The use of pathological and histopathological techniques in the diagnosis of peste des petits ruminants in India

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Summary

The authors report on an outbreak of peste des petits ruminants (PPR) among sheep and goats in the Province of Gujarat, India. Clinical signs observed during outbreaks were typical of PPR. Predominant signs were severe diarrhoea, dyspnoea, mucopurulent discharge from the eyes and nose, erosive rhinitis, necrotic ulcers in the mouth, on the dental pad, tongue, upper and lower lips, fever and depression. Common post-mortem findings included congestion, red hepatisation, raised patches of emphysema in the lungs, haemorrhages and froth exudates in the trachea, severe enteritis and streaks of haemorrhages in the intestine, enlargement and petechial haemorrhages in the spleen and oedema and inflammatory lesions in the mesenteric lymph nodes. Spectacular histopathological changes were observed in the lungs, intestine, spleen, mesenteric lymph nodes, liver and kidneys. Clinical, gross and histopathological lesions and haematological changes were suggestive of PPR, which was further confirmed by detection of PPR viral antigen in clinical samples, as well as post-mortem tissues using the sandwich enzyme-linked immunosorbent assay (s-ELISA).

Keywords

ELISA, Enzyme-linked immunosorbent assay, Goat, Gujarat, India, Peste des petits ruminants, PPR, Sandwich ELISA, Sheep.

Uso di tecniche patologiche e istopatologiche nella diagnosi della peste dei piccoli ruminanti in India

Riassunto

Gli autori descrivo un focolaio epidemico di peste dei piccoli ruminanti (PPR) in ovini e caprini della provincia di Gujarat, India. I sintomi clinici osservati durante i focolai epidemici erano tipici della PPR. I segni principali erano diarrea grave, dispnea, scolo mucopurulento oculare e nasale, rinite erosiva, ulcere necrotiche a livello di bocca, cuscinetto dentale, lingua, labbra superiori e inferiori, febbre e depressione. I riscontri post-mortem comunemente osservati includevano congestione, epatizzazione rossa e chiazze enfisematose rilevate a livello polmonare, emorragie ed essudati schiumosi nella trachea, enterite grave e striature emorragiche a carico dell'intestino, ipertrofia ed emorragie petecchiali a livello della milza, edema e lesioni infiammatorie nei linfonodi mesenterici. Sono state osservate rilevanti alterazioni istopatologiche

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a livello di polmoni, intestino, milza, linfonodi mesenterici, fegato e reni. Le lesioni cliniche, macroscopiche e istopatologiche e le alterazioni ematologiche erano attribuibili a PPR, che è stata ulteriormente confermata individuando l'antigene virale della PPR nei campioni clinici, oltre che nei tessuti post-mortem utilizzando una ELISA sandwich (s-ELISA).

Parole chiave
Caprini, Dosaggio immunoenzimatico, ELISA, Gujarat, India, Ovini, Peste dei piccoli ruminanti, PPR, s-ELISA.

Introduction

Morbillivirus infections have had a significant impact on both human beings and animals for centuries. They are highly contagious pathogens that cause some of the most devastating viral diseases of humans and animals worldwide (11). One of these is the peste des petits ruminants (PPR), which is a highly contagious, infectious and acute or a sub-acute viral disease of domestic and wild small ruminants.

Presently, PPR prevails in most African countries situated along a wide belt that extends between the Sahara and the Equator, the Middle East (Arabian Peninsula, Israel, Syria and Jordan) and the Indian subcontinent. Outbreaks of PPR are now known to be common in Afghanistan, Bangladesh, India, Pakistan and Nepal (1). In India, PPR was first reported in 1987 in the village of Arasur, located in the Villapurum District of Tamil Nadu (16). Since its first reported occurrence, PPR was thought to be restricted to southern area of India up until 1993, after which epidemics of PPR swept across large numbers of small ruminants from northern areas of India (12). Since then, the disease has been reported regularly from different parts of the country and is considered to be an endemic disease that causes significant losses to the small ruminant sector. The present investigation reports on an outbreak of PPR in sheep and goats in Gujarat.

Material and methods

Natural outbreaks of PPR in migratory/village flocks of sheep and goats were recorded in various areas of the Kachchh Region of Gujarat State. Sporadic deaths among young lambs were observed prior to recognition of the outbreak. Later, a sudden increase in mortality among sheep and goats of different age groups was recorded in the month of June with the onset of the monsoon weather. A total of 22 flocks in 12 villages in Mandavi and Abdasa Talukas in the Kachchh District were visited and relevant information was collected to ascertain the cause of mortality.

Clinical signs in affected animals were recorded. Blood, blood smears, nasal discharge and anal swabs were collected from these animals. Serum samples from convalescent animals were also collected for the detection of PPR virus (PPRV) antibodies. Following systematic necropsy examination, tissue samples from the liver, lung, kidney, lymph node, intestine, trachea, thymus, spleen, heart, brain, tongue and mesentery from four sheep showing gross lesions that died during the course of a field survey at the onset of the outbreak were also collected in Hanks balanced salt solution (HBSS) for the detection of PPRV antigen and in 10% buffered formalin for histopathological examination. Ante-mortem samples (nasal, anal and eye swabs and whole blood) for PPRV antigen detection were collected from sheep and goats showing symptoms and from those exhibiting lesions suggestive of PPR. Nasal, eye and anal swabs were collected using sterile wooden swabs in 500 µl of sterile phosphate buffered saline (PBS) (0.1 M, pH 7.4). Samples were transported on ice and stored at –20°C until use. Blood samples were collected in a heparinised vaccutainer and stored at 4°C until use.

A total of 110 samples were collected, as follows:

- 47 nasal swabs
- 10 eye swabs
- 15 rectal swabs
- 10 blood samples
- 4 spleen samples

- 4 liver samples
- 4 lymph node samples
- 4 lung samples
- 4 heart samples
- 4 intestine samples
- 4 tracheal samples.

Ante- and post-mortem samples were processed for detection of PPRV antigen using a sandwich enzyme-linked immunosorbent assay (s-ELISA). Kits were developed at the National Morbillivirus Referral Laboratory, Division of Virology, Indian Veterinary Research Institute (IVRI) in Mukteswar (17). A total of 176 serum samples from affected and in-contact healthy sheep and goats were also collected and tested for the presence of PPRV antibodies using a competitive ELISA (c-ELISA) using PPRV c-ELISA kits developed by the Division of Virology of the National Morbillivirus Referral Laboratory in Mukteswar (18). Standard protocols, in accordance with the instructions of the manufacturer, were followed for both the ELISAs. Tissues from carcasses were fixed in 10% buffered formalin for at least 24 h. They were processed using a standard paraffin embedding technique and 5 µ-6 µ sections were cut. Sections were stained using the haematoxylin and eosin (H&E) method as described by Luna (10). Haematological parameters, such as red blood cell (RBC) count, white blood cell (WBC) count and differential leucocyte counts were performed, in accordance with standard procedures. Blood smears were stained with the Leishman stain. Blood samples, nasal swabs and tissue suspensions were streaked on brain and heart infusion agar (Himedia Laboratories, Mumbai) for bacterial isolation.

Results and discussion

Clinical findings

The predominant signs in natural infection of PPR included dullness, high fever (41°C or 106°F), anorexia, mucopurulent nasal discharge (Fig. 1), eye discharge, lacrimation, congested mucus membrane, severe diarrhoea, along with soiled perianal region with faeces. In addition, a few animals also exhibited erosive rhinitis and dyspnoea. Erosive and ulcerative lesions on the tongue (Fig. 2) as well as on the lips and dental pad of affected animals were also observed. The clinical signs exhibited by the animals were similar to those reported by earlier workers either under natural or experimental conditions (2, 6, 9, 14).

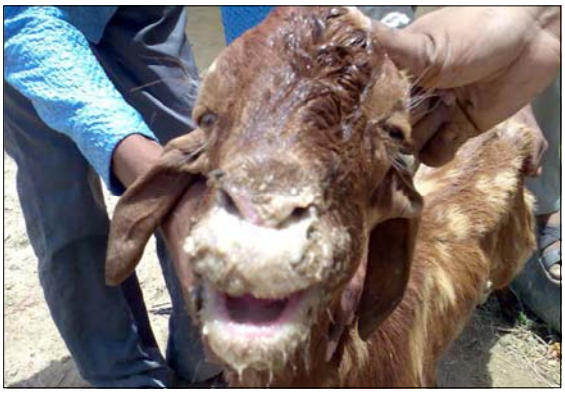

Figure 1
Goat with laboured breathing showing mucopurulent nasal discharge, oedema of the face

Figure 2
Erosive and ulcerative lesions on tongue of sheep

The mortality pattern showed sporadic deaths in the month of May, but high mortality among sheep was noticed in the last week of June and continued until the end of July. The data collected from various areas revealed that 90% of the sheep and goat population were affected. Mortality among sheep and goats was 27.75% (1 070 out of 3 855) and 21.25% (135 out of 635), respectively, which corroborates the findings of Purushothaman *et al.* (14). However, this was contrary to earlier reports where very high mortality was recorded in goats during outbreaks (20). The low mortality

recorded among goats compared to sheep during the present investigation might be due to the fact that flocks were mainly of sheep and each flock also had a few goats. Limited numbers of goats are kept in each flock compared to the large numbers of sheep. The susceptibility of goats and sheep to PPRV infection appeared to vary, with goats being more susceptible than sheep (7).

According to information received, mortality among sheep and goats was observed in the month of June with the start of monsoon weather when flocks started to migrate back to their respective homes. The origin of outbreak was unknown. However, it was revealed from interviews of farmers that the sheep and goats of a certain area generally grazed in one or two common grass fields. Interestingly, it was also noticed that the outbreak was also associated with intermixing of migratory/nomadic animals with local animals. Farmers are not fully aware about the dangers of mixing sick and healthy animals when they graze their stock. Many villages sell their animals at local markets and it was reported that diseased and in-contact healthy animals were often present at the same markets. The intermixing of migratory animals/nomadic animals with local animals, grazing of one or more flocks in one area and sales of diseased/in-contact healthy animals might have precipitated the outbreak. Similar observations were also made by Asmar *et al.* (4) in Saudi Arabia and Das *et al.* (6) in Bangladesh. Subsequent vaccination with PPRV live vaccine controlled the disease and no further mortality was recorded in August 2008.

Gross pathology

A detail necropsy was conducted on four sheep carcasses. In general, all dead animals were found emaciated and severely dehydrated. Most dead sheep showed erosive lesions on the lips and around the nose. Nostrils showed mucopurulent discharge and congested mucus membrane. The buccal cavity revealed erosive and ulcerative lesions on the tongue, gum, dental pads, hard palate, soft palate and pharynx (erosive stomatitis). The lung invariably showed pneumonic lesions in all necropsied sheep. The commonly affected lobes were apical and cardiac lobes which revealed red hepatisation and congestion. In two sheep, severe red hepatisation of most of the lobes was noted (Fig. 3). In addition to these, raised patches of emphysema were also observed. On cut sections, lungs showed large quantities of frothy exudates indicative of

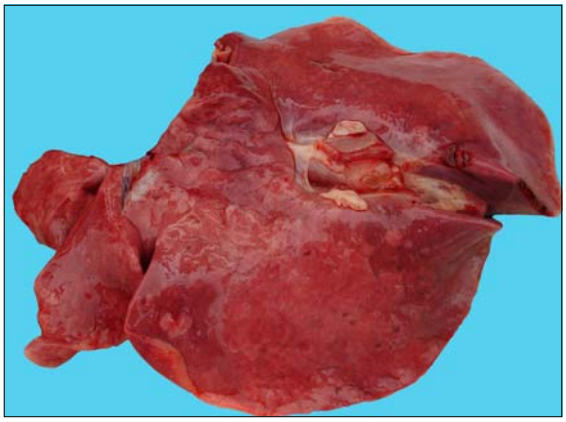

Figure 3
Lung with severe red hepatisation of different lobes

pulmonary oedema. Tracheal mucosa revealed multifocal to linear haemorrhages accompanied by the presence of frothy exudate. Intestines, especially the small intestine, showed moderate to severe haemorrhagic enteritis characterised by hyperaemic intestinal mucosa with intestinal contents. The intestinal mucosa was also found slightly thickened with linear haemorrhaging (Fig. 4). The abomasum presented erosions. Mesenteric lymph nodes (MLN) were enlarged, swollen and oedematous. The liver was slightly

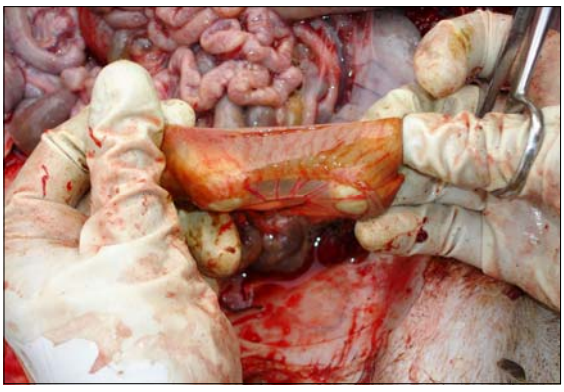

Figure 4
Intestine mucosa showing linear haemorrhages

yellowish in colour with the presence of focal pale areas of necrosis. The gall bladder was distended with bile. The heart was congested and contained white necrotic patches with prominent blood vessels. The spleen was enlarged and showed petechial to ecchymotic haemorrhages. The kidneys were highly congested. The gross lesions observed in the present study were also reported earlier in PPR-infected goats and sheep either individually or in groups (3, 8, 13, 14).

Blood smear examination

Blood smears prepared from affected animals were found negative for the presence of bacteria of significant bacterial infections.

Bacterial isolation

Cultural isolation of blood, nasal swabs and tissue suspension revealed no bacteria.

Haematological examination

The results of haematological examinations revealed marked leucopaenia and lympho-cytopaenia. Leucopaenia and lymphocyto-paenia were consequent to tropism of the virus to lymphoid tissue causing destruction of lymphocytes during virus multiplication. Rajak *et al.* (15) showed that PPRV causes marked immunosuppression as evidenced by leucopaenia and lymphopaenia.

Histopathological findings

The lesions present in the lungs were more severe and characteristic of broncho-interstitial pneumonia. The lesions were generally characterised by distention and dilatation of alveoli with oedema, congestion of alveolar capillaries and haemorrhages (Fig. 5). Infiltr-ation of mononuclear cells in alveoli, along with haemorrhages, interstitial pneumonia characterised by thickened interalveolar septa, caused by the infiltration of erythrocytes, mononuclear cells and increased histiocyte proliferation was evident at such locations. In addition to alveolar changes, lesions were also observed in bronchioles. There was desquamation of bronchiolar epithelium with the presence of epithelial cell debris in the lumen of bronchioles which was indicative of bronchopneumonia (Fig. 6). Severe congestion and alveolar haemorrhages were also noted. In

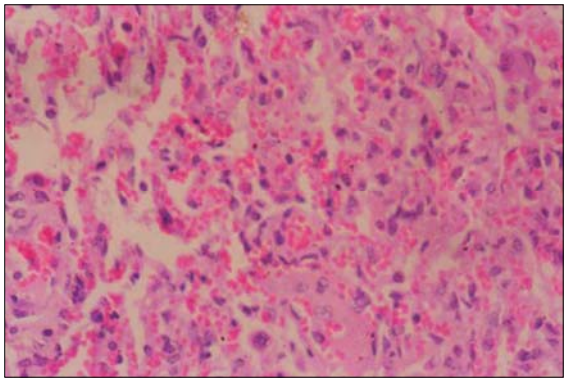

Figure 5
Section of lung showing congestion of alveolar capillaries and haemorrhages
(H & E 400×)

addition, multinucleated syncytia were observed in the alveolar epithelium (Fig. 7). Similar lesions were recorded by others (1, 5, 13). The intestine showed stunting and blunting of villi, with necrosis at villous tips and erosion and infiltration of inflammatory cells in the lamina propria. Congestion and haemorrhages were also evident. Goblet cell hyperplasia and depletion, as well as rarefaction of lymphoid cells, were evident in Peyer's patches. Lymphoid organs, especially the spleen and

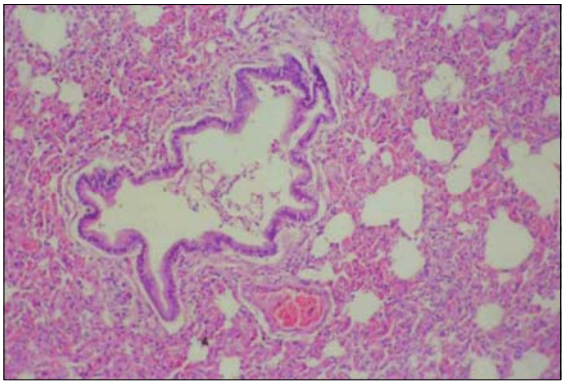

Figure 6
Section of lung showing bronchopneumonia, congestion and haemorrhages
(H & E 100×)

MLN, showed lesions of variable degrees of lymphoid cell depletion. Spleen showed acute necrosis of white pulp with depletion and rarefaction of lymphoid cells. MLN showed rarefaction and depletion of lymphoid cells, particularly in the cortical lymphoid follicles. The histopathological changes in kidneys were mostly vascular and degenerative in nature.

Kidney samples showed tubular degeneration, characterised by desquamation of tubular epithelium, tubular vacuolation, tubular necrosis and the presence of hyaline tubular cast in the tubular lumen. In places, tubular and glomerular haemorrhages were also observed.

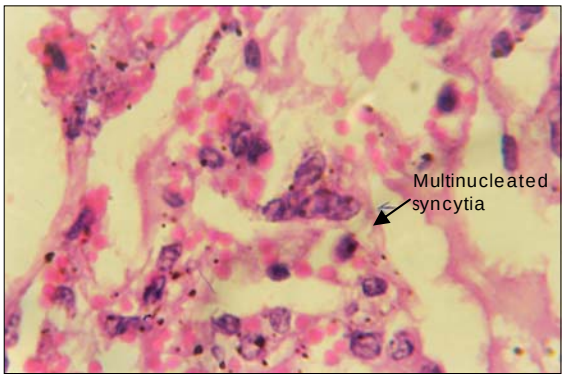

Figure 7
Lung section showing multinucleated syncytia in the alveolar epithelium

Sections of liver showed sinusoidal dilatation with haemorrhages. In the periportal area, there was infiltration of inflammatory cells, mostly neutrophils, and few mononuclear cells along with engorged blood vessels. The pathomorphological lesions confirmed earlier findings (3, 7, 17, 19). Multinucleated syncytial cells in alveolar lamina, together with mucosal lesions in the oral cavity, are pathognomonic for PPR and such lesions were detected in the present outbreak.

Detection of peste des petits ruminants viral antigen and antibodies

Of the various clinical and post-mortem samples screened for the detection of PPRV antigen, 50 out of 110 were found positive. Of 176 sera tested, 112 (63.64 %) were positive for the presence of PPRV antibodies. Among these serum samples, 156 were from sheep and only 20 from goats. In regard to species, the seroprevalence recorded was 64.10% and 60.00% in sheep and goats, respectively. The higher rate of seroprevalence might be due to the recovery stage of animals or could be due to the prior immunity in some of the animals against PPRV.

Conclusions

The diagnosis of PPR is based on clinical examination, gross pathology, histological findings and laboratory confirmation by virus isolations or genome detection. Most laboratory methods require collection of clinical and post-mortem materials of high quality and need to be shipped in a cold chain system from distant places. In such situations, morbid tissues can be collected in 10% formalin and processed for histological examination and hence, in the present investigation, pathological and histopathological techniques were used for the diagnosis of PPR. Although clinical and post-mortem findings may be sufficient for the diagnosis of PPR in endemic areas, histological and serological examinations are essential for definitive diagnosis. During the present outbreak, clinical and post-mortem findings and histopathological changes were suggestive of PPRV, which was confirmed by the detection of PPRV antigen in clinical and post-mortem samples using the s-ELISA. Results of c-ELISA using PPRV-specific monoclonal antibodies also revealed the presence of serum antibodies in recovered and in-contact clinically healthy animals. Finally, the disease was controlled by vaccination. It is concluded that the clinical findings, gross lesions, histopathological changes and detection of PPRV antigen and antibodies were useful for diagnosis of PPR and subsequently to control the disease by vaccination.

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