

Original Research Article

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## Outbreaks of Peste Des Petits Ruminants (PPR) in Goats in Punjab, India

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### ABSTRACT

Peste des petits ruminants (PPR) is an OIE notifiable, highly contagious and fatal disease of small ruminants causing significant economic losses to the farmers in term of morbidity, mortality, preventive and treatment measures cost. The present study report three outbreaks of PPR in two districts of Punjab state affecting migratory flocks of goats in 2016-17. Younger animals were more susceptible to PPR than adults. None of the affected animals were vaccinated against PPR. Presumptive diagnosis of the disease was done on the basis of history, clinical signs, mortality pattern, postmortem and histopathological findings. Mesenteric lymph node collected from dead animal was found positive for F gene by RT-PCR which is specific to PPR virus. PPR has become endemic in the country and there is urgent need to educate the farmers regarding the vaccination of small ruminants against PPR to prevent the future losses.

#### Keywords

Outbreak, PPR, Goats, ELISA, RT-PCR.

#### Article Info

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### Introduction

Peste des petits ruminants (PPR) literally means ‘Plague of small ruminants’ is a highly contagious and fatal OIE enlisted, transboundary viral disease of small ruminants caused by *Morbillivirus* belonging to family Paramyxoviridae. The disease is characterized by high fever, oculonasal discharge, stomatitis, diarrhoea and pneumonia (OIE, 2014) causing significant economic losses (Venkataramanan *et al.*, 2005). Infected animals can transmit the virus to close in-contact susceptible animals through exhaled aerosol or clinical excretions *viz.* lacrimal, nasal, saliva, feces (Rossiter and Taylor, 1994). The first clinical description of

PPR was made in 1942 in West Africa and characterized by necrotic and erosive stomatitis, enteritis and pneumonia (Ismail *et al.*, 1995). In India, first PPR outbreak was recorded in Tamil Nadu in the year 1987 (Shaila *et al.*, 1989). Since then several authors reported the outbreaks of PPR throughout the country and PPR has now become endemic in the country. The outbreaks are diagnosed tentatively on the basis of clinical signs and confirmed by the detection of PPR virus or antibodies to it. The present communication reports the outbreaks of PPR in migratory flocks of goats in Punjab and describes the clinical features,

histopathological changes and confirmation by ELISA and RT-PCR.

## Materials and Methods

Three outbreaks of PPR were attended and investigated from two districts *viz.* Ludhiana (two) and Sangrur (one) of Punjab state affecting migratory flocks of goats in 2016-17. Clinical examinations of the goat were carried out and temperature, pulse rate and respiratory rate were recorded. None of the affected animal was vaccinated against PPR. Blood and sera samples from affected goats were collected. Blood samples were analyzed for complete hematology. The sera samples were stored at  $-20^{\circ}\text{C}$  until they were tested for antibodies to PPR using commercially available ELISA kit. A detailed Post-mortem examination of the one goat was conducted following routine procedures. Tissue samples from the affected lung, mesenteric lymph nodes and spleen were collected in 10% neutral buffered formalin for histopathology. The tissue samples were dehydrated and embedded in paraffin and sections (4-5 mm thick) were cut and stained with haematoxylin and eosin (H&E) as per standard protocol by Luna (1968). Spleen (n=1), lung (n=1) and mesenteric lymph node (n=1) from dead animal, and the rectal (n=2), nasal (n=3) and ocular swabs (n=3) of the ailing animals were collected and stored in  $-20^{\circ}\text{C}$  and subjected to RT-PCR. For Polymerase Chain Reaction, RNA was extracted using RNA isoplus (Takara) and cDNA was synthesized using commercially available cDNA synthesis kit (Takara) as per manufacturer's guidelines. PCR reaction was carried out with a reaction mixture (25  $\mu\text{l}$ ) containing 2.5  $\mu\text{l}$  of 10XPCR buffer with magnesium chloride, 0.5  $\mu\text{l}$  Taq Polymerase (5 unit/ $\mu\text{l}$ ), 1  $\mu\text{l}$  (20pmol/ $\mu\text{l}$ ) each of forward (5'ATC ACA GTG TTA AAG CCT GTA GAG G 3') and reverse (5' GAG ACT GAG TTT GTG ACC TAC AAG C 3')

primers (Forsyth and Barrett, 1995), 5  $\mu\text{l}$  of cDNA, 1  $\mu\text{l}$  of dNTP (2.5mM each) and 9  $\mu\text{l}$  nuclease free water. Thermal cycling was performed in T Gradient Thermocycler with the following cycling parameter i.e. initial denaturation at  $95^{\circ}\text{C}$  for 5 minutes, denaturation at  $94^{\circ}\text{C}$  for 1 minute, annealing at  $60^{\circ}\text{C}$  for 1 minutes, extension at  $70^{\circ}\text{C}$  for 2 minutes for 30 cycles and final extension at  $70^{\circ}\text{C}$  for 5 minutes.

## Results and Discussion

Peste des petits Ruminants is endemic in India and regular outbreaks have been reported from different parts of the country (Singh *et al.*, 2004). In the present manuscript, three outbreaks of PPR were investigated in two districts of Punjab during the period 2016-17. Out of 632 goats at risk, 167 were critically ill and 61 died of disease with morbidity, mortality and case fatality rate were 26.42%, 9.65% and 36.53% respectively (Table 1). Although goats and sheep are the primary hosts for the virus, goats seem to be more susceptible to disease than sheep (Nanda *et al.*, 1996). Case fatality rate was highest in goats of 3-6 months age group as compared to adults. Similar findings were observed by Kulkarni *et al.*, (1996) who reported significantly higher case fatality rate in kids. This might be due to higher infection of young ones with subclinical coccidiosis causing immunosuppressive effect or *Escherichia coli* infection causing fimbrial adhesions with intestinal mucosa and thus enhancing the effect of PPR virus (Kumar *et al.*, 2001). The morbidity rate can reach 100% with a high case fatality rate in the acute form of disease (Pope *et al.*, 2013; Karim *et al.*, 2016). Affected goats showed high fever ( $105-107^{\circ}\text{F}$ ) ocular and nasal discharges (Fig.1) and diarrhea with soiling of hindquarters. The ocular and nasal discharge was first serous and later on become mucopurulent leading to crusting of nasal passages

and gluing of eyelids (Fig. 2). In the severely affected animals, yellowish-white deposits were seen over the tongue (Fig. 3).

In few of affected goats, discrete necrotic lesions developed on the commissure of lips (Fig. 4), gums and conjunctivitis was also observed. Moreover, affected animals also showed laboured breathing and coughing. Death usually occurred within 7-10 days of start of clinical signs. Similar clinical signs and symptoms were observed by earlier workers (Shaila *et al.*, 1989; Amjad *et al.*, 1996; Aruni *et al.*, 1998; Shankar *et al.*, 1998; Dhand *et al.*, 2002) who reported purulent oculonasal discharge, severe cough and diarrhoea at terminal stages. The above described clinical signs and mortality can vary considerably depending on the virulence of the viral strain and the immunological state of the affected animal (OIE, 2014). Peste des petits ruminants' virus is highly lymphotropic and infection often leads to a profound immunosuppression that causes leucopenia

and reduced antibody responses (Rajak *et al.*, 2005; Pope *et al.*, 2013). In the present study, hematological examination revealed severe leucopenia as reported by erstwhile workers.

The post-mortem was conducted on one dead kid. Grossly, pneumonic lesions were seen in lung (Fig. 5) involving cranioventral lobe of the lung. The mesenteric lymph nodes were enlarged and hemorrhages were seen on mucosal surface of colon. Histopathological examination showed interstitial pneumonia with lympho-mononuclear cell infiltration in lungs. Lympho-mononuclear cell infiltration was also seen in liver and kidney. Similar gross and histopathological changes have been described previously (Zahur *et al.*, 2009; Roy *et al.*, 2010). The affected animals were treated with broad spectrum antibiotics to check the secondary bacterial infection and anti-inflammatory drugs. Farmers were suggested to restrict the movement of animals and local veterinarian was advised for PPR ring vaccination.

**Fig.1** Muco-purulent nasal discharge in affected goat



**Fig.2** Muco-purulent ocular discharge and Gluing of eyelids in affected goat



**Fig.3** Yellowish white deposits on tongue



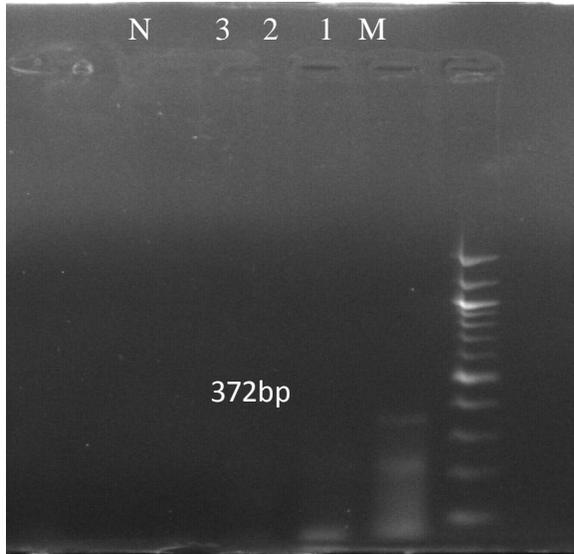
**Fig.4** Erosion of lip epithelium and conjunctivitis



**Fig.5** Pneumonic lesion in affected lung



**Fig.6** Lane: M: DNA ladder (100 bp), 1: Positive sample for F-gene PPRV, approximately at 372 bp, 2, 3: Negative samples, N: Negative Control



**Table.1** Epidemiological data related to outbreaks indicating morbidity, mortality and case fatality rate in goat

Outbreak No	Place	Species	Total animals at risk	Affected	Deaths	Morbidity (%)	Mortality (%)	Case fatality rate (%)
1	Ludhiana	Goat	32	32	1	100	3.13	3.13
2	Sangrur	Goat	200	100	40	50	20	40
3	Ludhiana	Goat	400	35	20	8.75	20.00	57.14
Overall			632	167	61	26.42	9.65	36.53

Confirmatory diagnosis of PPR is mainly based on immunocapture enzyme linked immuno sorbent assay (Singh *et al.*, 2004), polymerase chain reaction (Forsyth and Barrett, 1995) or virus isolation (Brindha *et al.*, 2001). In the present study, presumptive diagnosis was made from clinical symptoms, gross/ histo pathological lesions and ELISA. All serum samples collected from affected goats' revealed presence of antibodies to PPR by ELISA which may indicate recent or past infection. Confirmation of the disease was done by RT-PCR on nasal swabs, rectal swabs, lung tissue and mesenteric lymph nodes. Out of 11 samples collected from outbreaks, mesenteric lymph node sample was positive for F gene in which 372 bp product (Fig.6) was obtained which is highly specific to PPR virus (Forsyth and Barrett, 1995).

PPR has become endemic and outbreaks of disease have been reported throughout the country causing heavy morbidity and mortality. This outbreak resulted due to lack of education of the owner. Hence, there is urgent need to educate the farmers regarding the vaccination of small ruminants against PPR to prevent the future losses.

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