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Patho-morphological and histo-enzymic studies of experimentally induced peste des petits ruminants infection in goat

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Abstract
Peste des petits ruminants is an acute viral disease of small ruminants caused by genus Morbillivirus and family Paramyxoviridae. The present study was undertaken to observe gross pathology, histopathology and histoenzymic alterations in experimentally produced cases of PPR. Necropsy examination revealed broncho-interstitial pneumonia, linear haemorrhage in intestine and congestion in lymph nodes. Histopathological study revealed ulcer formation on the epithelium of the tongue and lips, depletion of lymphoid cells in lymph node and spleen with syncytia formation. Histoenzymic study revealed mild lactate dehydrogenase activity in lymph node, spleen and in the tip of the hyperplastic bronchial epithelium. While intense alkaline phosphatase activity was seen in the affected lung and follicles of lymph node.

Keywords: Peste des petits ruminants, experimental study, histopathology, histochemistry

Introduction
Peste-des-petits ruminants (PPR) is an acute contagious disease which affects mainly small ruminants i.e., sheep and goat.The disease has been considered as an emerging disease in our country causing an economic loss of around rupees four hundred crores annually. Several outbreaks of the disease has been reported in the recent years in Assam, causing a high mortality and morbidity in goat population in the state. PPR is caused by Morbillivirus belonging to the family of Paramyxoviridae. PPR is also known as Ovine rinderpest as it shows considerable similarity with Rinderpest in cattle.

Experimental production of a disease in animal is an approach to create a disease model to study the entire course of the disease.

Histopathology refers to the microscopic examination of tissue to study the different stages of diseases. While histoenzyme studies indicates the alteration of different enzymes secreted by organelles of a cell. Together these two technique provides information on the progression of lesion in tissues during a disease.

The prime aim of the present experiment is to study the gradual progression of disease, manifestation of clinical signs, development of lesion and consequence of the disease condition, which is usually not possible to observe in a natural case study.

Experimental production of PPR was performed by various workers, by inoculating viral stock through different routes like subcutaneous route (Kumar *et al*., 2004) [8] and intranasal and subcutaneous route (Truong *et al*., 2014) [16].

Manifestation of oral lesions (Kumar *et al*., 2004) [8], respiratory and gastrointestinal signs (Khan *et al*., 2005) [9]. and development of typical gross and histopathological lesions (Osman, 2005) [11] were also described.

Peste des petits ruminants has been produced experimentally using PPR virus isolates in Lamb testis cells (Osman *et al*., 2009) [12].

Experimentally produced PPR was confirmed by virus isolation, Real time-RT PCR, histopathology and IHC (Truong *et al*., 2014) [16].

Henderson *et al*. (1978) [4] and Drent *et al*. (1996) [2] could record lactase dehydrogenase activity in lungs.

Kuhn (1968) [7] and Edelson *et al*. (1988) [3] observed positive alkaline phosphatase activity in lungs. Tokuda *et al*. (1994) [15] detected intense alkaline phosphatase activity in the medullary region of lymph node.

Materials and Method

The experimental study was conducted in 12 healthy kids of 6 month age. The animals were confirmed to be negative for PPR using competitive ELISA, before the commencement of the experimentation. The kids were housed in the animal house in the department of Pathology, College of Veterinary Science, Khanapara-22. The animals were allowed to adjust with their new shed and environment for 7 days. During this period they were provided with sundried hay, Jackfruit leaves and a bran mixture composed of an equal amount of ground maize and wheat bran along with *ad lib* water. During the acclimatization period the animals were dewormed using Albendazole 10mg/kg body weight.

Both the group consisted of 6 number of animals and were kept in different shed to avoid transmission of infection.

Preparation of inoculum

Tissue consisting of lungs, spleen, intestines and lymph nodes was collected from naturally infected Peste des petis ruminants after confirming by PCR. The mixed tissue sample was minced and ground using a sterile pestle and mortar in 2 ml of phosphate buffer saline. The supernatant from mixed tissue mixture was filtered using a syringe filter of pore size 0.2 µm and was stored at -20. The supernatant was thawed and freezed several times at 4° C before administration in the animals.

Experimental inoculation of goats with PPR virus tissue suspension

2 ml of the inoculum was introduced into the body of the animals from the experimental group by intranasal route and as well by subcutaneous route.

Recording of clinical signs

All the animals from experimental group were examined twice a day for development of clinical signs.

Rectal temperature was examined by placing a thermometer in the rectum for 2 minutes and then recording the reading in degree Celsius. The presence of ocular and nasal discharges was examined by checking the eyes and the nostrils of each animal. The eyes were also examined for congestion of conjunctiva. The respiratory signs were examined by observing the respiratory parameters viz. respiration rate, type, rhythm and depth. The respiratory rate was examined by counting the number of breaths the animal took per minute. Respiratory type was determined by observation of the thoracic and abdominal movements during respiration. Rythm and depth were examined by auscultation of the chest region using a stethoscope. The oral cavity was examined for presence, location, size and distribution of oral lesions. Faeces were examined for colour, consistency and presence of mucus or blood. The findings were documented properly.

Examination for gross pathological examination of animals

Animals that became moribund during the course of the disease were sacrificed on 7 th day and 14th day. The exterior of the animal was examined for the presence of signs viz. the state of body conformation, or lesion on the skin, nose, mouth (lips, cheeks, gums, teeth, and tongue), eyes (eyelids, conjunctiva, cornea, sclera, and iris), ears, vulva and anus. Thereafter a detailed post-mortem was conducted for the presence of gross lesion on lung, lymph node, trachea, spleen, small and large intestine, liver, kidney and brain. All the findings were recorded accordingly.

Histopathology

For histopathological studies, representative tissue samples showing gross lesions were collected, trimmed into thin section of about 1cm, fixed in 10 % Formalin and tissues were processed through routine paraffin embedding technique and haematoxylin and eosin method (Luna, 1968) [10].

Histoenzymic study

For histoenzymic studies, tissue samples were collected from fresh carcass, preserved in deep freeze (-20^0 C) or liquid nitrogen and sectioned into 7-10 µm thickness with a rotary cryotome for further processing for detection of alteration of Lactate dehydrogenase and Alkaline phosphatase.

Processing and preparation of sample for Lactate Dehydrogenase

1. Frozen tissue sections were incubated with substrate stock solution at 37^0 C for 1 hr.
2. Tissue sections were transferred to 40% formol saline for 15 min.
3. The sections were washed under tap water.
4. The washed sections were counterstained with 2% Methyl green for 5 min.
5. Finally the sections were rinsed under tap water.

Processing and preparation of sample for Alkaline Phosphatase

1. Frozen tissue section were dipped in incubating medium and incubated at 37^0 C for 1 hr.
2. The sections were washed with distilled water.
3. The washed sections were counterstained with Methyl green for 5 min.
4. Finally the sections were washed under running tap water.

The processed tissue sections were mounted with an aqueous mountant i.e., Glycerol. The slides were then examined under a compound microscope to record the histoenzymic changes.

Results and Discussion

In the present investigation six goats were inoculated with the mixed tissue sample (lung, lymph node, spleen and intestine) from naturally infected cases of PPR. The treated goats developed clinical signs almost similar to that of the naturally infected animals with PPR. While the control goats did not develop any clinical signs of the disease.

Clinical sign

Following inoculation of the mixed tissue suspension by both intranasal route and subcutaneous route, the experimental group of animals did not manifest any signs of the clinical disease for about three days,which supports the earlier observation made by Osman (2005) [11] in experimentally produced PPR in goats.

By the end of third day, the body temperature began to rise which gradually subsided by fifth day. The rate of respiration was increased i.e., more than 35. This observation was found to be similar with the observation of Hammouchi *et al*. (2012) [5].

By fourth day the animals became less active and showed loss of appetite. The animals were found huddling together indicating the progression of fever. The mucous membrane were slightly congested with clear and watery nasal discharge along with coughing. This finding is in agreement with the findings of Hammouchi *et al*. (2012) [5]. Diarrhoea started

from seventh day onwards, which was characterized by watery, greenish faeces soiling the hind quarters of the animals (Fig.1). This observation is in agreement with the research findings of Osman (2005) [11] and Pope *et al*. (2013) [13].

By eight day the muco-purulent discharges from the nose dried off with plugging of the nostril, leading to difficulty in breathing.

Pasting of the eyelids, dyspnoea and severe diarrhea with presence of mucus was observed by ninth day. The animals could not stand as they became very weak due to severe diarrhoea and dehydration. Development of erosive lesions in the oral cavity was observed with drooling of saliva and inability to take feed. This was followed by formation of crust at the muco-cutaneous junction (Fig. 2) were observed in the affected animals. Similar findings were observed by Pope *et al*. (2013) [13].

Fig 1: Greenish Diarrhoea in PPR

Fig 2: Formation of Crust in the Mucocutaneous Junction

Gross lesion

The most characteristic lesions observed at post mortem examination were present in the gastrointestinal and respiratory system.

The lungs, were congested and consolidated affecting all the lobes but predominant lesions were noticed in the apical lobes (Fig. 3) and the mesenteric lymph nodes were found to be enlarged and edematous (Fig. 4). Similar changes were described by Osman 2005) [11] in experimental cases of PPR in goats.

The mucosal layer of the large intestine showed congestion and linear haemorrhage and petechial haemorrhage was found on the surface of the spleen. Almost similar changes were found by Patel *et al*. (2015) [14] in experimental PPR infection in goats.

Fig 3: Congestion and Consolidation in the Lungs

Fig 4: Enlarged Mesenteric Lymph Nodes and Congested Mesentery

Histopathology

Tissues were collected at 10th day and 12th day from experimentally produced PPR in goats.

As PPR virus is both lymphotropic and epitheliotropic, the histopathological changes were observed mostly in lymphoid organs, respiratory system and gastrointestinal system. Lymphoid organs viz., spleen and lymph nodes showed syncytia formation (Fig.5) depletion of lymphoid cells indicating initial replication of PPR virus and subsequent degeneration and necrosis of the cells. Similar findings were observed by Kumar *et al*. (2004) [8] and Khan *et al*. (2005) [9].

The pulmonary lesion indicated broncho-interstitial pneumonia characterized by thickening of inter alveolar septa with infiltrating cells viz., lymphocytes, macrophages and

inflammatory exudates with some neutrophils filling the alveolar lumen (Fig.6). Similar observation was made by Truong *et al*. (2014) [16] in PPR infection in goats. Degeneration and desquamation of bronchiolar and alveolar epithelial cells were also observed.

Eosinophilic intracytoplasmic inclusion bodies were observed in the hyperplastic bronchial/ bronchiolar (Fig.7) and alveolar epithelium. This finding is similar to the findings by Kumar *et al*. (2004) [8] and Aytekin *et al*. (2011) [1].

The intestinal mucosa showed degeneration and necrosis with neutrophilic infiltration and desquamation of intestinal villi along with depletion of Peyer's patches (Fig.8). Similar changes were recorded by Kumar *et al*. (2004); Khan *et al*. (2005) [9] and Truong *et al*. (2014) [8, 16].

The tongue revealed erosive lesion characterized by necrosis and loss of stratified squamous epithelial cells. This is in agreement with the observation of Truong *et al*. (2014) [16] in tongue lesion of PPR infected goats. Degeneration of renal tubular epithelium causing partial occlusion of the renal tubules were observed in the kidneys of the present investigation simulates the observation of Kumar *et al*. (2004) [8]. The atrophy of the glomeruli was also observed in the experimentally infected goats which are in close concert with the findings of Islam (2015) [6].

The liver showed degeneration of hepatocytes characterized by coagulative necrosis, fatty change and haemorrhage. Similar observation was also recorded by Islam (2015) [6].

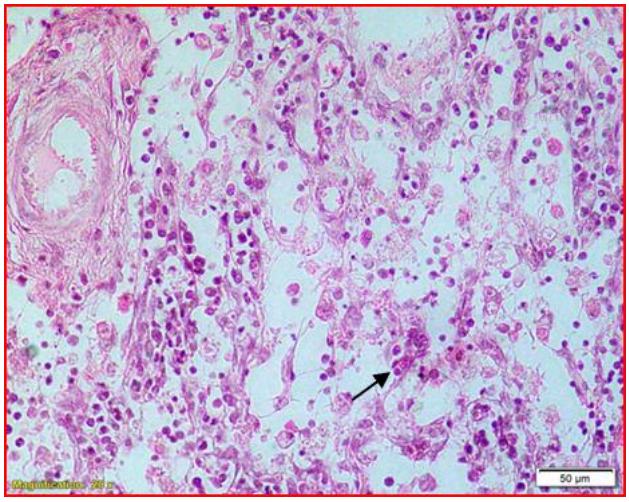

Fig 5: Showing depletion of lymphoid cells with syncytia formation (arrow) in spleen. H&e, x400

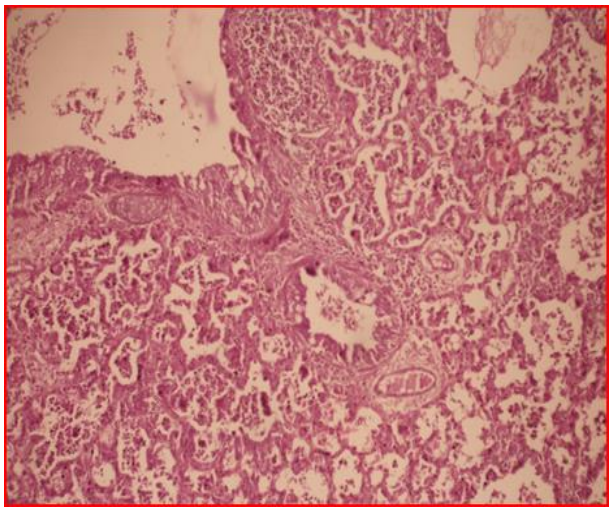

Fig 6: Broncho Interstitial Pneumonia. H&E, X100

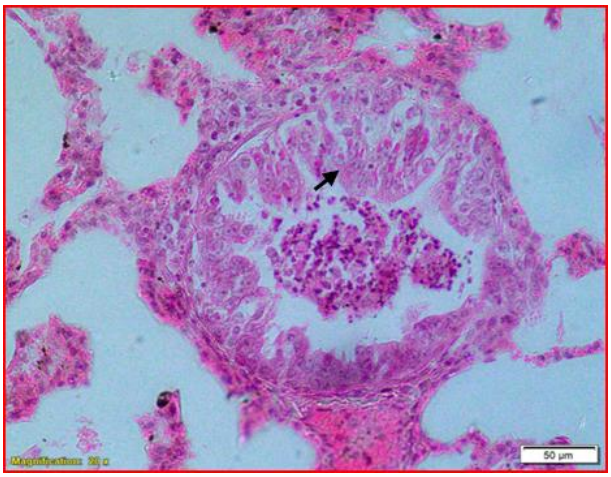

Fig 7: Hyperplasia of Bronchiolar Epithelium with Eosinophilic Intracytoplasmic Inclusion Bodies (Arrow). H&E, X 100

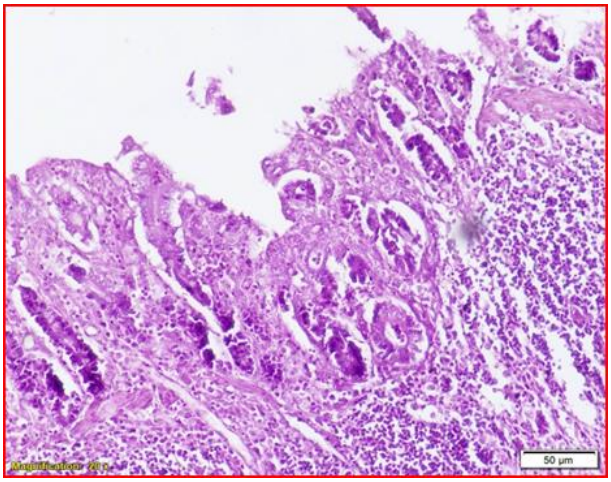

Fig 8: Intestine Mucosa Showing Degeneration and Necrosis with Cellular Infiltration. H&E, X100

Histoenzymic study

In the present study histoenzymic changes of two specific enzymes viz., Lactate dehydrogenase (LDH) and Alkaline phosphatase (AKP) were recorded in the lungs and lymph nodes in PPR affected animals. In normal condition LDH is present in the mitochondria, and shows intense colour reaction in the perinuclear area of the cells, but not in the degenerated cells as the enzyme also gets degenerated, so no peri nuclear activity could be seen. In the present study different pattern of histoenzymic activity was observed in the frozen sections of the affected tissue. Mild enzymic reaction was present in the degenerated and desquamated bronchiolar and alveolar epithelium of lungs. Mild to moderate degree of colour reaction was observed in the tip of the hyperplastic area of the bronchiolar epithelium (Fig.9) as compared to the deeper layer of cells indicating lack of normal blood circulation and nutrition to that area leading to degeneration. Henderson *et al*. (1978) [4] and Drent *et al*. (1996) [2] recorded the presence of lactate dehydrogenase activity in lungs indicating the pathological insult by various toxic agents. Frozen sections of lymph node exhibited mild to moderate enzyme activity in the degenerated lymphoid cells and several empty areas indicating lymphoid cell depletion.

Unlike Lactate dehydrogenase (LDH), Alkaline phosphatase (AKP) is a lysosomal enzyme, which remain securely inside the lysosome in a normal healthy cell, and donot show colour reaction. But in degenerated cells the lysosomal enzymes get leaked out of the cell and shows colour reaction in the

extracellular region. The enzyme reaction was observed more in an around the degenerated alveolar epithelial cells in the lungs in the present study. Kuhn (1968) [7] and Edelson *et al*. (1988) [3] could record alkaline phosphatase activity in lungs while identifying the alveolar type II cells. Intense colour change was present in the lymphoid follicles (Fig.10) and thickened trabaculae in the spleen lends support to the findings of Tokuda *et al*. (1994) [15]. Who observed severe alkaline phosphatase activity in the medullary zone of lymph node.

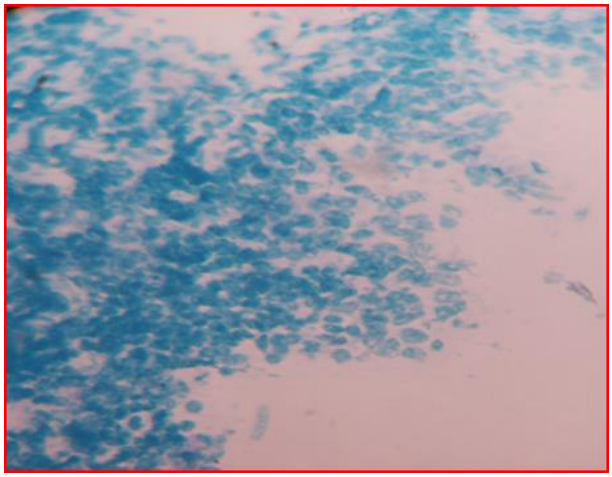

Fig 9: Showing Mild to Moderate Degree of Colour Reaction in the Tip of the Hyperplastic Bronchiolar Epithelium X400

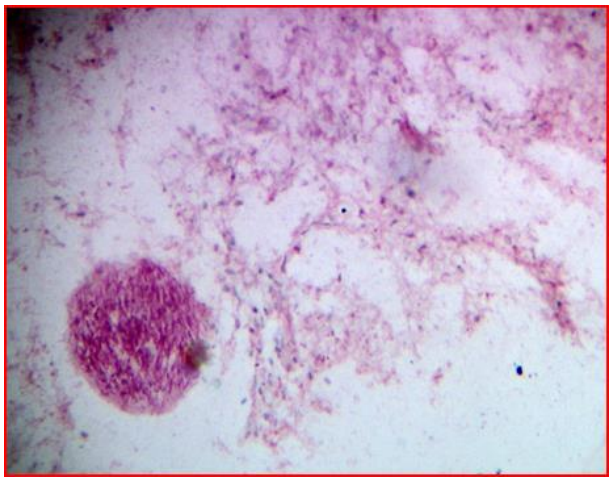

Fig 10: Showing Intense Alkaline Phosphatase Reaction in Denerated Lymphoid Follicle X 100

The experimental study of PPR in goats may prove to be helpful for research scholars who tend to work in similar and further prospect of the disease.

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