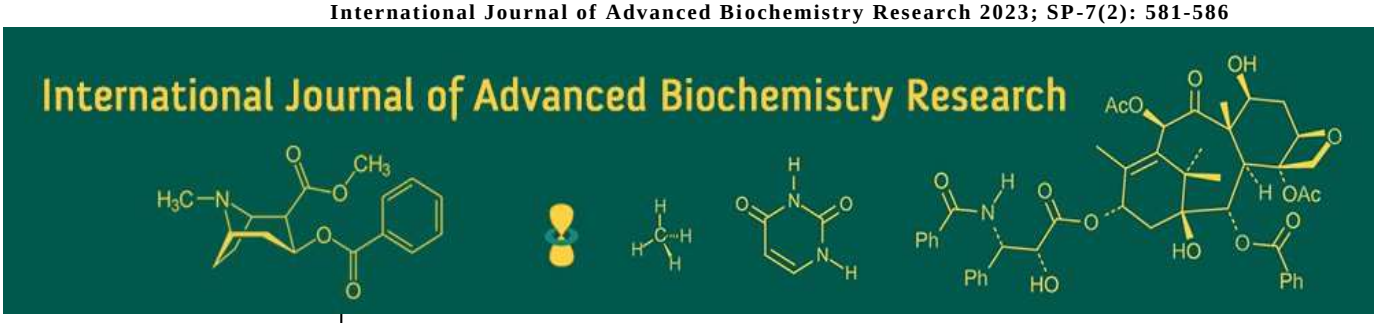

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M Islam
Department of Veterinary Pathology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

DC Pathak
Department of Veterinary Pathology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

S Das
Department of Veterinary Microbiology, College of Veterinary Science AAU, Khanapara, Guwahati, Assam, India

T Raman
Department of Veterinary Pathology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

S Sarma
Department of Veterinary Biochemistry, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Rita Nath
Department of Veterinary Biochemistry, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Anisha Sultana
Department of Veterinary Biochemistry, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Corresponding Author:
M Islam
Department of Veterinary Pathology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

Pathological and molecular studies of Peste des Petits ruminants in goats of Assam

M Islam, DC Pathak, S Das, T Raman, S Sarma, Rita Nath and Anisha Sultana

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Abstract
Peste des petits ruminants is an acute, febrile, emerging and economically important viral disease of goats having high morbidity and mortality rate. Present investigation was conducted to diagnose PPR in goats of Assam. On the basis of clinical and pathological alterations and confirmed by molecular techniques. The clinically affected animals showed predominant clinical signs like high fever, anorexia, dullness, mucopurulent occulo-nasal discharge, stomatitis and diarrhoea soiling the perianal region and hind quarter. Grossly, lungs showed congestion and consolidation in anterior and cardiac lobes, emphysema in the diaphragmatic lobes, frothy exudates in the lumen of the trachea, ulcerative lesion in gums, lips and tongue, and hemorrhagic enteritis. Microscopically, there were bronchopneumonia and interstitial pneumonia with presence of intracytoplasmic eosinophilic inclusion bodies in the bronchial epithelium. Lymphocytic depletion in lymph nodes and spleen were characteristic findings. Degenerative and ulcerative lesions in the tongue, gums and lips with intracytoplasmic inclusion bodies in the lining epithelium of lips were also common findings. In RT-PCR, out of 79 post mortem samples, 58 showed amplification of PPR viral nucleic acid at 463 bp for N gene using N gene specific primer.

Keywords: Assam, bp, goat, PPR, RT-PCR

Introduction
Peste des petits ruminants or 'plague of small ruminants is a febrile acute or sub acute and highly contagious viral disease of goat and sheep having high morbidity and mortality rates [1] which go up to 100 percent and 90 percent respectively. The disease in association with pasteurellosis may increase the mortality rate up to 100 percent. In natural infection, the disease primarily affects both the sheep and goats, but it is usually more severe in goats than sheep [2]. It is caused by PPR virus belonging to the genus Morbillivirus under the family Paramyxoviridae. The virus is antigenically closely related to the viruses of rinderpest, canine distemper, human measles and the marine Morbillivirus [3, 4]. Clinically the disease is characterized by pyrexia, dyspnoea, pneumonia, mucopurulent occulo-nasal discharge, errosive rhinitis, necrotic ulcers in mouth, on dental pad, tongue and lips [5]. At necropsy, there are erosive and ulcerative lesions in all parts of the digestive tract from mouth to colon and sometimes characteristic linear hemorrhages (zebra stripes) in the longitudinal mucosal folds of large intestine (rectum). Pneumonic lesions are present in lungs with patches of emphysema and edema. Frothy exudates were present in the tracheal lumen. The mesenteric lymph nodes and spleen become enlarged with presence of patchy to echymotic hemorrhages [6, 7]. Histopathologically, there is severe bronchopneumonia with epithelial hyperplasia, edema and presence of multinucleated syncytial cells in the alveolar epithelium. The intestinal sub mucosa reveals limited infiltration of lymphocytes and macrophages with goblet cell hyperplasia. The Lymph nodes and spleen reveals lymphocytic depletion with pyknoosis and karyorrhexis of the lymphocytes. Multinucleated syncytial cells in alveolar epithelium and mucosal lesion in oral cavity are the pathgnomonic lesions of PPR [6, 7]. Considering the endemicity of the disease in India in general and Assam in particular, prompt and accurate diagnosis of the disease is very much important. This paper puts forward a field investigation of PPR in goats of Assam on the basis of pathological and molecular diagnosis during natural outbreaks in different parts of Assam.

Materials and Methods

The study was conducted on natural outbreaks of the disease in different districts of Assam. During the MVSc research work, A total of 36 numbers of goat carcasses were necropsised from different outbreaks in different district of Assam.

Ethical approval: necessary ethical approval was undertaken before starting the research work with *vide approval no.:* 770/ac/CPCSEA/FVSc/AAU/IAEC/14-15/261 dtd. 20/06/2014.

Gross and Histopathological Examination

Post mortem of animals died of suspected PPR was performed and gross lesions if any in different organs were recorded systematically. For histopathology, representative tissue samples from lungs, liver, spleen, lymph node, kidneys, tongue, parts of intestine and brain were collected and fixed in 10% formalin for at least 24-48 hours. Tissues were processed in paraffin embedding technique and 4 µm thick sections were stained with routine haematoxylin and eosin staining method as described by Luna [8].

Molecular Detection of the Virus

A reverse transcriptase polymerase chain reaction (RT-PCR) was adopted for detection of PPR virus in tissue samples collected from necropsised animals. The reaction was carried out with a PPRV specific primer set (F-5' GAT GGT CAG AAG ATC TGC A 3' R-5' CTT GTC GTT GTA GAC CTG A 3') to amplify a 463-bp cDNA product from the N gene spanning. A lyophilized vaccine procured from Indian Immunologicals was used as a positive control. RNA was extracted from reconstitute vaccine and tissue homogenate from the field samples using TRIsol® reagent. The RT-PCR was performed with Qiagen one step RT-PCR kit. The thermocycling profile was as follows: initial denaturation was at 94 °C for 10 min, annealing at 50 °C for 30 sec., extension at 72 °C for 45 sec. followed by 35 cycle of denaturation and annealing. The final extension was done at 72 °C for 10 min. the RT-PCR product was analyzed by electrophoresis on 1.5% agarose gel stained with ethedium bromide.

Results

Clinical Signs

The prominent clinical signs recorded in all the outbreaks were anorexia, high rise of temperature (39.5- 41 °C), mucopurulent nasal discharge, rapid respiration, dyspnea, lacrimation, matting of eyelids, congested mucus membrane, erosive and ulcerative lesions on tongue as well as on the lips, gum and dental pads. Severe diarrhoea along with soiled hindquarters was observed in most of the cases.

Gross and Histopathology

Necropsy was conducted on 36 numbers of goat carcasses. In general, most of the carcasses were found dehydrated and emaciated with soiled hindquarters. The eye balls were sunken. The occulo-nasal discharges were seen as dried and adhered on the hair below the eyes and the nose. All the dead animals showed necrotic lesions with erosion and ulceration in the gums (Fig. 1), soft plate and hard palate, the cheeks near the commissures, lips and lateral and dorsal surfaces of the tongue. The erosions were shallow with a red, raw base. The ulcerated areas were sharply constant feature in all the necropsied carcasses. The mesenteric blood vessels were severely congested (Fig. 2). The inner surface of lips, gums, cheeks and the dorsal surface of tongue had necrotic foci, erosions and ulcerations. Erosion and ulceration in the oral mucosa were congested. Congestion in the ileo-cecal valve, the ceco-colic junction and the rectum was observed with linear hemorrhages on the crests of the folds of the mucosa of the colon and caecum (Fig. 3). Mesenteric lymph nodes were enlarged, swollen and edematous. The spleen was moderately enlarged. Enlargement of the liver was seen along with few necrotic foci on the surface and engorged gall bladder with bile. The pneumonic lesions were invariably observed mostly in the anterior and cardiac lobes with emphysema mostly in the diaphragmatic lobes of the lungs (Fig. 4) of all the necropsied carcasses. On cut section, large amount of frothy exudates were present in the bronchi (Fig. 5). The tracheal mucosa had congestion and the lumen contains frothy exudates.

Histopathologically, the lips showed necrosis, erosion and ulceration of the surface epithelium with sloughing off the lining epithelial cells and infiltration of mononuclear and polymorphonuclear cells in the submucosa. In some animals the stratum spinosum layer possessed few syncytial giant cells. Occasionally some of the epithelial cells of the stratum spinosum layer contained intracytoplasmic eosinophilic inclusion bodies (Fig. 6). The epidermal surface of the tongue showed degeneration, necrosis and sloughing out of the lining epithelial cells (Fig. 7). In between the fungiform and filiform papillae there was infiltration of large number of mononuclear and polymorphonuclear cells in the connective tissue core, which was distributed downward and either side of the papillae. Both the small and large intestines showed stunting and blunting of villi with degeneration, necrosis and sloughing off the villous epithelium (Fig. 8) and intense infiltration of mononuclear cells in the lamina properia and extending to the submucosa. The goblet cells were filled with mucin and appeared enlarged. Congestion and haemorrhage were also evident in the submucosa. The glandular epithelial cells showed cytoplasmic vacuolation. In few areas hyperchromesia of the glandular epithelial cells was also observed. The lumen of some mucosal glands was filled with mononuclear and polymorphonuclear infiltrating cells along with mucus. Hepatocytes showed both cytoplasmic and nuclear degeneration in the periportal areas with cytoplasmic vacuolation. Some of the hepatocytes showed focal coagulative necrosis. The Lungs showed congestion in the pulmonary capillaries and peribronchial areas with hemorrhages in the alveolar septa. There was presence of large numbers of haemosiderin pigment in the alveoli and interalveolar spaces. Presence of serofibrinous exudates along with mononuclear and polymorphonuclear cells and desquamated sloughed out lining epithelial cells in the lumen of the bronchi and bronchioles indicated bronchopneumonia (Fig. 9). In some areas there was there was thickening of alveolar wall with increase capillary bed, dilatation of capillaries and infiltration of mononuclear cells making the interalveolar septa thickened which was the indicative of interstitial pneumonia. Focal areas of alveolar edema were also observed. Intracytoplasmic eosinophilic inclusion bodies were present in the lining epithelial cells of bronchi, bronchioles and alveoli (Fig. 10). Edema was observed in both the cortical and medullary areas of lymph nodes (Fig.11) with severe depletion of lymphocytes and pyknosis and karyorrhexis of the lymphocytes. Few syncytial giant cells were also found scatteredly distributed in the lymph nodes

(Fig. 12). In spleen, severe depletion of lymphoid population in the lymphoid follicles was the constant finding. The white pulp was shrunken due to severe depletion of lymphoid cells and few lymphocytes were scatteredly distributed in that area. Some of the white pulps were completely destroyed and as a result there was formation of cystic cavity. The kidneys showed tubular degeneration and coagulative necrosis in the cortical area and interstitial congestion and hemorrhages in the medullary area. In the medullary area there was loss of cytoplasmic materials of the tubular epithelial cells making them granular. There was neuronal degeneration and necrosis followed by neuronophagia and vacuolation of the neuropil in the cerebrum. In the cerebellum, there was congestion in the grey matter and white matter.

Molecular Detection of the Virus by RT-PCR

All the post mortem samples collected from necropsied animals were subjected to RT-PCR for molecular diagnosis targeted against the N gene of the PPR virus. Three µl of cDNA was used for N gene amplification by PCR using specific primers. Three µl of PCR amplified product was run on 1.5% agarose gel in 1X TBE buffer at 80V for 45 minutes. Out of 79 post mortem tissue samples, 58 samples were showed positive for PPRV. The remaining samples failed to produce the targeted amplification of 463 bp (Fig. 13).

Discussion

Almost all the affected animals showed fever, anorexia, mucopurulent occulo-nasal discharge, diarrhoea and erosive and ulcerative lesions in the oral cavity which were constant feature. Similar findings were also reported by other workers in different parts of the world under natural or experimental studies in small ruminants [9-14].

Ulcerative stomatitis, congestion and consolidation of anterior and cardiac lobes of lungs, froth in the lumen of trachea and bronchi, necrotic foci on liver with engorged gall bladder, linear haemorrhage on the crests of the folds of intestinal mucosa, congestion of mesenteric blood vessels and enlargement of mesenteric lymph nodes in the current outbreaks were suggestive of PPR. Similar lesions were also reported by earlier several workers in PPR [9, 11, 13, 15, 16, 17].

The histopathological alterations observed in the present study were bronchopneumonia, interstitial pneumonia, edema, emphysema and intracytoplasmic inclusion body in the lining epithelium of bronchi and bronchiole of lungs. Severe lymphocytic depletion in lymph nodes and spleen, erosive and ulcerative lesion in gums, lips and tongue, together with intestine, coagulative necrosis of hepatic tissue and renal tubular epithelium were indicative of PPR and in accordance with earlier findings [9, 11, 16-20].

In the present study, out of 79 tissue samples 58 (73.41%) were found positive with an amplicons size of 463 base pair of N gene. The remaining samples failed to produce the targeted amplification of 463 base pair. Similar findings were also recorded by Chandra [21]. They also reported the target amplification of 463 base pair by using N gene specific primers.

Fig 1: Necrotic areas with erosions and ulcerations in gums of PPR affected goat.

Fig 2: Necrotic lesion with erosions and ulcerations in tongue of PPR affected goat on handling the area was sloughed out.

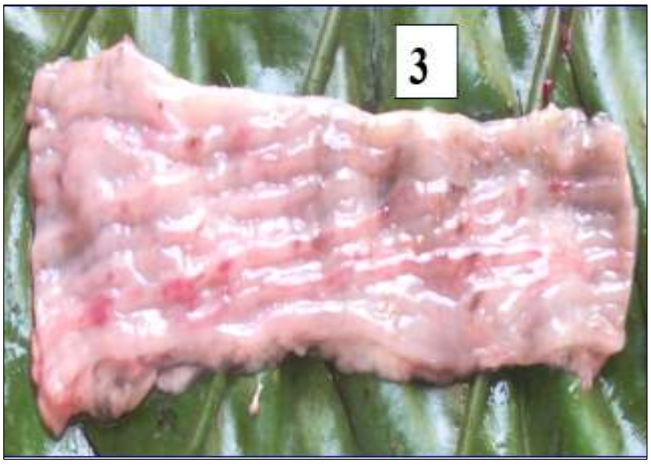

Fig 3: Linear haemorrhages on the creasts of folds of an intestine in PPR affected goat.

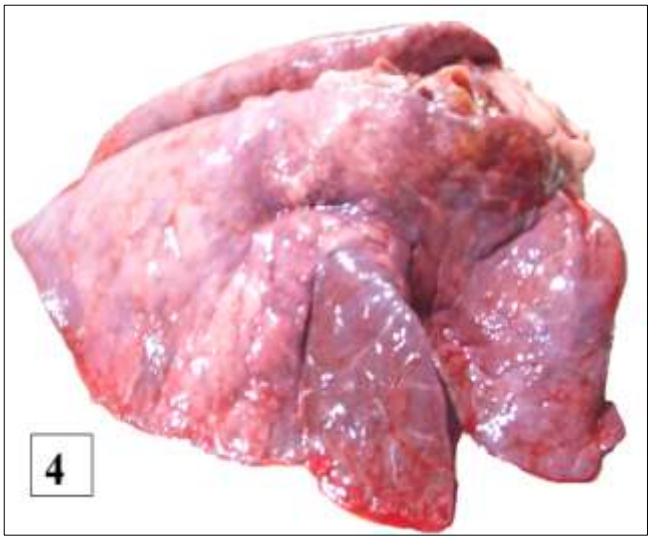

Fig 4: Lung showing congestion and consolidation of anterior and cardiac lobes and patches of emphysema in diaphragmatic lobes.

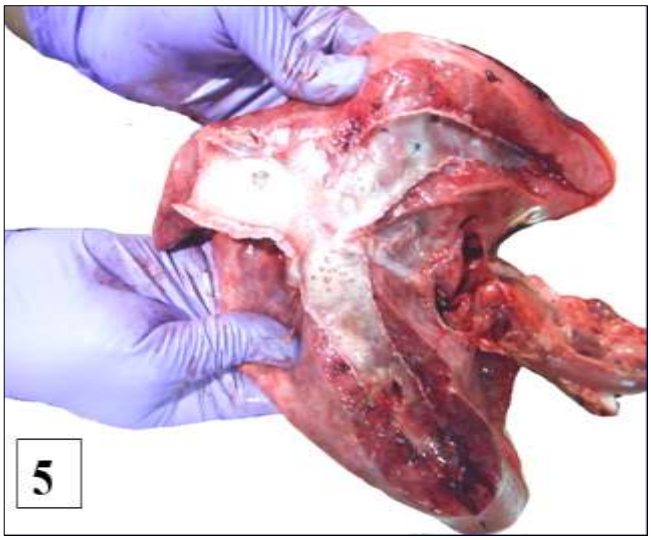

Fig 5: Cut section of lung showing presence of white frothy exudates particularly in the lumen of bronchi.

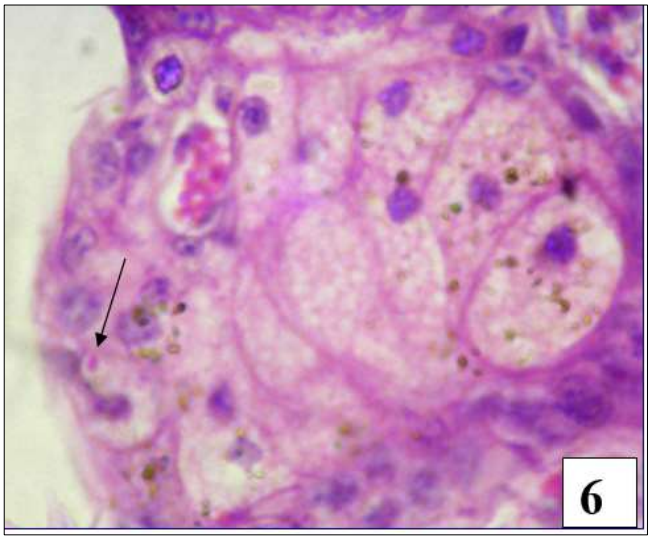

Fig 6: A section of lip of PPR affected goat showing intracytoplasmic eosinophilic inclusion body in cells of stratum spinosum layer. H&E, X400.

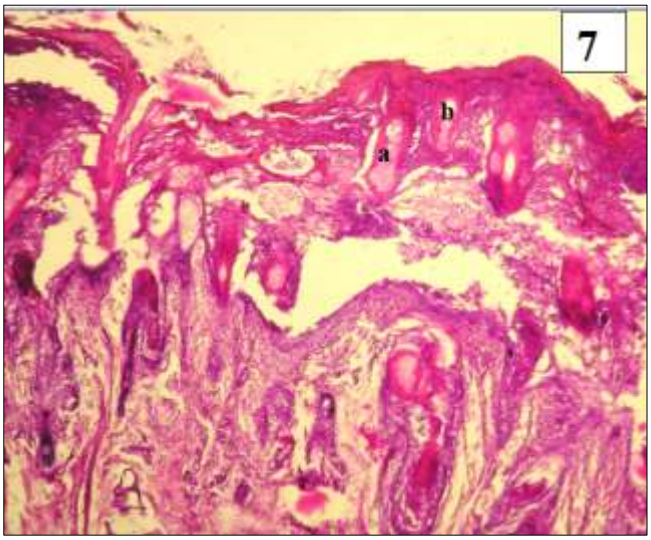

Fig 7: A section of tongue of PPR affected goat showing sloughing of superficial layer of the epidermis with (a) fungiform and (b) filiform papillae. H&E, X40.

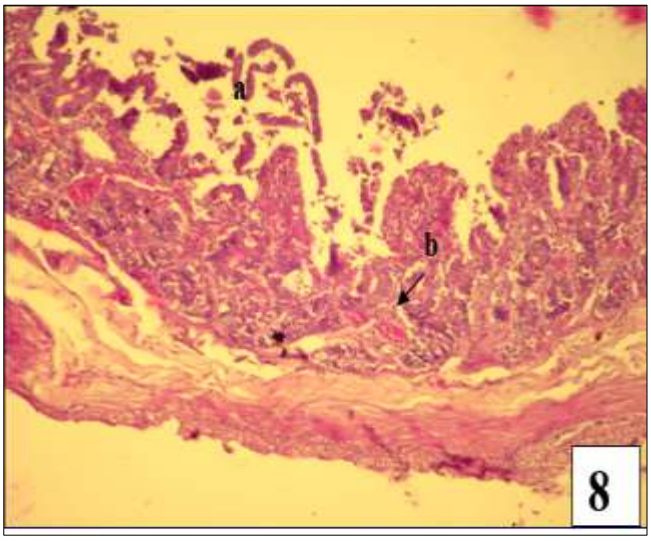

Fig 8: Photomicrograph of a section of a small intestine of PPR affected goat showing (a) necrosis and sloughing of villus epithelium (b) congestion in submucosa. H&E. X100.

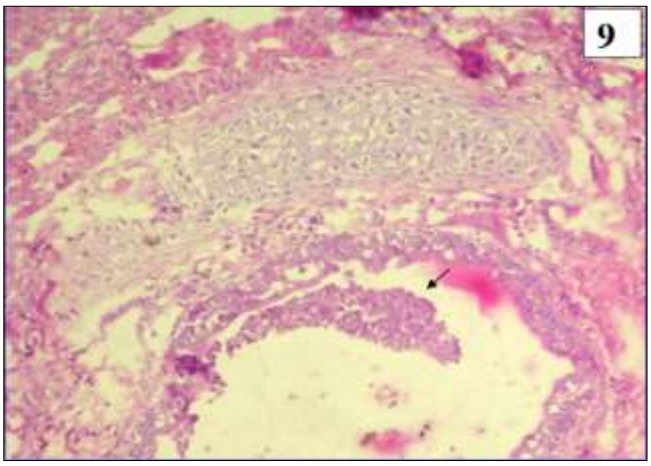

Fig 9: Photomicrograph of a section of lung of PPR affected goat showing presence of mononuclear and polymorpho-nuclear infiltrating cells and sloughed out necrosed epithelial cells in to the lumen of bronchi. H&E, X100.

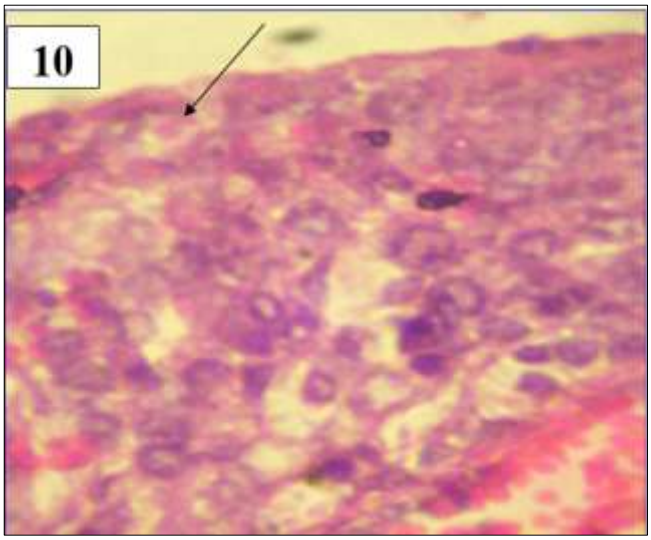

Fig 10: A section of lung of PPR affected goat showing intracytoplasmic eosinophilic inclusion body in the lining epithelium of bronchiole. H&E, X1000.

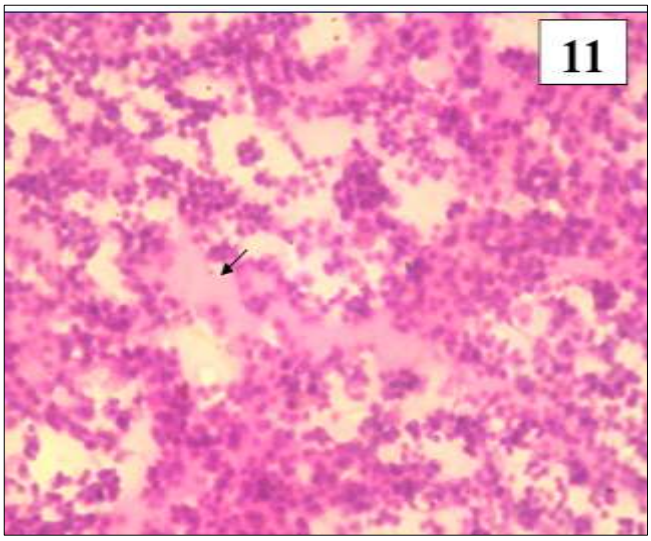

Fig 11: Photomicrograph of a section of a lymph node of PPR affected goat showing edema. H&E, X400.

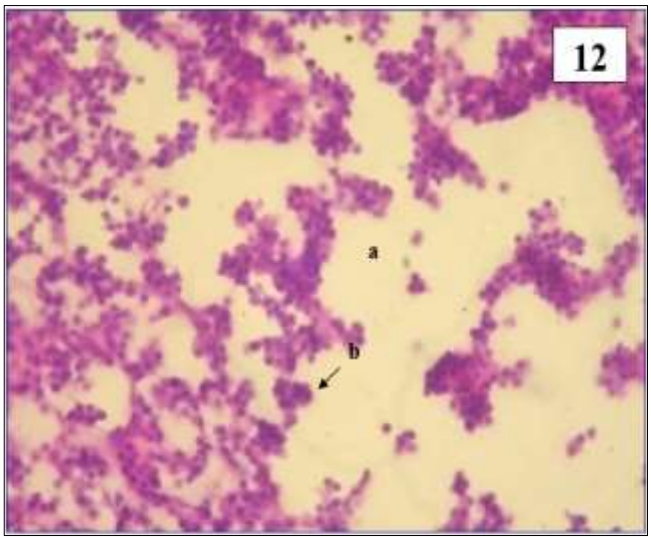

Fig 12: Photomicrograph of a section of a lymph node of PPR affected goat showing (a) severe depletion of lymphocytes and (b) presence of syncitial giant cells. H&E, X400

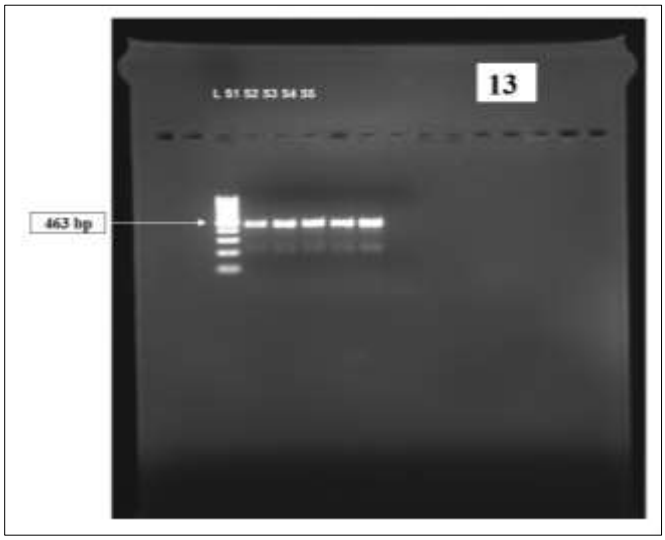

Fig 13: Representative photograph showing PCR amplification of n gene using cDNA prepared from extracted tissue RNA. Standard 100bp (Fermentas) ladder (L) was used. S1= positive control, S2, S3, S4 and S5= infected tissue samples.

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Conflicts of Interest: The authors declare no conflict of interest.

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