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Clinical, Pathological and Molecular Diagnosis of *Peste des Petits Ruminants* in Saudi Arabia

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ABSTRACT

Background: *Peste des Petits Ruminants* (PPRV) is one of the major notifiable diseases of the world organization for animal health (OIE) and its existence can have a catastrophic. So far, the epidemiological situation of the virus a in Saudi Arabia is not clear. The study was designed to assess the viral infection in indigenous sheep and goats.

Methods: A herd was struck by the virus. Clinical signs were recorded and postmortem examination was carried out on dead animals. Tissue samples from oral cavity, abomasum, small and large intestine, spleen and mesenteric lymph nodes as well as lungs were fixed in 10% neutral buffered formalin and processed routinely. Paraffin wax-embedded sections were stained with haematoxylin and eosin. Similarly tissues were also collected and stored at -80°C for PCR test.

Result: PPRV was detected by RT-PCR and the fragment size of the amplified products was 191 bp of M gene. The clinical signs were a sudden onset of fever with excessive salivation associated with mucopurulent discharges from the nose and eyes. At necropsy, fibrinonecrotic stomatitis associated with hepatized lung especially in the cranioventral lobes. The large intestine showed streaks of congestion “zebra stripes”. Histologically, lungs showed interstitial pneumonia characterised by thickening of alveolar septa and presence of syncytial cells. Small and large intestine revealed fibrinonecrotic enterocolitis characterized by massive necrosis of mucosa accompanied by mononuclear cell infiltration as well as severe congestion of the submucosal blood vessels.

Key words: Histopathology, PCR, PPR.

INTRODUCTION

Peste des petits ruminants (PPRV) is a highly contagious disease among sheep and goats as well as wildlife ruminants. The virus has been reported and endemic in the Middle East, Africa and Asia (Balamurugan *et al.,* 2010; Banyard *et al.,* 2010; Alemu *et al.,* 2019). The host affinity of the virus and different risk factors are responsible for the transmission of disease in different regions of the world (Kivaria *et al.,* 2013; Chota *et al.,* 2019; Herzog *et al.,* 2020). The disease occurs with a higher incidence in the rainy season and the infection is mostly transmitted by close contact between animals (Lefevre and Diallo, 1990). It is of great economic importance based on mortalities, morbidity, loss of meat, milk and offspring (Murphy *et al.,* 1999; Diallo, 2006). Singh *et al*. (2009) believed that the economic losses due to such disease may reach US$ 39 million/year. Nowadays, more than 1 billion goats and sheep in Asia and Africa are exposed to being infected with PPR (Bello *et al.,* 2018). Therefore, the disease is considered one of the major threats to about 22 million small ruminants where mortality may reach 100%. Several studies indicated that outbreaks were more common and severe in goats (Balamurugan *et al.,* 2012;Troung *et al.,* 2014; Islam *et al.,* 2021). The clinical signs of disease are pyrexia, anorexia, stomatitis, diarrhoea, occulonasal discharge and respiratory manifestations (Dhar *et al.,* 2002; Muthuchelvan *et al.,* 2006). The gross findings were congested lungs and gastrointestinal tract, especially the discontinuous streaks of congestion, which is referred to as Zebra stripes or Zebra markings, oedematous and congested retropharyngeal and mesenteric lymph nodes, linear hemorrhages in the intestinal mucosa (Ugochukwu *et al.,* 2019).

Histopatholgy is characterised by pseudomembranous stomatitis and enteritis associated with bronchointerstitial pneumonia (Troung *et al.,* 2014; Ugochukwu *et al.,* 2019). A specific syncytial cells in oral mucosa and lung as well as intranuclear and intracytoplasmic inclusion bodies in respiratory and alimentary tract (Begum, 2021;Islam *et al.,* 2021). PCR technique using F-gene primers has been developed (Forsyth and Barrett, 1995) and became a highly sensitive tool for diagnosis (Oshiek *et al.,* 2018; Bhoyar *et al.,* 2019). Phylogenetic studies indicate that PPR virus strains can be classified into 4 distinct lineages, three of them occur in Africa and the fourth is found in Africa and Asia (Shaila *et al.,* 1996; Alidadi *et al.,* 2019). In Saudi Arabia, Peste des Petits Ruminants (PPRV) is currently considered as one of the

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main transboundary viral diseases that constitute a threat to livestock production (Abu Elzein *et al.,* 1990; Sharwai *et al.,* 2010; Mahmoud *et al.,* 2017; Mahmoud *et al.,* 2021). Outbreaks are still repeated annually in the country and there is a big loss among all ages. Morbidity is up to 90% and mortality may reach 100% in young animals (Housawi *et al.,* 2004; Al-Afaleq *et al.,* 2004; Al-Dubaib, 2009; Boshra *et al.,* 2015; Hemida *et al.,* 2020). Therefore, the proper understanding of the epidemiology, pathology and molecular characterization of the strain of the virus in Saudi Arabia still needs further investigation. The present article is planned to study clinical signs, pathological descriptions and the molecular identification of PPR.

MATERIALS AND METHODS

Animals

An outbreak among sheep and goats has appeared in winter 2021 (Al-Ahsa District). A total of 230 animals was examined (80 sheep and 150 goats) at Department of Pathology, King Faisal University, Saudi Arabia. These animals had not been vaccinated and there was also a history of the new entry of animals. The clinical signs were a sudden onset of fever with stomatitis and excessive salivation associated with mucopurulent discharges from the nose and eyes. Diarrhoea developed 3-4 days after the beginning of the fever. Mortality reached up to 90% particularly at young ages (< 1 year).

Postmortem and sample collection

Postmortem examination was carried out on dead animals. Tissue samples from the oral cavity, abomasum, small and large intestine, spleen and mesenteric lymph nodes, as well as lungs, were fixed in 10% neutral buffered formalin and processed routinely. Paraffin wax-embedded sections were stained with hematoxylin and eosin (HE) (Suvarna *et al.,* 2019). Similarly tissues were also collected, transferred to the Central Biotechnology Laboratory at the College of Veterinary Medicine, King Faisal University, Saudi Arabia and stored at -80°C for PCR test.

RNA extraction

QIAamp Viral RNA Mini Kit (QIAGEN, USA) was used to extract total RNA according to the manufacturer's recommendations. Briefly, 140 µl of the samples lysed by adding 560 µl of AVL buffer containing carrier RNA and then incubated at room temperature for 10 min. After complete lysis, absolute ethanol (560 µl) was added and mixed through pulse vortexing for 15 seconds. 630 µl Aliquots transferred sequentially to a QIAamp spin column followed by centrifugation at 8.000 rpm for 1 min. The binding RNAs were washed by AW1 (500 µl) buffer followed by AW2 buffer (500 µl). The RNAs eluted in 50µl of AVE buffer and stored at -80°C until used.

Molecular identification of PPRV

The presence of PPRV was tested in extracted RNAs using a One-step RT-PCR Kit (QIAGEN, USA). The RT-PCR reaction mixture was composed of 5 µl RNA, 5 µl 5x Qiagen one-step RT-PCR buffer, 5 µl Q buffer, 1 µl of a dNTPs mix, 1 µl (50 pmol) of each forward primer; 5′-CTTGATACTCC CCAGAGATTC-3′ and Reverse primer; 5′-TTCTCCCATGAG CCGACTATGT-3′ (Balamurugan, 2006), 1 µl of the enzyme mix (containing RT and PCR reaction enzymes) and 6 µl of RNase free water. The RT-PCR reaction was optimized at 50°C for 30 min, then 95°C for 15 min, followed by 40 cycles starting with denaturation at 95°C for 30 seconds, primers annealing at 57°C for 30 seconds and 72°C for 30 seconds and final extension step at 72°C for 10 min. The amplified PCR products were electrophoresed in 1.2% agarose gel containing 0.5µg/ml ethidium bromide and documented using an ultraviolet gel documentation system (BIORAD).

RESULTS AND DISCUSSION

PPR virus is one of the major notifiable diseases of the world organization for animal health (OIE) and its existence can have a catastrophic impact on the region of farming development (Hemida *et al.,* 2020). So far, the epidemiological situation of the virus in Saudi Arabia is not clear. During winter, 2021, The virus infects a large population of sheep and goats in different areas in Al Ahsa region. The animals were not previously vaccinated and also had a history of the new entry of animals. Previous studies stated that disease might be due to the change in the environmental conditions, illegal movement of animals through trade and husbandry practices (Kumar *et al.,* 2014; Alemu *et al.,* 2019). In the present study, PPRV was detected by RT-PCR in gel electrophoresis (Fig 1). The fragment size of the amplified products was 191 bp of M gene. This indicated the circulation of PPRV in the study districts. This finding was reported by previous studies (Balamurugan *et al.,* 2012; Chitradevi *et al.,* 2018; Shanmugavadivu *et al.,* 2021). The clinical signs, necropsy findings and histopathology were quite similar in both sheep and goats. The clinical signs were sudden onset of fever with

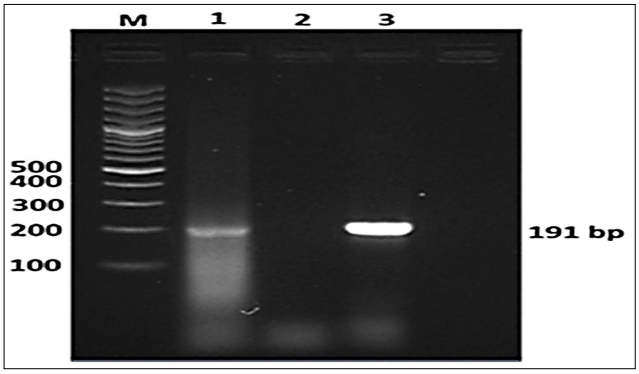

Fig 1: Agarose gel electrophoresis of RT-PCR amplified products of M gene region of PPRV field samples. Lanes M, Gene Ruler 100 bp plus DNA ladder (ThermoFisher Scientific); Lane 1,3 field samples and Lane 2, water served as negative control.

fibrinonecrotic stomatitis characterized by tiny, whitish, necrotic foci on the inner side of lips and gums stomatitis (Fig 2a). This was associated with excessive salivation and mucopurulent discharges from the nose and eyes. These findings were in agreement with previous studies (Truong *et al.,* 2014; Islam *et al.,* 2021). Diarrhoea developed 3-4 days after the beginning of the fever. The hindquarters soiled soft/water faeces and the perineal region was hyperemic and revealed whitish-grey necrotic foci (Fig 2b). Similar findings were recorded by several authors (Housawi *et al.,* 2004; Hemida *et al.,* 2020). The mortality rate was 90% particularly in young ages and these results were in accordance with previous studies (Mahmoud *et al.,* 2017). The trachea, bronchi and bronchioles were filled with froth exudate.The lungs were congested and hepatized especially in the cranioventral lobes (Fig 2c). These findings were quite similar to those observed by several authors (Troung *et al.,* 2014; Islam *et al.,* 2021). The most common and constant

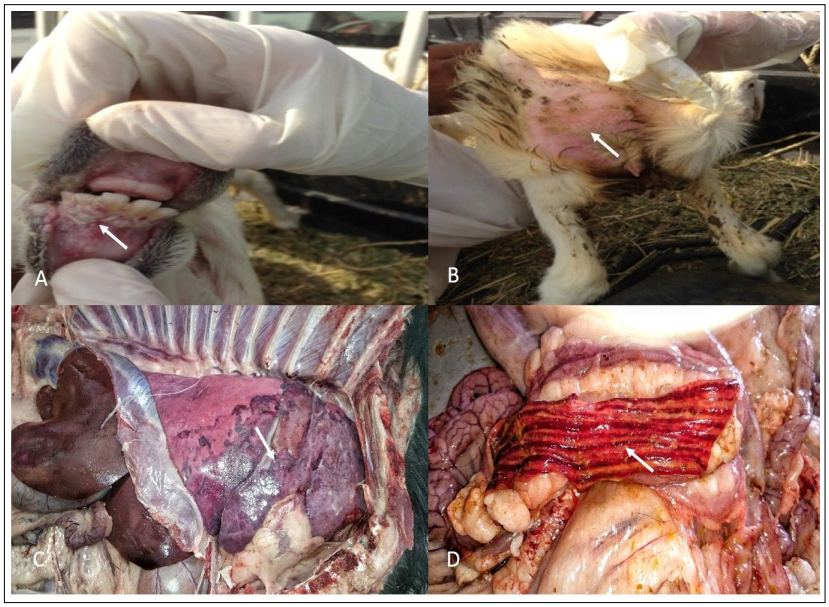

Fig 2: A-Fibrinnecrotic stomatitis (arrow). B- Severe diarrhea. C- Bronchpneumonia in cranial lobes (arrow). D- Zebra stripes in colon (arrow).

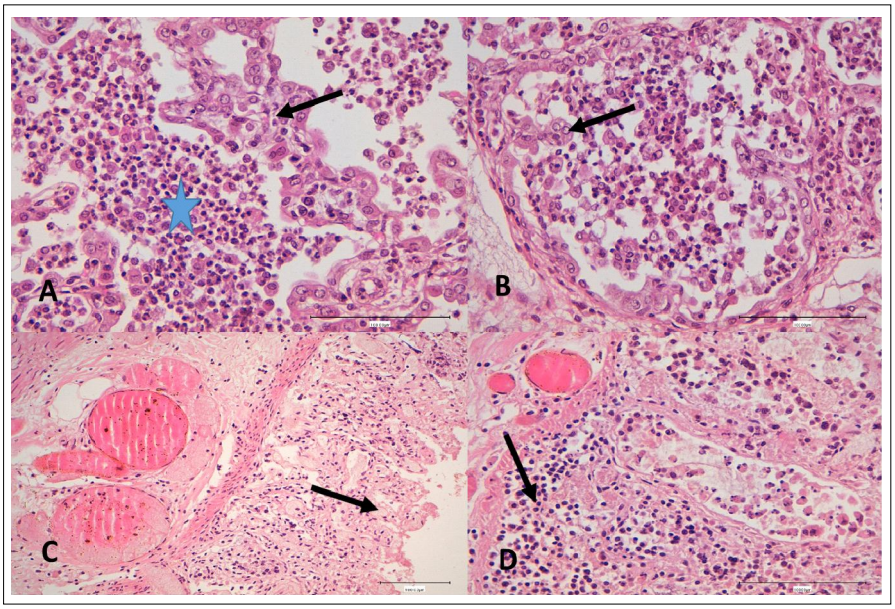

Fig 3: a- Bronchointerstitial pneumonia, alveoli filled with mononuclear cells (star) and interlobular septa appeared thickned (arrow) H&E, scale bar = 100 µm. b- Syncytial cells inside alveoli (arrow). H&E, scale bar= 100 µm. c- Fibrinonecrotic enteritis, destruction of mucosal surface (arrow). H&E, scale bar= 100 µm. d- Mononuclear cells infiltration along with lamina propria of intestine (arrow). H&E, scale bar= 100 µm.

findings in the intestine were severe enteritis as well as streaks of congestion in the colon and caecum (zebra stripes) (Fig 2d). These findings were very much consistent with several previous studies (Mahmoud *et al.,* 2017; Robi, 2019; Ugochukwu *et al.,* 2019). Histopathology revealed bronchointerstitial pneumonia characterized by thickening of alveolar septa (Fig 3a). The alveoli showed edema and alveolar macrophages. This finding was parallel to Troung *et al.,* (2014) and Begum *et al.,* (2021). In our findings, some syncytial cells appeared inside alveoli which were considered pathognomonic for PPRV (Fig 3b). This lesion was consistent with several previous studies (Kumar *et al.,* 2014; Begum *et al.,* 2021; Islam *et al.,* 2021). Small and large intestine revealed fibrinonecrotic enterocolitis characterized by massive necrosis of mucosa accompanied by mononuclear infiltration as well as severe congestion of the submucosal blood vessels (Fig 3 c,d). This lesion was consistent with several previous studies (Housawi *et al.,* 2004; Kumar *et al.,* 2014; Troung *et al.,* 2014; Islam *et al.,* 2021).

CONCLUSION

The occurrence of the virus is considered an emerging pathogen in this district and affect susceptible host during adverse weather. Therefore, the state animal husbandry along with other stakeholders must keep a strict vigil on the development of disease among animals in susceptible areas. Also, quarantine of new animals before introduction to the herd and immediate implementation of mass vaccination, slaughter and proper disposal of affected animals is very important to prevent any further losses of disease.

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Conflict of interest: None.

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