10 µl of antibiotic- antifungal solution (Sigma, USA) and incubated at 37°C for 30 minutes and 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 RNA iso Plus, Japan). The extracted RNA pellet was eluted in 25 µl of nuclease-free water and stored at -80°C.The purity 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 Script™ First Strand Synthesis cDNA). 8 µl of RNA template, 1 µl of random hexamers and 1 µl of dNTPs (2.5 mM) were mixed in 0.2 ml PCR tube and incubated at 65°C for 5 min and then cooled immediately on ice. To this mixture 4.0 µl of PrimeScript Buffer (5x), 0.5 µl of RNA inhibitor (40 U/µl), 1.0 µL of PrimeScript RTase (200U/µL) and 4.5 µL RNase-free water was added.The PCR tubes were placed in the thermocycler (QIAGEN) with the thermal conditions as 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 using F-gene-specific primers (Forsyth and Barret, 1995). The PPR vaccine was used as positive control and nuclease-free water was used as negative control. The master mix was prepared by adding 5.0 µL of cDNA, 2.5 µL of 10x PCR buffer (with $MgCl_2$), 1.0 µL of dNTPs (2.5 mM each), 0.5 µL of Taq polymerase (5 U/µL), 1.0 µL each of forward and reverse primer (20 pmol/µL each). The final volume was made 25 µL with nuclease-free water. The reaction mixture was placed in thermocycler with following thermal cyclic conditions 1 cycle of 95°C for 5 minutes, 30 cycles of 94°C for 1 minute, 50°C for 1 minute, 70°C for 7 minutes. PCR products (10 µL) were visualized in 1.5% agarose gel using gel documentation system (SYNGENE).

Isolation of PPR virus

Vero cell line available at PPR Laboratory of IVRI, Mukteshwar (India) was used for isolation of the virus. Vero cells were propagated in Eagle's minimum essential medium (EMEM, Sigma, USA) containing 10% bovine calf serum (BCS) and antibiotics *viz*., Penicillin G and streptomycin 100 unit and 100 µg/ml, respectively. Incubated at 37°C with 5% CO_2 for 25-36 h. 25cc flask with 80-85% confluency were selected for virus infection. Then EMEM was discarded and fresh serum free EMEM was added. One ml of PCR positive PPRV inoculum was added to the flask for adsorption of virus. It was incubated at 37°C for 1 h with intermittent shaking of flask at 15 min interval. After 1, fresh EMEM with 2-3% BCS was added and incubated at 37°C with 5% CO_2. The flask was observed daily for CPE and after 10^{th} day of post-infection, cells showed 80% CPE. CPE was observed after three passages. The cell culture supernatant of sample showing CPE was subjected to RT-PCR for further confirmation.

A total of 150 samples (ocular, nasal and rectal swabs) from sheep as well as goats were collected and subjected to RT-PCR for 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 372 bp (Fig 1) and all the samples from sheep were found negative for PPR virus. From 3 samples, PPRV was isolated from one sample using Vero cell line. The presence of virus was further confirmed by RT-PCR. Studies have been conducted by various researchers to detect PPR virus. Luka *et al*. (2012) conducted F-gene based molecular studies on PPR suspected oculo-nasal and blood samples. They found that 67 samples out of 383 were positive. Similarly, Chowdhury *et al*. (2014) detected viral RNA by RT-PCR in 69 out of 84 nasal swabs 59 out of 84 blood samples and 21 out of 21 lymph node samples in Bangladesh. Kaur *et al*. (2018) also conducted F-gene based molecular studies on PPR suspected oculo-nasal swab samples and found 28 positive samples out of 100 samples. They also subjected virus isolation in B95a cell line and observed one sample showed the cytopathic effects (rounding of cells, syncytia formation and aggregation of cells) out of four samples tested. Ullah *et al*. (2016) also observed isolation of PPR virus from fecal samples in Vero cells which were positive in RT-PCR. Fallahi (2017) studied on diagnosis of PPR virus from oral and nasal samples of sheep and goats and observed two isolates which were confirmed by using RT-PCR. Similar findings were reported by Bahadar *et al*. (2009) in nasal and ocular samples.

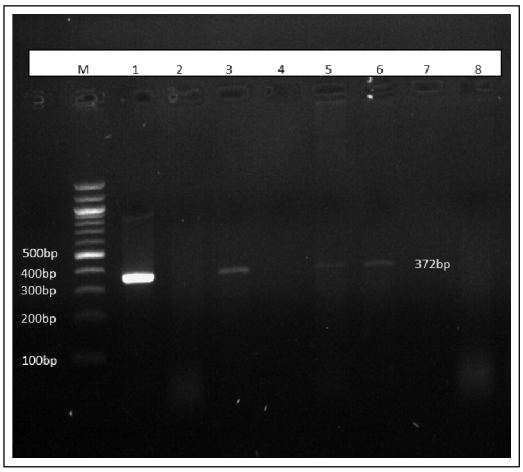

Fig 1: PCR for detection of PPR virus.
Lane M- Marker; Lane 1-Positive control; Lane 3,5,6 Positive samples; Lane 2,4,7 negative samples, Lane 8-Negative control.

CONCLUSION

Isolation and molecular characterization of PPRV was done. A total of 150 samples from sheep and goat (nasal, ocular and rectal) were collected and screened for PPRV. Out of 75 samples from 25 goats, 3 (4%) were found positive by F gene specific PCR. Out of 25 goats 3 (12%) goats (aged between 1-2.5 years) were found positive for PPRV. Out of

3 samples positive, 2 samples were ocular swabs and 1 was nasal swab. Out of 3 PCR positive samples, 1 was isolated in Vero cell line.

Conflict of interest: None.

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