An outbreak of peste des petits ruminants in sheep and goats at Salem district of Tamil Nadu, India


Central University Laboratory, Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai-600 051, India.

Received: 09-06-2015 Accepted: 14-04-2016 DOI:10.18805/ijar.9644

ABSTRACT

An unknown disease outbreak was reported in four villages of Salem district viz., Aranganoor, Athishipatty, Avadathur and Amanikondalanpatty in Tamil Nadu State and subsequent mortality among the Mecheri breed of sheep and Tellichery breed of goat was investigated. The clinical signs observed among the animals were high fever, anorexia, profuse diarrhoea with bilateral mucopurulent ocularnasal discharge. Bran like necrotic ulcers in the mucosa of oral cavity, tongue, dental pad was seen on necropsy. Samples were collected for laboratory based diagnostic assay and the investigation using RT-PCR confirmed the etiology as PPR. The Nucleoprotein gene of the virus was sequenced and phylogeny of the N gene sequence showed that the virus belongs to lineage IV PPRV.

Key words: Diagnosis, Histopathology, Outbreak, Pathology, Peste des petits ruminants, Polymerase chain reaction.

INTRODUCTION

India has vast resources of sheep and goats which play a vital role in improving the socio-economic conditions of small and marginal farmers and landless labourers in rural areas. The sheep and goat population in Tamil Nadu is 172.66 lakhs which contributes 56.13% of total livestock population of Tamil Nadu (All India Report, 2012). The most common factor which hampers the growth of sheep and goat industry is the outbreak of infectious diseases, among which Peste des Petitis ruminants (PPR) is very significant. Peste des petits ruminants is caused by Peste des Petitis ruminants, a member of the morbillivirus genus of paramyxovirus. The PPRV genome consists of a single stranded negative sense RNA of approximately 16 kilo bases containing six genes, encoding 6 structured and 2 non structural proteins. The virus exists as one serotype, but genetically divides into four distinct lineages (Senthilkumar et al., 2014). In India, the disease was first reported in Southern India (Shaila et al., 1989) and subsequently in northern India during 1994 (Nanda et al., 1996) and later across the Indian sub-continent (Dhar et al., 2002; Nanda et al., 1996; Roy et al., 2010; Sreeramulu, 2000; Taylor et al., 2002; Senthil Kumar et al., 2014). Recent estimates on the potential impact of PPR have suggested that 63% of the small ruminant populations of Southern Africa, central Asia, South-east Asia, China, Turkey and Southern Europe at risk to PPR as determined by the Food and Agriculture Organization (Libeau et al., 2014). In India, the economic losses due to PPR in goats and sheep is Rs, 8895.12 crores, of which Rs, 5477.48 and 3417.64 crores respectively are due to disease in goats and sheep (Singh et al., 2014). This study was undertaken as a part of the investigation into the PPRV outbreak during 2012. The study was undertaken to know about the clinico-pathological findings in sheep and goats during a natural outbreak of the disease and detection of virus genome in tissues by Polymerase chain reaction.

MATERIALS AND METHODS

History of the outbreak: Outbreak of an unknown disease was reported during the year 2012 in four villages of Aranganoor, Athishipatty, Avadathur and Amanikondalanpatty of Salem district in Tamil Nadu State. The mortality rate observed were 17.51% in sheep and 6.13% in goats within a period of one week. Sheep and goats between 6 months and 9 months of age and of both sexes were affected. The clinical signs observed were bilateral serous to mucopurulent ocular nasal discharge (Fig.1), diarrhoea, rise in pyrexia (Temperature 105°F), erosions in oral mucosa (Fig.2) and moderate scabby lesions on the lips. A detailed investigation was undertaken to find out the etiology of the disease. The carcasses were dehydrated (Ten goats and Twenty sheep were necropsed) and revealed severe multifocal to coalescing ulcers on the oral mucosa, gums and dorsum of tongue. Lungs revealed severe diffuse...
congestion and edema with marked consolidation of cranial lobes. In a few cases fibrinous pleurisy and pneumonia were noticed in sheep. Abomasum revealed severe congestion of mucosa with mild erosions and ulceration. Intestine mucosa was diffusely haemorrhagic with characteristic linear hemorrhages in rectal mucosa of goats. Ileocaecal junction also appeared hemorrhagic. Mediastinal and mesenteric lymph nodes were enlarged and edematous.

**Samples collection:** Samples including nasal swab, ocular swab, oral swab and faecal swabs were collected from clinical cases. Tissues samples such as lungs, liver, spleen, mesenteric and mediastinal lymph node, brain, abdomen and intestine were collected from dead animals in 10 per cent formalin or neutral buffer formalin and processed for histopathology as per the routine haematoxlin and eosin method (Bancroft and Gamble, 2008). Tissue samples like lung, spleen and mesenteric lymph node were also collected in phosphate buffered saline (PBS) for use in counter immune electrophoresis (CIE) and molecular diagnostic assays. Counter Immuno Electrophoresis was performed to detect the presence of PPRV antigen as described earlier (Roy et al., 2010).

**Polymerase chain reaction (PCR):** RNA was extracted from 10 percent tissue suspensions using TRIZOL (Invitrogen, UK) following manufacturer protocol. cDNA was synthesized using first strand cDNA synthesis kit (Invitrogen, UK) as per manufacturer’s instructions. The primers specific for PPRV- N gene (Forward primer – 5’ TCT CGG AAT CGT CCT CAT CAG A CTG 3’ and reverse primer -5’ CCT CCT CCT GTT CCT CCA GAA TCT 3’) following a region 350 bp (Couacy – Hymann et al., 2002). The reaction mixture contained - 12.5 µl and ampligon Red Dye mastermix (Invitrogen, UK) ,10 pM forward and reverse primers each and DEPC water up to 25 µl. PCR was performed in an Eppendorf thermocycler with the following conditions: 95°C for 5 min followed by 34 cycles each of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, synthesis at 72°C for 30 sec and final extension at 72°C for 10 min. The PCR product was electrophoresed on 1.5% agarose gel in Tris EDTA buffer stained with ethidium bromide and visualized under Bio – Red gel doc system, for desired size of band.

**Sequence analyses:** The PCR products were sequenced by sanger dideoxy sequencing method with commercial sequencing services(Eurofins, Germany). The partial N gene sequence data was aligned with the representative isolates of all four lineages of PPRV using the Clustal-W algorithm incorporated in MEGA 6.0. The phylogenetic analysis was carried out using neighbour joining method following the Kimura 2-parameter nucleotide substitution model. Neighbour joining tree was constructed using the distance matrices generated in MEGA 6.0 with 1000 bootstrap replicates to test the robustness of the tree topology.

**RESULTS AND DISCUSSION**

In the present outbreak high mortality of 17.5% was recorded in sheep as compared to earlier record of 13.4% mortality in adults and 41.4% mortality in kids during a natural outbreak of PPR in goats (Kumar et al., 2004; Kulkarni et al., 1996). The most prominent pathological lesions were seen in the lymphoid organs as described earlier (Kumar et al., 2004). Clinical signs and the gross lesions observed in the present outbreak were consistent with PPR outbreak as reported earlier (Kul et al., 2007; Chaukan et al., 2011). Histopathological observations in goat tissues included extensive serofibrinous bronchointestinal pneumonia, characterized by diffuse large infiltration of lymphocytes, macrophages with numerous syncytia containing up to 6-8 nuclei. Serofibrinous exudate was seen...
in the interstitial and in alveoli. The bronchi and bronchiolar epithelium revealed marked hyperplasia or in a few areas necrosis and desquamation of epithelium was noticed. Intracytoplasmic eosinophilic inclusion bodies were recorded in the bronchiolar epithelial cells and syncytia (Fig-4). Intestine revealed severe diffuse haemorrhagic enteritis characterized by necrosis and desquamation of epithelium with the presence of erythrocytes in exudates, infiltration of lymphocytes and a few neutrophils in the lamina propria and sub mucosa. Depletion of lymphoid cells in Peyer’s patches was also noticed. Spleen and lymph node showed variable degrees of lymphoid cell depletion. Liver revealed congestion of veins and sinusoids with diffuse mild vacuolar degenerative changes in hepatocytes with periportal infiltration of lymphocytes. Kidneys revealed degeneration and necrosis of tubular epithelial cells. Similar histopathological lesions were noticed in tissues collected from sheep infected with PPR except that the presence of syncytial and inclusion bodies were not very consistent. The predominant lesion in sheep was supplicative to fibrinous type of bronchointerstitial pneumonia.

The tissue samples of lung and spleen were positive for PPRV by CIE test using antiserum raised against PPRV. This confirmed that the infection was due to Morbillivirus (PPRV or RPV). For confirmatory diagnosis, RT-PCR was done with N gene specific primers. Amplification of 350 bp (Fig.3) product of N gene confirmed the identity of PPRV. The genetic data generated confirmed that the clinical disease observed in goats was caused by PPRV and the virus belonged to lineage IV that is prevalent in India (Fig.5). Only one PPRV sample from a sheep clinically infected was sequenced in this study. The virus was found to be of the lineage IV PPRV. Previous studies (Senthil Kumar et al., 2014) failed to observe host specific (Sheep/Goat) PPRV lineages; however there are earlier reports describing the prevalence of PPRV specific antibodies (Singh et al., 2004 and Balamurugan et al., 2012) more in sheep than goats of southern India. However, in this study the single sheep isolate did not cluster separately with other isolate from sheep. When partial N gene based phylogenetic tree was constructed. At this moment the higher pathogenicity in sheep during this outbreak could not be correlated with partial N gene sequence data. However complete genomic sequencing of a larger amount of isolates from sheep and goats may shed light on whether the viral determinants play a role in the difference in pathogenicity of PPRV. In conclusion, in the present outbreak high mortality was noticed in the sheep rather than goats due to PPRV lineage IV infection.
REFERENCES


