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Research Article

Clinico-pathological and molecular based investigation of PPR virus in goats

Muhammad Moazzam Jalees[1], Hafiz Muhammad Ishaq[2], Abu Baker Siddique[3*], Muhammad Fakhar-e-Alam Kulyar[4,5], Samina Shabbir[6,7], Prerona Boruah[8], Wangyuan Yao[9] and Zeeshan Ahmad Bhutta[10*]

[1]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur-63100, Pakistan

[2]Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

[3]Department of Microbiology, Faculty of Life Sciences, Government College University, Faisalabad 38040, Pakistan

[4]College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, PR China

[5]Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

[6]State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China

[7]Institute of Plant Protection, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, Guangdong, China

[8]School of Biotechnology and Bioinformatics, DY Patil University, Navi Mumbai, 400614, India

[9]Department of Microbiology and Plant Pathology, University of California-Riverside, Riverside, CA, 92521, USA

[10]Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, 28644, Republic of Korea

*Correspondence: zeeshan@chungbuk.ac.kr; absiddique@gcuf.edu.pk

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ABSTRACT

Peste des petits ruminants (PPR) is a deadly viral illness that affects small ruminants, especially goats. The Peste des petits affects both small and big ruminants and is a highly contagious illness. The disease is extremely infectious and has a fluctuating risk of recurrence in Punjab, Pakistan, with a reported fatality rate of 35-60%, the disease is of enormous economic importance and prevalent throughout Asia. After a PPR epidemic in Pakistan's Bahawalpur area, the current study was undertaken on a private farm. The goats were categorized into different groups based on breed age and sex during the investigation. Different breeds had greater rates of morbidity, death, and case fatality than non-descriptive animals. Morbid animals showed characteristic clinical signs and symptoms of PPR. The morbidity, mortality and case fatality were very high. Different characteristic clinical signs and postmortem lesions were observed in PPR infected animals. At microscopic level, various microscopic lesions in heart, kidneys, lungs, liver, spleen, and intestine were observed. The results of this study suggested that frequent and regular investigation of PPR infection is vital to control and limit the spread of viruses and implement suitable control plans to lower the risks of viruses in small ruminants.

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Introduction

PPR, known as Peste des petits, is a severe and highly infectious illness of sheep and goats that affects both wild and farmed small and big animals (Banyard and Parida 2015). It is estimated that in Pakistan the prevalence of PPR is nearly about 43.55% in goats and sheep (Ahaduzzaman 2020). The virus in question is a member of the Paramyxoviridae family's Morbillivirus genus. PPR progression or pathogenesis includes entry of the virus through the respiratory system and its replication in regional lymph nodes; later, the virus is disseminated to GIT, respiratory tract, bone marrow, and spleen. Viremia develops at 2-3 days post infection (dpi); the virus is also observed within the organs with abundant epithelial and lymphoid tissue due to its epitheliotropic and lymphotropic properties (Truong et al. 2014). High fever, anorexia, severe diarrhea, ulcerative necrotic

stomatitis, purulent nasal and ocular discharges, and respiratory distress characterize the morbid animal (Rahman et al. 2020); this condition is linked to pneumonia and coughing leading to mortality. Morbidity and mortality in non-endemic locations may vary depending on population susceptibility, which in severe circumstances might reach 90-100% (Hussain et al. 2003). The condition is complicated by simultaneous bacterial, viral, and parasite illnesses, and fatality rates can reach 100% (Kitching 1988); however, mortality rates in endemic regions have been reported as low as 24% (Khaliq et al. 2020).

As sheep and goats are reared by poor farmers and meat is exported due to HALAL meat concerns, the main obstacle is the presence of irresistible disease within the herd population due to the lack of proper vaccination. That is why, this highly infectious disease has considerable economic importance (Albina et al. 2013). This disease is endemic throughout the Asian regions (Banyard et al. 2016). In Asia, 35-60% mortality rate has been reported (Dhar et al. 2002). Moreover, this endemic is transboundary in nature along with countries which are bordering Afghanistan, India, Pakistan, Iran, and China, which are more prone to evidence-based risk (Munir et al. 2015; Mohebbi et al. 2018). It has been classified as a class one disease by the FAO and the OIE, and it must be eliminated (OIE and FAO 2015). The effects of PPR in goats and sheep along with large ruminants were reported, even though this disease also affects camels (Rahman et al. 2020). The pathogenicity linked with the PPR virus is associated with the respiratory and digestive systems. Mass vaccination and prevention are the ways to control PPR; a deep knowledge of disease progression and pathogenesis is the most crucial point of concern. Many PPR outbreaks have recently been reported in Pakistan, with greater morbidity and mortality rates, particularly among small ruminants (Khan et al. 2018). Assessing the genetic relatedness between the prevalent PPR virus strains is essential, which is very important to control the disease.

Moreover, identifying homogenous strains within the population proves to help produce the appropriate vaccine, which ultimately counters the vaccine failure effects and impotent steps in the disease control strategy. So, the assessment of genetic relations between prevalent virus strains is carried out using the basic molecular diagnostic test, i.e., RT-PCR. The present study was conducted to identify the PPR virus, isolated from the field outbreak, based on N-protein genes (Khaliq et al. 2020) and histopathology.

Materials and Methods

Area of Study and farm management

The current research was carried out on a private small ruminant farm in district Bahawalpur (Pakistan), which had a total of 5390 animals, comprising 1219 sheep and 1264 goats. Kajli sheep and Beetal goats weighed between 40 and 50 kg, whereas Dorper sheep and Boer goats weighed 75 and 100 kg. All the animals were kept in the same way, with ad-libitum water and seasonal forage supplemented with prepared concentrates. Animals were vaccinated against PPR, pleuropneumonia, and enterotoxemia. Before this outbreak, 25 goats (Beetal) and 40 sheep (Kajli) were procured from the local market and kept for quarantine period for a week. PESTDOLL-S® was used to vaccinate them and then allowed to enter the herd.

Gross examination and sample collection

Following the mortalities of goats and sheep, a postmortem was performed, and identical images of lesions in goats and sheep were observed. Gross examination was recorded, including lesions and biometrical observations, and categorized as mild, moderate, and severe depending upon the severity of the disease. Morbid organs (liver, spleen, lungs, and intestinal tract) were collected and preserved in 10% formalin, processed, sectioned, and stained as described by Mayer and Klein (1961).

Molecular detection of PPRV nucleic acid through RT-PCR

For RT-PCR analysis, tissue samples from the spleen, lungs, liver, bronchial, and mesenteric lymph nodes were taken. The collected organs were homogenized in PBS (20%) suspension and allowed for 10-minute centrifugation at 800g. The supernatant was taken and treated with gentamycin (500µg/mL) and then stored at -20°C. The positive control group was vaccinated with a live PPR (Freeze-dried) vaccine purchased from VRI (Veterinary Research Institute, Lahore, Pakistan). Qiagen RNeasy Kit (Germany) was used to extract RNA from the vaccinal virus and tissue suspension, following the manufacturer's instructions. RT-PCR was used to identify the viral nucleic acid (One-Step RT-PCR kit, Qiagen).

A 352-base pair fragment of the PPRV nucleoprotein gene was amplified with NP-3 and NP-4 gene primers (Table 1). In brief, the 5µL of RNA was employed in a mixture having dNTP mixture (2µL), One-Step RT-PCR mixture (2µL), Q-Solution (10µL) and 5 × Qiagen buffer (10µL), while each primer with a final concentration of 0.6µM and water. The isolated RNA was reverse transcribed by incubating at 50°C for 30 minutes. After that, the PCR process began with denaturation and Taq polymerase activation at 95°C for 15 minutes. This was followed by amplification (40 cycles), which corresponded to 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds, and a final extension for 10 minutes at 72°C. The amplified PCR products (10µL) were stained with ethidium bromide and analyzed by gel electrophoresis (1.5 percent agarose gel); after this, results were recorded on the gel doc system.

Data analysis

The data were analyzed using Minitab statistical software and the Chi-square test, with a significance level of $P<0.05$.

Results

Morbidity mortality and case fatality

On the bases of sex:

Morbidity, mortality, and case fatality were 34.2, 13, and 38.1%, respectively, among 283 male goats. Morbidity, mortality, and case fatality were found to be 45.7, 17.9, and 39.3%, respectively, in 256 female goats.

On the bases of age

Five age-based groups were made ranging from less than 1 year to greater than two year. Out of 143 goats which were under 1 year of age, morbidity, mortality, and case fatality were 60.8, 28.6, and 47.1%, respectively. Out of 161 goats, 1 year old, morbidity, mortality, and case fatality were 36.6, 15.5, and 42.3%, respectively. The group of 83 goats of 1.5 years showed 33.7, 9.6, and 28.5% morbidity, mortality, and case fatality, respectively. The goats in the herd (85), age ranging from 1.6 to 2 years, morbidity rate was 27%, mortality rate was 6%, and case fatality was 26%. In the last group which comprised 67 goats of more than two years of age, 25.3, 7, and 29.4%, morbidity, mortality, and rates of case fatality, respectively, were examined.

On the bases of the breed

Out of 167 Beetal goats, morbidity, mortality, and case fatality were 54.4, 28.1, and 51.6%, respectively. Boer breed showed 46.8% morbidity and 14.8% mortality, with the lowest case fatality rate of 14.8%. Indigenous breeds, i.e., Beetal goats showed significantly ($P < 0.001$) higher morbidity and mortality rates by 54.4 and 28.1%, as compared to exotic breeds, i.e., Australian Boer goats (46.6 and 14.8%). Case fatality was also highest (51.6%) among the Beetal. On the other hand, the lowest case fatality was recorded among the Boers.

Clinical signs and symptoms

Morbid animals showed consistently elevated temperature of the body (104-105°F) for the period of 3-5 days. Animals showed anorexia, dryness of the muzzle, and depression with a dull coat. Goats affected by PPR showed lacrimation, nasal discharge, along with catarrhal conjunctivitis. Serous nasal discharge was observed at an early stage, which became mucopurulent over time. Furthermore, abdominal breathing and rales with respiratory distress were also observed in the affected animal. Severe blood-stained or watery diarrhea followed by emaciation, dehydration, dyspnea, and finally death (Fig. 1).

Gastro-intestinal tract

Necropsy lesions

The gross lesions in morbid animals were cheilitis (inflammation of lips) with ulcers and erosions in oral mucosa, hyperemic gums, and erosive lesions were also observed on the pharynx, upper third of the esophagus, and hard palate. Striations of hemorrhages in the duodenum and terminal ileum, as well as extensive ulceration of Payer's patches and necrotic enteritis, were seen in the GIT. In the large intestine, congestion at the ceco-colic junction, around the ileocecal valve, rectum, and posterior colon was also observed (Zebra stripes). Congestion along with enlargement of spleen, liver, and mesenteric lymph nodes were also examined. The respiratory tract showed bronchopneumonia, with petechiae and erosions on larynx and nasal mucosa. Trachea also showed severe congestion along with froth accumulation. Lungs showed deposition of fibrinous material and congested apical lobe.

Histopathological lesions

Histopathological lesions that are distinctive and linked with intestines were stunting, blunting, and sloughing of villi along with necrotic tips of the villi; mononuclear cellular infiltrates along with neutrophils were also observed in lamina propria. The proliferation of fibrocytes and diffused type edema of the submucosal layer ultimately increased the submucosal thickness of the small intestine. Degenerated and necrosed glandular epithelial cells of the upper duodenum, jejunum, and ileum were also observed. Complete or partial destruction of Peyer's patches (lymphoid follicles) was characterized by karyorrhexis and lympholysis (Fig. 2).

Marked lymphocytic depletion in parafollicular white pulp areas and reticuloendothelial cell hyperplasia were also observed. Dilated sinusoids filled with plasma cells, macrophages, and a few neutrophils were also observed (Fig. 3). Similar type of lesions was observed in tonsils, retropharyngeal and mesenteric lymph nodes. The liver showed multifocal coagulative necrosis, sinusoids dilatation, hepatocytic vacuolation, congestion, and engorged blood vessels. Hepatocyte nuclei were pyknotic at that site. The area around the portal vein exhibited inflammatory cell infiltration, predominantly neutrophils, along with a few mononuclear cells (Fig. 4). Kidney showed severe necroses, degenerated, desquamated, and vacuolated renal tubular epithelium, along with glomerular hemorrhage. Hyaline casts were also observed in the tubular lumen. Our findings related to kidney damage are in agreement with Khaliq et al. (2020), as they also reported congested and degenerated lesions in the kidney (Fig. 5). Severe congestion and myocarditis in heart of goats infected with PPR virus was observed in goats infected with the PPR virus (Fig. 6). Similar results were observed by Beghum et al. (2021). Gross lesions linked with the respiratory tract were broncho-pneumonia with small petechiae and erosions on the larynx and nasal mucosa, congested trachea with froth accumulation, and severe congested apical lobe of the lungs. Fibrinous material deposition was also observed. In the lungs, histopathological lesions included severe interstitial pneumonia of bronchioles. Bronchiolar epithelium was desquamated, with the accumulation of epithelial cell debris in the lumen of the bronchioles, which clearly indicated broncho-pneumonia. The fibrino-edematous fluid accumulation, macrophages, numerous neutrophilic infiltrations within the alveoli, and thickened interalveolar septa indicated interstitial pneumonia. Mononuclear cell infiltration within alveoli, along with hemorrhages, was evident. Serofibrinous exudate indicated fibrinous pneumonia, with few neutrophils and macrophages within alveolar Lumina. Intranuclear eosinophilic inclusion bodies were also evident (Fig. 7).

Discussion

The present study was conducted in Bahawalpur district (Pakistan) at a sheep and goat farm to investigate the PPR outbreak during mid of July. The PPR outbreak investigations were made on goats (Boer and Beetal) along with sheep of Dorper and Kajli breeds. The animals were placed into 5 groups based on their age (less than 1 year to more than 2 years).

Inclusive rates of mortality and morbidity in the recent study were observed as 48.77 and 21.50%,

respectively. Khaliq et al. (2020) also conducted an epidemiological study and observed the 24% mortality rate associated with the PPR virus during an outbreak. Morbidity, mortality, and case fatality were expressively greater (P<0.001) in Beetal goats (54.4, 28.1, and 51.6%, respectively) as compared to exotic breed (Boer) (46.8, 14.8 and 14.8%, respectively). Higher rates of mortality and morbidity were linked with virus of PPR, in indigenous goat breeds (Beetal), as compared to exotic breed (Boer), have also been observed by Khan et al. (2018), as they examined considerably (P<0.001) greater mortality rates along with higher morbidity of indigenous breeds by 81.10 and 37.24%, respectively, while Boer goat breed (exotic) showed morbidity and mortality rates by 5.01 and 2.23%, respectively, thus variation in susceptibility of PPR among different breed, might be linked with some genetic factors, location, breed population and inappropriate vaccination.

On the bases of age, under a year aged group showed 60.8, 28.6, and 47.1%, morbidity, mortality, and case fatality, while more than 2 years aged group showed 29.4, 25.3 and 7, case fatality, morbidity, and mortality rates, respectively. These results in our study are also similar to the results of Soundararajan et al. (2006), as they also observed higher mortality rates in young goats (37.32%), as compared to adult ones (6.44%). Mohanto et al. (2018) also reported higher susceptibility of PPR among young male goats (less than one year of age) as compared to adult ones. This higher susceptibility among young ones may be attributed to poor immune systems, malnutrition, and poor management.

Among sex, our study showed higher susceptibility for PPR in female goats as compared to male goats, as female goats showed higher case fatality, morbidity, and mortality rates of 39.3, 45.7, and 17.9%, respectively, while male goats showed 34.2, 13, and 38.1%, respectively. These results of our study are also in agreement with the study of Mohanto et al. (2018), as they also observed higher susceptibility of PPR among female goats (45.96%) than male goats (38.81%). This higher susceptibility of PPR among female goats might be linked to genetic factors.

Morbid animals showed elevated body temperature (104–105°F), anorexia, dull hair coat, serous to mucopurulent nasal and ocular discharge with catarrhal conjunctivitis, respiratory rates, and abdominal breathing. Severe watery and blood-stained diarrhea was also observed, leading to emaciation and dehydration (Fig. 1). A similar finding was also reported by Mohanto et al. (2018).

The PPR viral DNA was isolated from organs (liver, lungs, kidney, intestine, spleen, bronchial, and mesenteric lymph nodes) collected from the mortalities. The N protein genes were targeted for the identification of the PPR virus. The NP3 and NP4 gene primers were employed to amplify the PPRV nucleoprotein gene length (352-bp fragment). Khan et al. (2018) also used the same primers for molecular identification of the PPR virus in their study.

The gross lesions in morbid animals were cheilitis with ulcers and erosions in oral mucosa and hyperemic gums, and erosive lesions were also observed on the pharynx, hard palate, and upper third of the esophagus. Characteristic lesions noted in GIT were specific hemorrhagic streaks in the duodenum and terminal part of ileum, necrotic enteritis, and severe ulcerated Payer's patches (Sahoo et al. 2020). Congestion at the ceco-colic junction, around the rectum and ileocecal valve, and the congestion in zebra stripes in the posterior colon were also observed. Congestion, necrosis, and enlargement of the spleen, mesenteric lymph nodes, and liver were also examined. The respiratory tract showed bronchopneumonia, with petechiae and erosions on the larynx and nasal mucosa. Trachea also showed severe congestion along with froth accumulation. Lungs showed deposition of fibrinous material and congested apical lobe, edema, and consolidation (Fig. 7). Similar type of lesions has also been reported by Balamurugan et al. (2014), Begum et al. (2018), Iniobong et al. (2019) and Khaliq et al. (2020).

Characteristic histopathological lesions linked with intestines were stunting, blunting, and sloughing of villi along with necrotic tips of the villi. Infiltration of mononuclear cells along with neutrophils was also observed in lamina propria. Proliferation of fibrocytes and diffused type edema of submucosal layer, which ultimately increased the submucosal thickness of small intestine was also observed. Degenerated and necrosed glandular epithelial cells of upper duodenum, jejunum, and ileum were also observed. Complete or partial destruction of Peyer's patches (lymphoid follicles), which was characterized by karyorrhexis and lympholysis (Fig. 2). Infiltration of inflammatory cells in the mucosa of the small intestine and epithelial necrosis in PPR infected animals, have also been reported by (Fayyad et al. 2020). Blunting and Stunting of intestinal villi, epithelial cells necrosis, and mononuclear infiltrates along with lymphoid depletion of Payers patches, also reported by Khan et al. (2018).

Marked lymphocytic depletion of white pulp's parafollicular areas and the hyperplasia of reticuloendothelial cells were also observed. Dilated sinusoids were filled with plasma cells, macrophages, and a few neutrophils. Lesions in the tonsils, mesenteric, and retropharyngeal lymph nodes were similar to those in the spleen. Sahoo et al. (2020) have also reported marked lymphocytic depletion in the white pulp area of the spleen, infiltration of mononuclear cells within bronchiole and alveoli, bronchio-interstitial pneumonia, and with necrosis, desquamation, and degeneration of bronchiolar epithelial cells. Their findings related to intestinal pathology also agree with our findings, as they reported lymphoid depletion of Payer's patches of ileum, along with congestion, edema, and mononuclear cellular infiltration in the submucosa.

Liver showed coagulative necrosis (multifocal areas), dilatation of sinusoids, along with vacuolation of hepatocytes, congestion, and engorged blood vessels. Hepatocyte nuclei were pyknotic at that site. The area around the portal vein exhibited inflammatory cells infiltration, predominantly neutrophils, along with few mononuclear cells (Fig. 4). Our findings were similar to Pruvot et al. (2020), as they also observed hepatocellular degeneration in a PPR outbreak study.

Kidney showed severe necroses, degenerated, desquamated, and vacuolated renal tubular epithelium, along with glomerular hemorrhage. Hayline casts were also observed in the tubular

lumen. Presence of intranuclear viral inclusion bodies was also seen there (Fig. 5). Hemorrhages, congestion, and degenerative changes in PPR infected kidneys were also observed by Khaliq et al. (2020).

Lungs showed alveoli filled with a mixture of sero-fibrinous edematous fluid and fibrinous exudate (interstitial pneumonia), moderate leukocytic infiltration, and alveolar macrophage accumulation. In bronchioles and alveoli, basophilic intranuclear viral inclusion bodies and areas of congestion were noted (Fayyad et al. 2020). Bronchiolar epithelium was desquamated, with the accumulation of debris of epithelial cells in bronchioles lumen, which clearly indicated broncho-pneumonia. The fibrino-edematous fluid accumulation, along with macrophages and numerous neutrophilic infiltrations within the alveoli, thickened interalveolar septa, clearly indicated interstitial pneumonia. Serofibrinous exudate was indicative of fibrinous pneumonia, along with few neutrophils and macrophages in the lumina of alveoli. The existence of intra-nuclear eosinophilic inclusion bodies was also apparent (Fig. 7). Sahoo et al. (2020) also observed a similar type of microscopic lesion.

Table 1: Primers used for amplification of desired genes

Sr #	Genes targeted	Primers sequence
1	Np-3	(5′-TCTCGGAAATCGCCTCACAGACTG-3′)
2	Np-4	(5′-CCTCCTCCTGGTCCTCCAGAATCT -3′)

Fig. 1: Photograph showing conjunctivitis, rhinitis, chellitis depression and mucosal diarrhea in goats infected with PPR virus

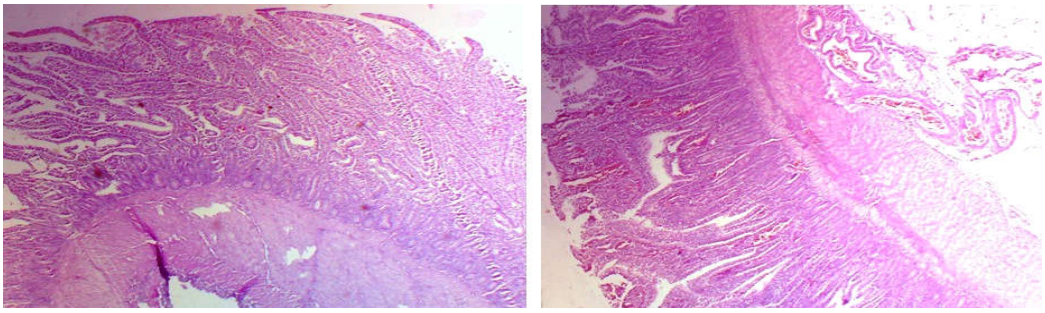

Fig. 2: Photomicrograph showing stunting, congestion edema and monuclear cellular infiltrates within lamina propria in intestine of goats infected with PPR virus

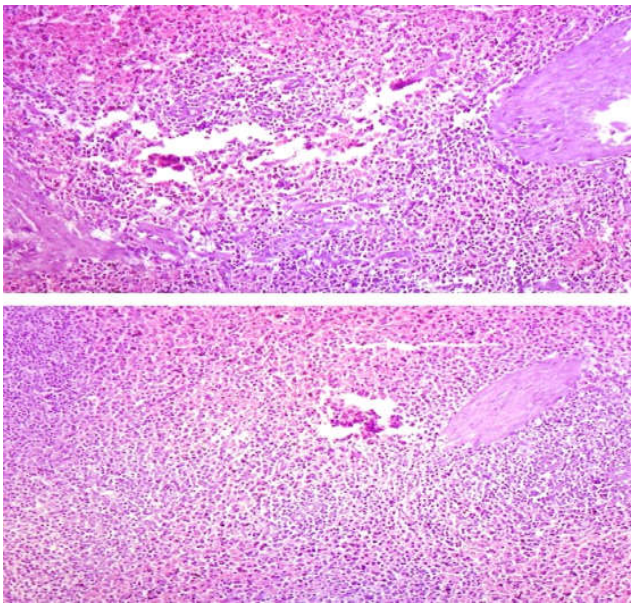

Fig. 3: Photomicrograph showing different histopathological lesions in spleen of goats infected with PPR virus

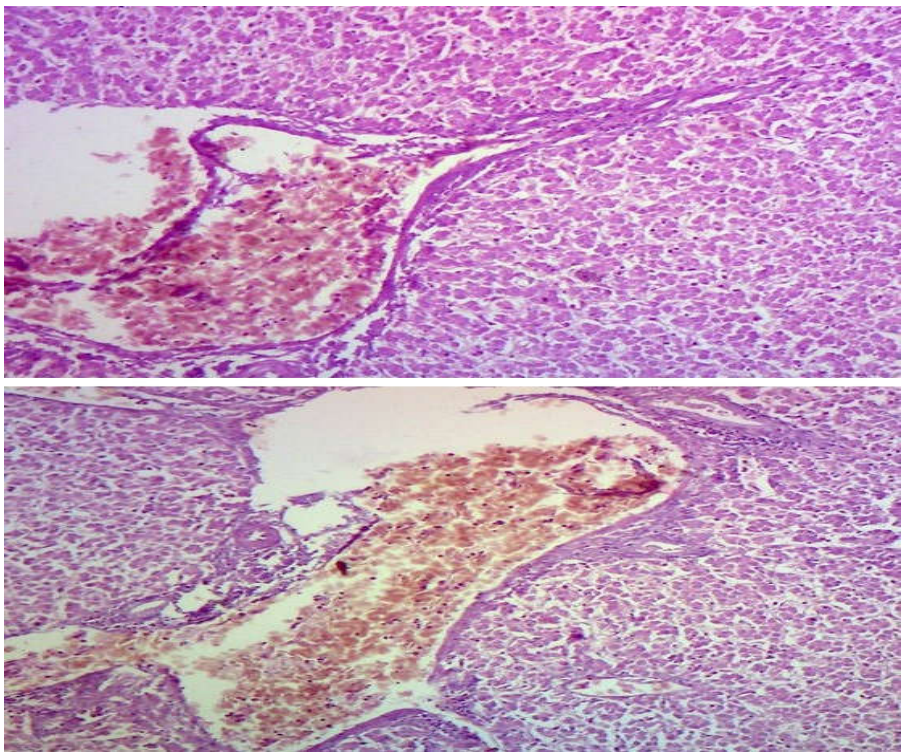

Fig. 4: Photomicrograph showing necrotic and degenerated hepatocytes and congestion in liver of goats infected with PPR virus

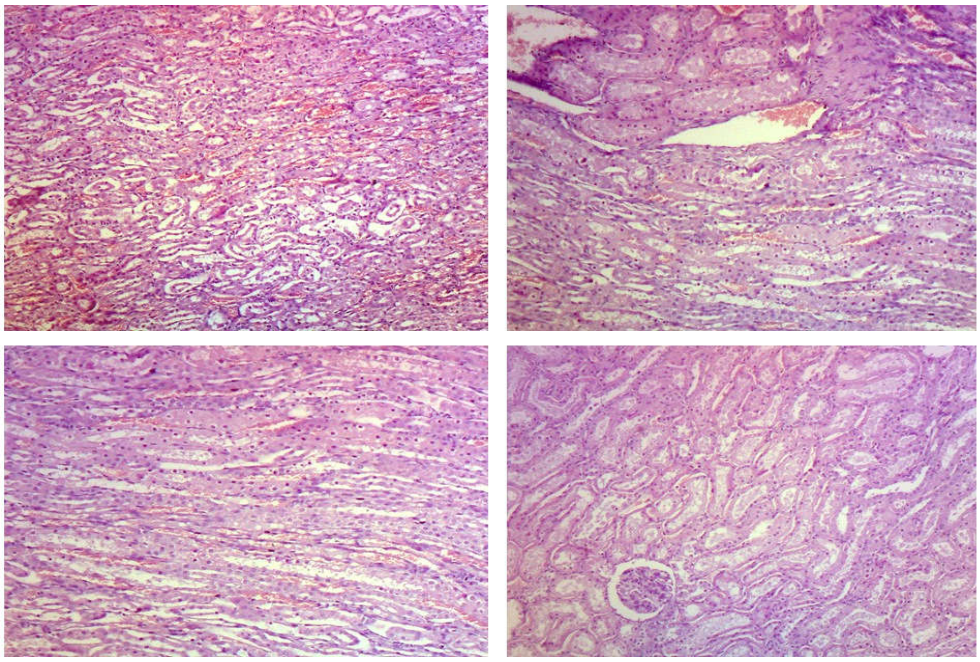

Fig. 5: Photomicrograph showing severe congestion, hemorrhages, necrosis of and tubular epithelial cells in kidneys of goats infected with PPR virus

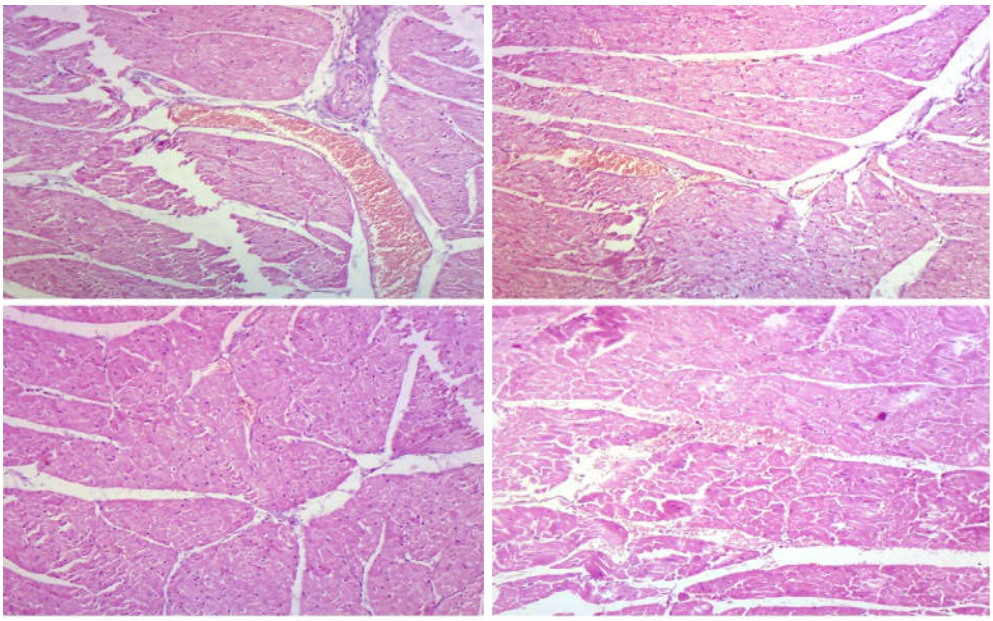

Fig. 6: Photomicrograph showing severe congestion and myocarditis in heart of goats infected with PPR virus.

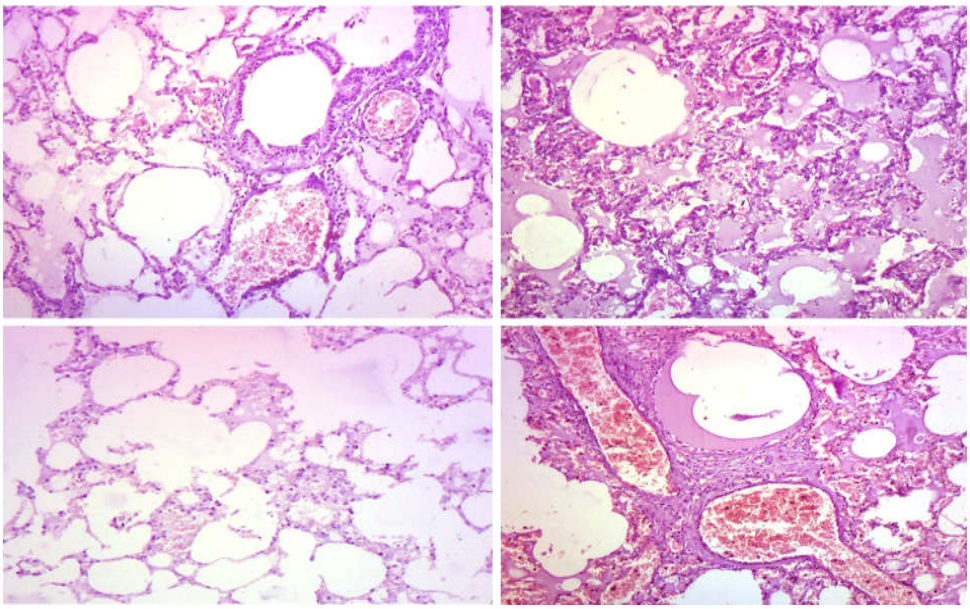

Fig. 7: Photomicrograph showing inflammation, congestion, intranuclear viral inclusion bodies, and edema in lungs of goats infected with PPR virus.

Conclusions

After observing the clinical signs and symptoms of PPR in the infected goats and after postmortem reports, it can be concluded that goats died of PPR, and the PPR virus was also established by the histopathological studies and molecular diagnostic test (RT-PCR).

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