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Short Communication

Patho-morphological, Immunohistochemical and Virological Investigation of Natural Outbreak of Peste des Petits Ruminants (PPR) in Osmanabadi Goat

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Abstract

Patho-morphological and immunohistochemical investigation of a natural outbreak of PPR was carried out in Osmanabadi goat in Nagpur (Central India). The flock morbidity and mortality were 100% and 60% respectively. Gross examination revealed erosive stomatitis, cranioventral consolidation of an apical lobe of lungs and hemorrhagic enteritis with characteristic streaks of hemorrhages in the terminal ileum. Microscopically, lungs revealed diffuse infiltration of mononuclear cells, multinucleated syncytia formation and characteristic intracytoplasmic and intranuclear eosinophilic inclusions in the bronchiolar epithelium and alveolar macrophages. Lymphoid organs revealed marked depletion of lymphocytes. PPR viral antigen was detected in the cytoplasm of the bronchiolar epithelium, alveolar macrophages and, epithelial cells of the intestine with immunohistochemistry. Phylogenetic analysis confirmed the involvement of the lineage IV of PPRV.

Keywords: Goat, Immunohistochemistry, Lineage IV, PPR, Phylogenetic analysis

Introduction

Peste des Petits Ruminants (PPR) is an economically important and highly contagious viral disease of small ruminants. About 62.5 % of populations of total small ruminants are at risk due to PPR globally Libeau *et al.* (2014). The disease is considered as a major obstacle to the development of sustainable agriculture across the developing countries and global elimination of PPR by 2030 is targeted by the World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO) Banyyard *et al.* (2014). PPR frequently manifests as stomatitis, oculo-nasal discharge, pneumonia, and diarrhea; clinically and pathologically similar to rinderpest in cattle Bundza *et al.* (1988). It is caused by a Peste des Petits Ruminants Virus (PPRV), belonging to the genus *Morbillivirus,* family *Paramixoviridae.* The first outbreak of PPR was reported from the south Indian state, Tamilnadu in 1987 Shaila *et al.* (1989). Since then, many outbreaks are being reported from different regions of the country and now the disease is prevalent in all parts of India. The phylogenetic analysis has divided the isolates into 4 genetic lineages Shaila *et al.* (1996). Lineage I to III are found in Africa, while all Asian and maximum Middle Eastern isolates belong to linege IV. Outbreaks of PPR involving Asian lineage (IV) have been reported in Africa and European part of Turkey emphasizes the need of continuous surveillance of the disease in endemic areas Banyard *et al.* (2010). The present study describes the pathomorphological, immunohistochemical, and phylogenetic analysis of a natural outbreak of PPR in Osmanabadi goat.

Materials and Methods

An outbreak in Osmanabadi goat having flock size of 100 with a history of fever, anorexia, dyspnoea and diarrhea occurred at goat farm of Nagpur Veterinary College, Nagpur during the month of January 2019. Animals were treated with an antibiotic. The age group of the flock was 4 months and they were purchased recently from the nearby area of Nagpur. Morbidity and mortality rates were 100% and 60% respectively. Detailed postmortem examination was conducted at the Department of Pathology and gross lesions were recorded. Representative tissue samples of lungs, trachea, intestine, mesenteric lymph nodes and spleen were collected in 10% neutral buffered formalin for histopathology and also collected on ice for molecular diagnosis. Tissue samples were processed routinely for paraffin embedding sections and H&E staining (Bancroft and Gamble, 1998). RNA was isolated from spleen and mesenteric lymph nodes by Trizol method and cDNA was synthesized as per manufacturers protocol (Promega, USA). RT-PCR was carried out for detection of N gene using previously published primers (Couacy-Hymann *et al.* (2002). The PCR products were separated on 2% gel electrophoresis. PCR products sequenced commercially (Eurofins, India). The sequence was aligned and a phylogenetic tree was constructed in MEGA 6. For IHC, paraffin-embedded sections of lungs, intestines, and mesenteric lymph nodes were taken on APES (Sigma, USA) coated slides. The sections were deparaffinized by 3 changes of xylene 5 min each and rehydration in descending grades of ethyl alcohol (100%, 90%, 80%, 70%, 60%, and 50%) The antigen retrieval was carried out by boiling sections in sodium citrate buffer (pH- 6.0) for 20 min in the microwave oven. The endogenous peroxidase activity was quenched by immersing the slides in 3% hydrogen peroxide in distilled water solution for 30 min and then washed thrice with PBST for 5 min. The sections were incubated with 5 % normal goat serum for 1 hr to block nonspecific binding. The slides were washed thrice with PBST and incubated at 4°C overnight with primary anti-PPR monoclonal antibodies diluted 1:100 in 1% bovine serum albumin. Secondary anti-mouse IgG antibody HRPO (Sigma, USA) diluted at 1:200 was added to the sections and incubated for 1 hr at room temperature. Diaminobenzidine (DAB) substrate solution was applied on the sections and counterstaining was done with Mayer's hematoxylin for 30 sec.

Results and Discussion

Morbidity and Mortality

The flock morbidity and mortality were 100% and 60% respectively which in line with previous reports wherein morbidity and mortality rates between 5-90 and 50-80% in the susceptible population are recorded (Lefevre and Diallo (1990) and Chowdhury *et al.* (2014). Immune status, age, production system and concurrent diseases influence mortality and mortality particularly in endemic areas. The goats that died within 48 hours of the onset of clinical signs were in good body condition and those that died after 3-4 days were dehydrated and emaciated. In the present case, animals died in the acute stage after showing illness for 2-3 days. Young goats of 3-12 months are more susceptible than adults. This may be due to the persistence of maternally derived antibodies for up to 3 months Libeau *et al.* (1992). The young animals which survive from infection and sub clinically infected animals develop

antibody which may give protection from subsequent exposure to PPRV animals as well as their young ones through passive immunity.

Gross Findings

Gross examination of the buccal cavity revealed multifocal erosions (Figure 1a) on the mucosa of gums, dental pads and palate. Lesions of respiratory tracts included severely congested tracheal mucosa with the presence of froth due to pulmonary edema, partial consolidation of lobes especially the apical lobe of lungs which had dark red in color and firm consistency (Figure 1b). In the gastrointestinal tract, abomasal mucosa was severely congested and large intestine was also congested with characteristic streaks of hemorrhages in mucosa of terminal ileum (Figure 1c). The lesions were severe at the ileo-caecal valve. Mesenteric lymph nodes were swollen, congested and edematous. Gross findings are in accordance with findings of previous studies Bundza *et al.* (1988); Debasis *et al.* (2002); Pawaiya *et al. (*2004) and Chowdhury *et al.* (2014).

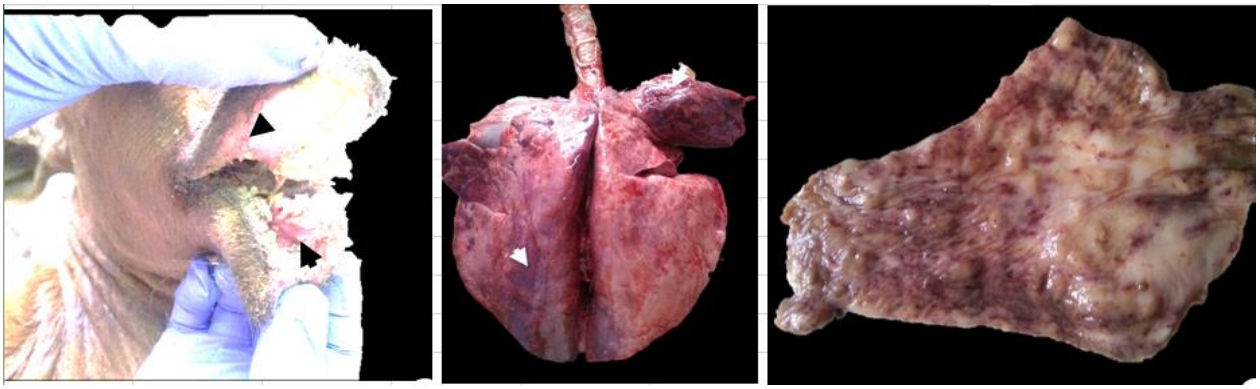

Figure 1a-c: a: Multifocal erosions on mucosa of gums (arrow); **b:** Consolidation of apical lobe (arrow) and focal area of dark gray discoloration of lungs (arrow head); **c:** hemorrhagic streaks in terminal ileum.

Microscopic Findings

Section of lungs revealed interstitial pneumonia with severe diffuse infiltration of mononuclear cells in interstitium and alveoli (Figure 2a), characteristic intracytoplasmic eosinophilic inclusion bodies in the bronchiolar epithelium (Figure 2b), intranuclear inclusions in alveolar macrophages (Figure 2c), syncytial giant cells (Figure 2d) and hyperplasia of type II pneumocytes. The entire alimentary tract revealed hemorrhagic gastroenteritis with loss of villi, mononuclear cell infiltration in the lamina propria, and necrosis of intestinal glands. Intracytoplasmic eosinophilic inclusion bodies were present in necrosed epithelial cells of the intestine. Spleen, mesenteric lymph nodes and Peyer's patches revealed depletion of lymphocytes (Figure 2e).

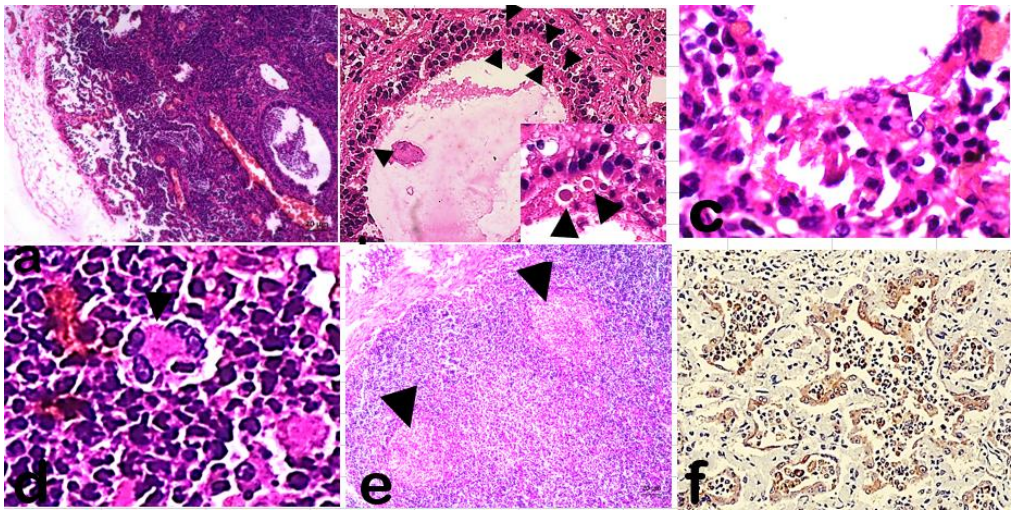

Figure 2a-f. Fig 2a: Bronchointerstitial pneumonia characterised by diffuse infiltration of mononuclear cells (×100, H&E). **Fig 2b:** Intracytoplasmic eosinophilic inclusion bodies (arrows) surrounded by clear halo (inset) in bronchiolar epithelium (×200, H&E); **Fig 2c:** Intranuclear inclusion in alveolar macrophages (white arrow) (×400,

H&E). **Fig 2d**: Syncytial cell in lungs (arrow) (×400, H&E). **Fig 2e:** Depletion of lymphocytes (arrow) in follicles of mesenteric lymph nodes (×100, H&E). **Fig 2f:** PPR antigen in alveolar macrophages and pneumocytes of lungs (IHC, DAB ×100)

PPR virus has an affinity for respiratory, GIT mucosa and lymphoid organs. Necrosis of oral mucosa, syncytial formation in respiratory epithelium, presence of intracytoplasmic eosinophilic inclusions in bronchial and bronchiolar epithelium of lungs and intestines are considered as pathognomonic microscopic lesions of PPR. Proliferative and necrotizing broncho-interstitial pneumonia usually occurs in natural and experimental PPR infections which is grossly results in the consolidation of the apical lobe of the lungs Aruni *et al* (1998). Intracytoplasmic and intranuclear eosinophilic inclusions in the respiratory and gastrointestinal epithelium are characteristic of morbillivirus. Viral inclusions were present in the lungs and intestines of animals died in the acute phase of infection. Inclusions were absent in animals that died 4-5 days of onset of clinical signs but depletion of lymphocytes in spleen and mesenteric lymph nodes were severe which resulted in severe immunosuppression. These findings are corroborated with earlier findings Yener *et al.* (2004) and indicate that animals had survived the acute phase of PPR and were in the recovery phase.

Immunohistochemistry

PPRV antigen was localized in the cytoplasm and nuclei of the alveolar and bronchiolar epithelium, macrophages of lungs (Figure 2f), cytoplasm of epithelial cells of ileum and rectum, and macrophages of spleen. The PPR antigen was detected in alveolar macrophages, pneumocytes and epithelial cells of the large intestine. In field condition, the PPR infection is usually complicated by secondary bacterial infection by *Pasteurella multocida* and *Manhemia haemolytica* due to lack of pulmonary defense mechanism. Microscopic lesions in the lungs and intestines were predominantly of non-suppurative inflammation which indicates a pure viral infection. This may be due to the flock received antibiotic treatment which might have prevented the secondary bacterial infection. This is well correlated with the mortality rate which was 60% in contrast to many field outbreaks wherein about 90-100% is reported.

Viral Nucleic Acid Detection and Phylogenetic Analysis

Viral RNA could be detected in the spleen and mesenteric lymph nodes by RT-PCR. The primer specific for N gene yielded PCR products of 351 bp (Figure 3).

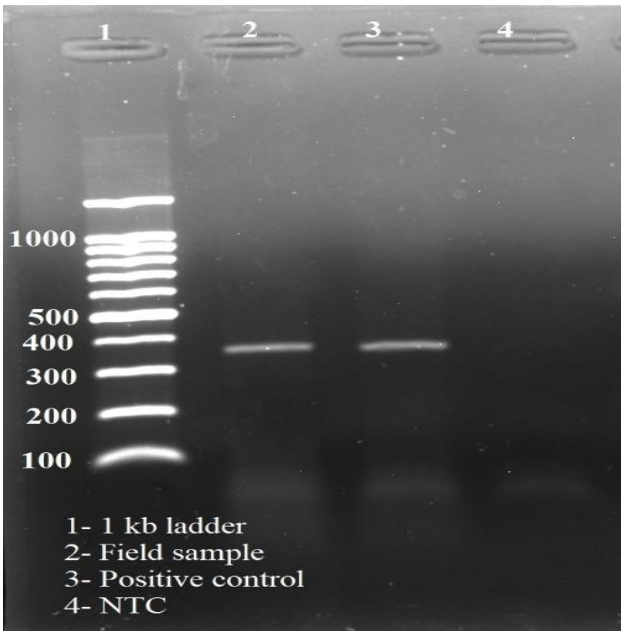

Figure 3: Gel electrophoresis of PCR product of N gene of PPRV showing 351 bp amplicon

Phylogenetic analysis revealed that the outbreak was caused by lineage IV of PPRV and clustered with virus circulated in India (Figure 4). This finding is in line with earlier report by Kumar *et al.* (2014) and Muthuchelvan *et al.* (2014).

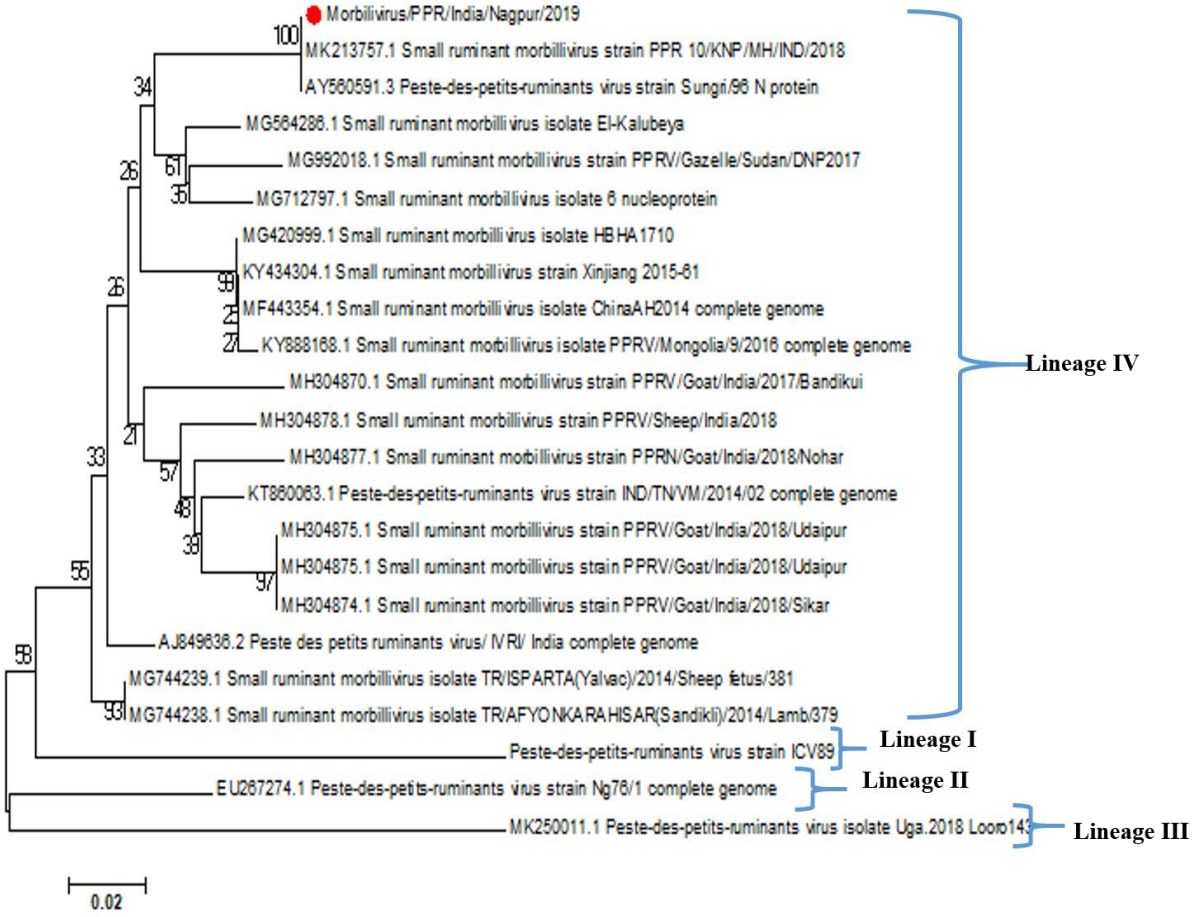

Figure 4: Phylogenetic tree based on partial N gene sequence of PPRV. The Neighbor joining tree was constructed using 1000 bootstrap values

Respiratory syncytial virus (RSV) and Parainfluenza-3 (PI-3) infection also induces lesions similar to PPR *viz.* syncytia with intracytoplasmic inclusions in respiratory epithelium and characteristic lesions may be absent in GIT in animals those die in per acute cases therefore RT-PCR and IHC are useful for the definitive diagnosis of PPR.

The risk factors associated with the occurrence of PPR include the introduction of recently purchased animals without following an adequate quarantine period, contact of the susceptible population with infected animals during grazing or during close confinement, changes in weather conditions, maintenance of cold chain of vaccination or inadequate protection following vaccination. PPR outbreak has been reported mostly during the rainy and winter seasons. The present outbreak occurred in the month of December, peak winter season in central India where minimum temperature ranges between 8-10°C in Nagpur region of Central India and animals remain in close contact with each other. Such condition may favor the transmission of the PPR virus. In India, a vaccine prepared from PPRV/Sungri/96 strain is used for mass vaccination in goats and sheep. This vaccine is safe for use but thermolabile. The flock was vaccinated against PPR but animals may not be having protective antibody titer because of improper vaccination/vaccination failure. The vaccine is to maintain the cold chain as these vaccine strains are labile to high temperature and proper vaccination must be carried out. Experimental studies have shown that each animal should receive a minimum recommended dose (OIE) of vaccine i.e., 10 ^{3}TCID50.

Conclusion

As PPRV is enzootic in the country proper vaccination should be carried out and a quarantine period must be followed before the introduction of new animals into flocks. Molecular characterisation of virus involved in field outbreak is essential to know the circulating strain of the virus.

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Conflict of Interests

There is no conflict of interest.

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