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RESEARCH ARTICLE

Clinicopathological and molecular investigation of a PPR outbreak in an organized goat farm in Tripura

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Abstract

Morbillivirus caprinae, belonging to the genus *Morbillivirus* and family Paramyxoviridae, is the cause of peste des petits ruminants (PPR), an acute and infectious viral disease that affects mainly small ruminants, especially goats. The disease is characterized by fever, necrotic stomatitis affecting the inner part of the lower lip and adjoining gum, severe gastroenteritis, inflammation of the bronchi, and often fatality. One privately owned organized goat farm on the outskirts of Agartala procured sixty Beetal and Sirohi goats from Rajasthan. Following a few days of introduction, the animals began exhibiting various symptoms, including fever, anorexia, cough, nasal mucus discharge, and diarrhea. The affected animals died within two to three days of the onset of clinical signs. Morbidity reached approximately 100%, and mortality was recorded to be 50%. Samples collected from ailing animals and necropsied specimens were subjected to histopathological examination followed by polymerase chain reaction (PCR) employing primer pairs specific for the nucleocapsid (N) gene of *Morbillivirus caprinae*. The pathological lesions were suggestive of PPR, which was later confirmed by PCR.

Keywords: Peste-des-petits ruminants, diagnosis, histopathology, polymerase chain reaction, Tripura

Introduction

One of the most significant viral diseases of goats that infrequently affects sheep is peste des petits ruminants (PPR), also referred to as "goat plague" because of its high fatality rate in goats (Kumar *et al*., 2014; Fayyad *et al*., 2020). PPR is severe and extremely contagious in goats, resulting in substantial financial losses to the farmers (Fayyad *et al*., 2020). PPR is a World Organization for Animal Health (WOAH-OIE) listed notifiable disease with high morbidity (up to 100%) and up to 90% mortality rate (Singh *et al*., 2009). Incubation period of PPR ranges from 2-7 days (Kumar *et al*., 2014). *Morbillivirus caprinae* is enveloped, with its genome being negative-sense single-stranded RNA in nature. The virus is classified under the *Morbillivirus* genus of the family Paramyxoviridae. PPR was first reported in the year 1942 from Ivory Coast, West Africa (Gargadennec and Lalanne, 1942). However, in India, it was first reported in 1987 from Tamil Nadu (Shiala *et al*., 1989). PPR is endemic in India and prevalent throughout the country (Sharma *et al*., 2015). Characteristic clinical features of the disease are a high rise in body temperature, mucopurulent nasal discharge, leukopenia, bronchopneumonia, ulceration and erosion of oral and nasal mucosa, necrotic stomatitis involving the gum and lower lip, and acute gastroenteritis (Balamurugan *et al*., 2014). In this study we report an outbreak of PPR in newly procured goats by an entrepreneur from Ajmer, Rajasthan, to Agartala, Tripura, which was confirmed by clinicopathological examinations and the molecular detection method.

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Materials and Methods

History

An entrepreneur procured sixty Sirohi and Beetal goats from Ajmer, Rajasthan, and introduced them to a newly established goat farm on the outskirts of Agartala, the capital city of Tripura. The animals began exhibiting a

variety of symptoms, including fever, anorexia, coughing, respiratory distress, diarrhoea, mucopurulent nasal discharge, and crusts over the nostrils within two to three days after being introduced. The mortality was as high as 50%, while the morbidity rate was 100%.

Samples

Nasal and rectal swab samples in 4 mL of sterile PBS (pH 7.4) were collected from ailing animals. The swab and tissue samples were immediately chilled by putting them on ice and transported to the laboratory under refrigeration. The swab samples were processed as described earlier (Pandey *et al*., 2022) and preserved at -20°C. Necropsy examination of a few carcasses of goats was performed. After a thorough necropsy examination, gross lesions were observed, and tissue samples from different internal organs were collected and fixed in 10% formalin for histopathological examination. Subsequently, paraffin-embedded sections of tissues (5 µm) were prepared for staining with H&E stain as described previously (Luna, 1968).

RNA extraction and cDNA synthesis

The swab samples and tissues were processed for RNA extraction following the manufacturer's protocol (NucleoSpin® RNA, NG, Germany). Complementary DNA (cDNA) was synthesized from the extracted RNA using the PrimeScript first strand cDNA Synthesis Kit (Takara-Bio Inc., USA).

Polymerase chain reaction (PCR)

PCR was performed using primer pairs previously described by Dadas *et al*. (2012), which amplify 368 bp of the N gene of *Morbillivirus caprinae* (forward primer: 5´-ACAGGCGCAGGTCTCCTTCCT-3'; reverse primer: 5´-CACTGATTTCGACAGAGGGTG-3'). PCR reaction mixture was prepared using 5 µL of cDNA as template, mixed with 25 µL of EmeraldAmp®GT PCR Master Mix (Takara-Bio Inc., USA), forward and reverse primers (0.4 µM each), and nuclease-free water (NFW) to 50 µL. PCR amplification was carried out in a T100™ Thermal Cycler (Bio-Rad Laboratories, Inc., USA) as per conditions earlier described by Dadas *et al*. (2012). PCR product (5 µL) was electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and visualized and documented using a GelDoc Go Gel Imaging System (Bio-Rad Laboratories, Inc., USA).

Results and Discussion

During necropsy examination, carcasses were found to be dehydrated and emaciated. The perineal area of animals was soiled with faeces. The oral mucosa showed multiple areas of ulcerative lesions, especially in the gums and inner aspect of the lips. Grossly, the tracheal lumen revealed the presence of frothy fluid with a mild to moderate degree of congestion. A few lobes, especially the apical lobe, were diffusely congested and consolidated. On incision, a large quantity of frothy fluid oozed out. In the digestive tract, abomasal mucosa showed varying-sized ulcers. Congestion observed in the intestinal mucosa ranged from a moderate to severe degree. Mesenteric lymph nodes were congested, oedematous and enlarged.

Microscopically, lung sections showed broncho-interstitial pneumonia; the inter-alveolar septa were thickened due to infiltration of mononuclear cells (Figure 1A). Hyperplasia of type II pneumocytes occasionally occluding alveolar spaces was noticed. The macrophages and syncytial cells demonstrated the presence of both intranuclear and intracytoplasmic inclusion bodies (Figure 1B). Inflammatory cells and necrotic lining epithelium were present in the lumen of the bronchus and bronchioles. The intestine showed congestion and haemorrhages. Mesenteric lymph nodes were congested with a mild to moderate degree of lymphoid depletion (Figure 1C).

At the end of PCR, the reaction mixture was electrophoresed on 1.5% agarose gel and the expected band size of 368 bp was visualized and documented (Figure 1D) under UV light in the GelDoc Go Gel Imaging System (Bio-Rad Laboratories, Inc., USA).

PPR is one of the most important viral transboundary diseases of small ruminants, especially goats. It causes significant economic losses to the farmers (Banyard *et al*., 2010; Parida *et al*., 2015). It can also affect sheep; however, clinical manifestations in goat is more acute and severe in nature (Kumar *et al*., 2014; Nanda *et al*., 1996). The causative agent is a negative-sense single-stranded RNA virus, *Morbillivirus caprinae* of genus *Morbillivirus*, and family *Paramyxoviridae* (Pope *et al*., 2013). PPR is reported from all over the country, including the northeastern region of India, and is considered to be endemic (Balamurugan *et al*., 2012, 2014).

The gross changes, such as ulcerative lesions in the oral mucosa, respiratory, and digestive tracts, observed in the present study were similar to earlier reports of other workers (Patel *et al*., 2015; Singh *et al*., 2017; Singh *et al*., 2018). The pneumonic changes observed in the present study were possibly due to descending infection further complicated by secondary opportunistic bacterial infection, as seen in previous studies (Nwoha *et al*., 2013).

The microscopic alterations observed in the present investigation included the presence of syncytial cells and inclusion bodies in the macrophages, hyperplasia of type II pneumocytes, and broncho-interstitial pneumonia with thickening of inter-alveolar septa. Similar microscopic lesions were also described earlier by several workers (Maina *et al*., 2015; Patel *et al*., 2015; Singh *et al*., 2018). The current investigation found necrosis of the epithelium of the trachea, bronchi and bronchioles along with inflammatory cells in the bronchi, in accordance with earlier findings (Aktas *et al*., 2011; Maina *et al*., 2015). The histopathological alterations noted in this present study were indicative of PPR.

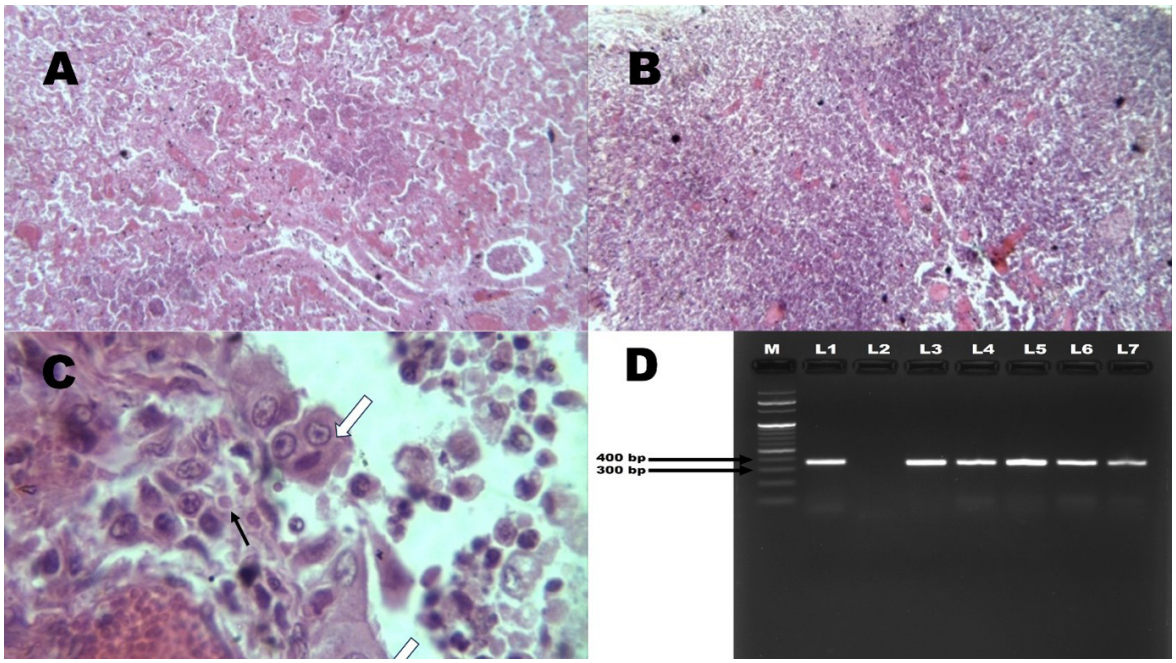

Figure 1 A: Section of lung showing severe broncho-interstitial pneumonia with mononuclear cell infiltration in the alveoli and bronchiolar lumen, and hyperplasia of type II pneumocytes. H&E 100X; **Figure B:** Section of lung showing eosinophilic intracytoplasmic inclusion body (black arrow) and syncytial cells (white arrow). H&E 1000X; **Figure C:** Mesenteric lymph node section showing mild to moderate lymphoid depletion and congestion. H&E 100X; **Figure D:** Result of PCR using primers targeting 368 bp of nucleocapsid gene, confirming presence of PPRV, M: 100 bp DNA Ladder (Himedia, Cat. No. MBT049), Lane 1: Positive Control, L2: Negative Control, L3-L7: PCR products from samples.

Complementary DNA (cDNA) synthesized from extracted RNA of the samples was subjected to PCR using primers targeting the nucleocapsid (N) gene, which yielded 368 bp of PCR product, confirming the disease to be PPR. The N gene is widely used as a target for diagnosis of PPR from clinical samples (Couacy-Hymann *et al*., 2002; Alwan and M. Al Saad, 2022; Bisht *et al*., 2023). A real-time RT-PCR assay using primers and probes specific for the N gene was developed by Batten *et al*. (2011) for detection of PPR. In another report, primers specific for N gene were used for the development of a reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) for PPR (Mahapatra *et al*., 2019). Kumar *et al*. (2014) used F, H, and N gene-based phylogenetic analysis of isolates and found that results based on N gene is ideal for clustering of virus isolates based on geographical location. Previously PPR outbreak was seen in Tellicherry goat breed in an organized farm in Tamil Nadu with mortality rates of 100% and 87.5% in kid and adult goats, respectively (Roy *et al*., 2010). In another study PPR was reported to be causing mortality and morbidity rates of 52% and 100%, respectively, in Tellicherry breeds (Jaisree *et al*., 2017). Observations in both the studies are consistent with the present outbreak in Tripura as the mortality reached up to 50% with morbidity rate of 100%.

Sixty Sirohi and Beetle goats from Ajmer, Rajasthan, were procured by an entrepreneur from different private, unorganized farmers and carried by road using an animal carrier vehicle to Agartala, Tripura. The vaccination history of the animals was unknown. Within two to three days of the introduction of the animals to the farm, there was a severe outbreak of PPR among the animals, which was confirmed by clinicopathological examination and the molecular detection method. The origin of the infection might be a few infected animals, which later spread the disease to other susceptible animals when they were in close contact during transportation. Since the vehicle was regularly used for transportation of animals across states and was not properly disinfected before transportation of the animals, its role as an inanimate source of infection cannot be ignored. As reported previously, transportation, especially a long-distance one, is very stressful for goats, as it exposes them to unfamiliar and harsh conditions such as lack of sufficient feed and water, bumpy roads, and loading and unloading (Yadav *et al*., 2024). Another study concluded that goats can experience an extreme stress after being transported continuously for longer than 20 hours, which leads to deterioration in immunity and antioxidant capacity, significant increase in inflammatory response and deviation in rumen fermentation indices (Li *et al*., 2024). In this study, it is also believed that the animals' stress from extended transit contributed significantly to the disease's spread among them. The present study highlights the need for verifying the vaccination history of animals, proper cleaning and disinfection of the carrier vehicles, and maintaining proper

quarantine measures before procurement, transport, and introduction of animals, respectively. The transportation of the animals should be done in the least stressful manner possible by avoiding overcrowding, providing ample drinking water and feed, etc.

Conclusion

Sixty Sirohi and Beetal breeds of goats procured from different unorganized farmers of Ajmer, Rajasthan, to Agartala, Tripura, by an entrepreneur witnessed a severe outbreak of PPR within two to three days of introduction. This was confirmed by clinicopathological and polymerase chain reaction. With a morbidity of 100% and a mortality rate reaching 50%, the outbreak of PPR caused significant financial loss to the farmer. Before procurement of the animals, their vaccination history was not verified, and they were transported by road a long distance from Rajasthan to Tripura. This study emphasizes the need for the procurement of animals with known vaccination history, proper cleaning and disinfection of the vehicle they are to be transported in, and the importance of maintaining proper quarantine measures before the introduction of new animals. The transportation stress also plays a major role in disease progression by lowering the animal's immunity. That's why animals should be transported in the least stressful way possible by avoiding overcrowding, providing ample water and feed, making frequent stops on the journey, etc.

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