An outbreak of Peste des Petits Ruminants Among Thelichery Breed of Goats

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ABSTRACT

An outbreak of Peste des petits ruminants (PPR) and mortality among Thelicherry breed of goats was investigated. Specimens collected from the affected animals were negative by haemagglutination test with chicken erythrocytes, but positive when tested in counter immunoelectrophoresis with PPR virus (PPRV) specific antiserum. Virus isolation was done in Vero cell line. The cytopathogenic effect was characterized by fusion of cells with a clock face appearance. Pathological lesions included focal areas of erosion in the abomasal mucosa and intestinal lamina propria, conjunctivitis, congestion, and haemorrhages in the Peyer's patches, lungs, and spleen. Syncytia formations in the lungs, containing intranuclear eosinophilic inclusion bodies, were noticed. Specific diagnosis of the disease as PPR was confirmed by Polymerase chain reaction with N and M gene specific primers. Possible reasons for high mortality (100% for kids and 87.5% for adult goats) in Thelichery breed of goats during a natural outbreak of PPR and diagnosis of the disease using a panel of tests with merits and demerits have been discussed.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute viral disease of sheep and goats characterized by fever, catarrhal inflammation of ocular and nasal mucous membrane, erosive stomatitis, gastroenteritis, and pneumonia.[16] The disease is prevalent in most African, Middle Eastern countries, and theIndian subcontinent. [14] The disease in India was first recorded among sheep in Tamilnadu State during the year 1987.[15] Since then, the disease has been recorded in sheep and goats from different parts of India.[9,11] The disease causes considerable economic loss to the farmers, and diagnosis of the disease is not straight forward.

PPR virus (PPRV) is a morbilli virus under the family paramyxoviridae and closely

related to rinderpest virus. Both the viruses produce almost similar clinical signs in sheep and goats. Thus laboratory diagnosis is highly essential to confirm the etiological agent. Present paper describes (1) clinico-pathological lesions in Thelichery goats that resulted in 100% mortality in kids and 87.5% mortality in adults during a natural outbreak of the disease; (2) isolation of the virus in Vero cell line; and 3) detection of the virus specific antigen in infected tissues by polymerase chain reaction

In a private goat (Thelichery breed) farm, all the 20 kids and 28 out of 32 adult goats died within a period of 1 week's time. Disease investigation was undertaken to find out the cause of mortality.

Postmortem examination was conducted in six adult goats and five kids. Gross lesions were recorded. Representative tissue samples from lungs, liver, spleen mesenteric lymph node (MLN), brain,abomasum, and intestine were collected in 10%, neutral buffered formalin for histopathological studies. Tissue samples from spleen, MLN, lungs, and ocular swabs in phosphate buffered saline (PBS) were collected on ice for PPRV isolation or demonstration of PPRV specific antigen by counter immuno electrophoresis (CIE), haemagglution (HA) test, and Polymerase Chain Reaction (PCR).

Antiserum Production

A live attenuated vaccine prepared with a local strain of PPR virus (belongs to lineage 4) was given to 1-year-old unvaccinated sero negative goats and challenged 3 weeks later with a local strain of PPRV (belongs to lineage 4) with a dose of 103 Sheep infective dose 50. Serum was collected 3 weeks later which showed serum neutralization titre of 2 6 and used as antiserum for conducting serological tests like CIE.

CIE

CIE was done as described earlier.[12] in microscopic slides, known antiserum (as described above), and samples (clear suspension of 20% lung homogenate, 20% MLN homogenate, 20% spleen homogenate, and ocular swabs samples) were charged into alternate wells. Known positive and negative controls antigen were incorporated simultaneously. Sample wells were connected to cathode and antiserum to anode. A constant current of 4 milli amperes was passed for about 45 minutes and results were read under diffuse light.

HA Test

An HA test with 1% chicken erythrocytes was done as described17using clear suspension of 20% lung homogenate, 20% MLN homogenate, 20% spleen homogenate, and ocular swabs samples prepared in Minimum essential medium.

PCR

For PCR, cDNA was synthesized as described by Forsyth and Barett.5 For reverse transcription, RNA-10µl was mixed with random hexamer - 2 µl and kept at 700C for 5 min, snap cooled on ice for 1 min. RT buffer (5 x) – 4 µl , RNase inhibitor – 1 µl , 10 mM d NTP – 2 µl, M – MulV Reverse transcriptase – 1 µl were added and subjected to thermal programme at 25ºC for 10 minutes, 42 ºC for 60 minutes followed by 72 ºC for 10 minutes. This cDNA was stored at 20 ºC until use. All the reagents were obtained from Revert Aid First strand cDNA synthesis kit (Fermantas, USA)

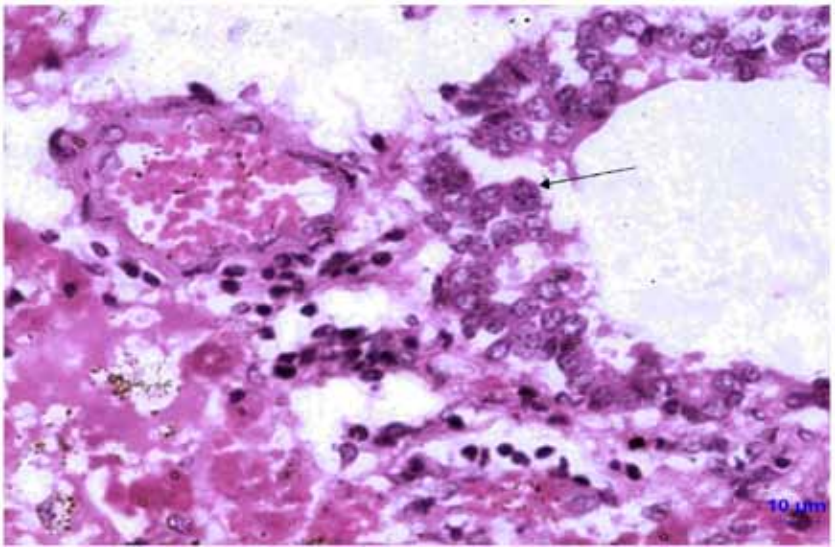
M gene Specific PCR

For carrying out M gene specific PCR, the reaction mixture contained cDNA – 5 µl, forward primer (Ind F: 5' CTT GAT ACT CCC CAG AGA TTC 3') – 10 p mol (1 µl); Reverse primer (Ind.R: 5' TTC TCC CAT GAG CCG ACT ATG 3' – 10 p mol (1 µl); Eppendorf master mix (2.5 U) – 20 µl (Magnesium Chloride 1.5 mM, dNTPS – 200 µM, Taq DNA polymerase 1.25U); and Nuclease free water – 23 µl. PCR was performed in Perkin – Elmer PCR thermocycler in the following cycle condition. One cycle 95º C for 3 minutes, 29 cycles, 94º C for 30 seconds, 60ºC for 30 seconds and 72ºC for 30 seconds, and final extension at 720C for 10 minutes.

N Gene Specific PCR

For carrying out N gene specific PCR,

Figure 1. *Lungs: Alveolar changes include the presence of large alveolar macrophages and formation of variable sized syncytical cells.*



cDNA was synthesized as described above. For PCR amplification, the reaction mixture contained cDNA and Eppendorf master mix as described above. Forward primer (Mf – morb: 5' GCA, GAG GAA GCC AAA CTA GTC TCG GA 3') 20 p mol (2 µl); Reverse primer (Mr-ppr3: 5' CTC AGC CGA TCT TTG ADC CTC ACG AG 3') – 20 p mol (2 µl); and nuclease free water – 21 µl. PCR was carried out in Perkin –Elmer PCR thermocycler with the following cycle condition. One cycle, 94 ºC for 5 minutes; 35 cycles, 94 ºC for 1 minute; 55 ºC for 1 minute and 72 ºC for 1 minute; and final extension was done at 72 ºC for 7 minutes.

A volume of 8 µl of PCR product was analyzed after electrophoresis on a 1.5% agarose gel containing 2.5 ug/ml of ethedium bromide and Amplicons were visualized under UV light. Amplicons were stored at - 200 C for further use.

For isolation of the etiological agent, pooled tissue suspension of spleen, mesenteric lymph node, and extract of oculonasal swabs were filtered through 0.22 µm membrane filter and inoculated into Vero cells monolayer culture. Simultaneously, uninoculated Vero cells monolayer were kept as controls. Inoculated cells were harvested by freeze and thaw after 5 days. After centrifugation, the supernatan was again inoculated into vero cell monolayer culture. The blind passage was done until cytopathogenic effects (CPE) were observed.

For bacteriological investigation, swab samples were collected aseptically from heart blood and lungs. Samples were streaked onto blood agar, MacConkey agar, and Nutrient agar. The bacterial colonies were identified by morphological and biochemical tests.[3]

A total of 48 out of 52 kids and goats died in a period of 7 days. Clinical signs as recorded by the farmers include anorexia, dullness, mucopurulent nasal discharge, lacrymation, diarrhea, and leg weakness in some cases. Dead animals were emaciated. Oral mucosa were congested in almost all cases and in few areas were eroded. Conjunctivitis was noticed in some cases. Abomasal mucosa were congested and erosion was noticed in scattered areas. Small intestine was congested and hemorrhages were seen in scattered areas. Peyer's patches were hemorrhagic. Lungs were severely congested and consolidated. Spleen and lymph nodes were enlarged and congested. Intestinal lamina propria showed mild to moderate

***Figure 2**. Vero cells infected: Cellular aggregations, syncytia formations giving a clock face appearance*

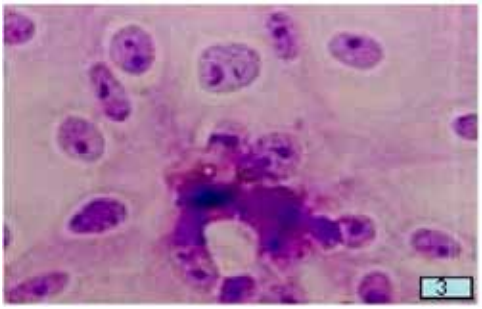
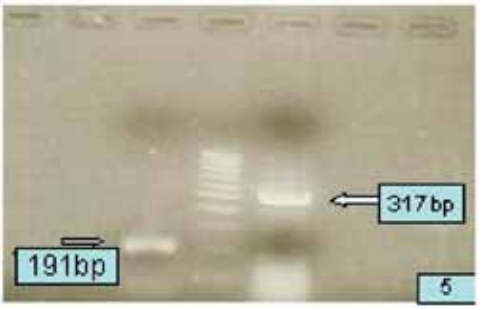


***Figure 3.**: PCR results: 1st lane 191bp M gene specific amplicon, 2nd lane - Molecular weight marker, 3rd lane - 317bp N gene specific amplicon*



infiltration of mononuclear cells and plasma cells. Abomasum showed erosion in the mucosa and infiltration of mononuclear cells in the lamina propria. Lymph nodes showed a variable degree of lymphoid cell depletion. The spleen showed congestion with blood pigments and a variable degree of lymphoid depletion. The lungs were congested, and showed a marked infiltration of mononuclear cells in the bronchi and interstitial areas. Alveolar changes included the presence of large alveolar macrophages and the formation of variable sized syncytical cells (Fig.1). Some syncytical cells contained intranuclear eosinophilic inclusion bodies.

In HA test, samples were negative for hemagglutination using 1% chicken erythrocytes. In CIE, ocular swab samples, lymph node, and spleen samples were positive for PPRV. In PCR, when samples were tested with N gene specific primers resulted in the production of 317 bp amplicon of expected size (Fig-3). Similarly, PCR by M gene specific primers yielded amplicon of 191 bp of expected size (Fig-3).

In Vero cell monolayer, after three blind passages, the isolate produced cytopathogenic effects (CPE) from the fourth passage. The CPE was characterized by aggregations and rounding of cells, clock faced syncytia (Fig-2) and cell death. In bacterial examination, E. coli were isolated from different organs.

Some Asian strains of Rinderpest and PPR viruses produce clinically indistinguishable diseases. Rinderpest usually causes high mortality, but the disease has been eradicated from India. The first outbreak of PPR was observed in 1987 among sheep and published in 1989.[15] Subsequently the disease has been recorded among sheep and goats [9.11] and prevalent in India. In the present outbreak high mortality (100% for kids and 87.5% for adult goats) was recorded compared to the earlier record of 13.4% for adults and 41.4% for kids during a natural outbreak of PPR in Goats,[9] which necessitated having a differential diagnosis. Clinical signs recorded in the present study were comparable with the earlier reports of PPR in goats.[8,9]

The most prominent pathological lesions were seen in the lymphoid organs as described earlier for PPR.[8] The lung is considered as an important target organ for most Morbilliviruses.[18] In the present study, syncytia and intranuclear inclusion bodies were observed in the lungs. Rinderpest virus a closely related Morbilli virus, rarely produces syncytia in the lungs, but mostly in stratified squamous epithelial cells and lymphoid tissues.[2,18] The lung lesions in the current outbreak were suggestive of PPRV involvement as suggested by Kumar et al.[8] However, by clinical signs, postmortem, and histopathological lesions, it is very difficult to differentiate the closely related PPRV and RPV, although RP has been eradicated. Confirmatory diagnosis is needed to implement control measures.

PPRV causes hemagglutination, but in the present study, hemagglutination could not be observed, possibly because of low concentration of virus in suspected specimens.The actual reason is not known. The present samples were positive for PPRV by CIE test using antiserum raised against PPRV. Since PPR and RP viruses have antigenic sharing, the antibody used in the CIE test is not highly specific. This confirmed that the infection was due to a Morbillivirus (PPRV or RPV). Further, the virus was cultivated in Vero cell line and the virus produced CPE characterized by fusion of cells, giving rise to clock face or cartwheel appearance of nuclei, which is considered to be common in PPRV.[13]

For confirmatory diagnosis, PCR was done with M and N gene specific primers. M gene is highly conserved4 whereas N protein is the major cross reacting protein for PPRV and RPV.[10] M gene specific primers can detect as low as 101 TCID 50/ml of virus.[6] N gene specific primers specifically amplify only PPRV but not RPV. Amplification of 317 bp N gene and 191 bp M gene confirmed that the etiological agent for the current outbreak is only PPRV, as the primers are specific. Most often diagnosis at field-level laboratories is done by HA test, but present study highlighted the weakness of HA test for diagnosis of PPR and thus, suspected outbreaks should be investigated not only by HA test, but also with other tests like CIE or other sensitive tests like RT/PCR or antigen capture ELISA. The differential diagnosis can easily be done by PCR with N gene specific primers. High mortality in the present natural outbreak of PPR is possibly due to breed variation as the affected animals were Thelicherry breed of goats and possible complication with secondary bacteria like E. coli as reported earlier that heat labile enterotoxin of E. coli increases the clinical severity and mortality rate of PPR.[1,7]

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