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Peste Des Petits Ruminants Virus Infection of Small Ruminants in Al-Sharkia Governorate, Egypt

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ABSTRACT

Peste des petits ruminants (PPR) is a contagious, economic viral disease of small ruminants. In Egypt, PPR disease is highly widespread in the last years with high morbidity and mortality. An outbreak was recorded in Al-Sharkia governorate, Egypt on a flock of small ruminants consisted of (34 goats and 11 sheep) with clinical signs suggested to be PPR virus (PPRV) infection following introduction of new animals. The clinical investigation of infected animals revealed high fever, erosions on lips and oral mucosa, ocular and nasal discharges, severe conjunctivitis, and severe offensive diarrhea that was bloody in some cases. Morbidity, mortality and case fatality rates of infected flock were 88.2, 23.5 and 26.7%, respectively. On necropsy, erosions and ulceration were noted in buccal cavity, lungs were congested and consolidated, and lymph nodes of lungs and intestine were edematous. In addition, there was congestion and enlargement of spleen and liver with distended gall bladder. Intestines were hemorrhagic with zebra stripping, the pathognomonic histopathological lesions of PPR, were found in ceco-colic junction. Detection of PPRV N gene by One-Step conventional reverse transcriptase PCR (RT-PCR) assay using NP3 and NP4 primers was applied. Out of 24 samples collected from diseased animals and dead carcass, RT-PCR confirmed PPRV infection in 13 samples (five oculonasal swabs, three buffy coats, two mesenteric LNs, two lungs, one spleen). Sequencing and phylogenetic analysis was carried out on the PCR product of NP gene revealed that, the circulating virus is closely related to circulating PPRV strains throughout Egypt and African countries. The authors planned further understanding the PPRV molecular epidemiology to develop strategies to eradicate the disease with a proper vaccine.

Key words: PPR, Egypt, Sheep, PCR, Sequencing.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute highly contagious viral disease affecting both domesticated and wild small ruminants, experimental studies suggest that the infection in cattle, pigs and rats is subclinical (Balamurugan et al. 2012). The disease causes serious economic losses due to high morbidity and mortality rates (Dhar et al. 2002; Chemweno et al. 2019). The disease is caused by peste des petits ruminants' virus (PPRV) that is closely related to rinderpest virus (RPV), measles virus (MV) and canine distemper virus (CDV) in genus *morbillivirus*, family *paramyxoviridae* (Parida et al. 2015). After that the disease has been widely spread in Sub-Saharan Africa, Middle East and Southwest Asia (Altan et al. 2019; Halecker et al. 2020). In Egypt, PPRV was firstly detected in 1987, then many reports described the disease among Egyptian sheep and goats (ElAshmawy et al. 2018).

The disease is transmitted mainly through close contact between infected and susceptible animals via scattered aerosols as well as oral route or contact with secretions and excretions of infected animals (Parida et al. 2019). Clinically, PPR characterized by fever, nasal and ocular discharges, mouth sores, pneumonia and diarrhea (Khan et al. 2018). The characteristic pathological features of the disease are ulcerative stomatitis, zebra stripes in ceco-colic junction and broncho-interstitial pneumonia (Begum et al. 2018; Bamouh et al. 2019).

Although the genome of PPRV encodes for 6 structural proteins, a nucleoprotein (N), the most appropriate gene for molecular characterization as it is more sensitive and N messenger RNA is produced more during PPRV infection (Kwiatek et al. 2007; Kumar et al. 2014; ElAshmawy et al. 2018). The nature of circulating PPRV has a crucial role in devising more appropriate diagnostic and control strategies. Therefore, the present study aimed to detect and

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molecularly characterize the PPRV in infected small ruminants flock based on suspected clinical manifestations.

MATERIALS AND METHODS

Ethical Approval

Collection of samples was for the routine diagnosis of the disease under the usual veterinary service work in Egypt, no permits were required for collection and all collections were performed according to the national standards within the country.

Field Study

The present study was conducted on a group of nine clinically diseased sheep and goats in addition to two dead goats that were admitted to the clinic of Veterinary Medicine Faculty, Zagazig University, Egypt in September 2020. The data collected from the owner revealed that affected animals belonged to a private local flock consisted of 45 animals (34 goats and 11 sheep) aged 9-18 months, and located in Abou-Kabeir, Al-Sharkia governorate, Egypt. Al-Sharkia governorate is located in the northern part of the country. This study was conducted during September around the period of major festivals, Eid Al Adha, when large numbers of sheep and goats are moved along the country. Notably, the flock data showed that diseased cases appeared 12 days after the introduction of newly purchased animals from the local market without quarantine. Moreover, flock vaccination history revealed absence of PPRV vaccination. The general clinical examination was performed according to (Constable et al. 2017), routine P.M examination was applied, and gross changes were recorded according to (Jackson and Cockcroft 2002).

Collection of Samples

A total of 24 samples (nine buffy coats, nine oculo-nasal swabs and six tissue samples) were collected from suspected living 5 goats and 4 sheep for PPRV identification by RT-PCR. Nasal swabs were obtained on saline containing 10% pen-strop- amphotericin B to avoid bacterial and fungal contamination. Lymphocyte separation medium (Iymphoprep®, Axis shield, Scotland) was used for easy and rapid separation of lymphocytes from whole blood. While the two dead goats after the characteristic PM picture of PPR was shown, six samples (two spleen, two lungs and two mesenteric lymph nodes) were taken for histo-pathological examination and PPRV detection.

Histo-pathological Examination

For histo-pathological examination, sections from the same collected tissue samples in addition to hard palate and intestine were immediately fixed in 10% neutral buffered formalin. Then tissues were subjected to ascending alcohol concentrations for washing and dehydration, clearing in xylene, and embedding in paraffin. Thick tissue sections of 4–5µm were further stained with hematoxylin and eosin according to the protocol of (Bancroft and Gamble 2002), then were observed under light microscope.

Laboratory Diagnosis of PPRV

Extraction PPR Viral RNA

RNA extraction was performed by using the QIAamp viral RNA mini kit (Qiagen, Germany) according to instructions of manufacturer. RT products were stored at -20°C until used.

RT-PCR

It was carried out for confirmation of PPR in suspected diseased and dead animals by detecting the viral nucleic acid using Qiagen one step RT-PCR kit (Qiagen, Germany) and primers directed to the highly conserved sequence of nucleoprotein gene of PPR virus. Oligonucleotide primers of RT-PCR protocol targeted N gene sequence at accession number JN647718.1. Forward primer sequence: (NP3) 5- GTC- TCG- GAA- ATC- GCC- TCA- CAG -ACT- 3-´. Reverse primer sequence: (NP4) 5- CCT- CCT- CCT- GGT- CCT- CCA- GAA- TCT 3-´, primers amplify at 351 bp (Soltan and Abd-Eldaim 2014). The thermal profile was shown in (Table 1).

Sequencing and Phylogenetic Analysis

Sequencing in both directions was applied to 351 bp PCR band in an automated Applied Biosystems 3130 genetic analyzer (HITACHI, Japan). The obtained sequence was exposed to analysis using NCBI-BAST and compared with other PPRV sequences available in GeneBank. The phylogenetic analysis was obtained by the using of neighbor-joining method in MEGA6 (Tamura et al. 2013).

Statistical Analysis

Data collected were subjected to Fisher's Exact Test of Independence was performed to test relationship between two categorical variables (Species type and morbidity of PPRV). Data were expressed as count and percentages.

Also, Fisher's Exact Test of Independence was run to test whether the species type and positive PPRV samples detected by RT-PCR are independent. Results <0.05 were considered statistically significant. All analyses were performed by SPSS version 24.0 (IBM. Corp., Armonk, NY).

RESULTS

Clinical Findings

The data collected from the flock owner revealed that the overall number of diseased and dead animals was 37 and 8, respectively. The epidemiological outbreak feature was shown in (Table 2). The infected animals were suffered from fever, erosions in the oral mucosa, ocular and nasal discharges, conjunctivitis, increased respiratory rate, severe offensive diarrhea that was bloody in some cases (Fig. 1).

Post-mortem Findings

Necropsy findings revealed erosions and ulceration of buccal cavity, congested consolidated lungs and edematous lymph nodes of lung and intestine. In addition, there was congestion and enlargement of spleen and liver with distended gall bladder and zebra stripes in ceco-colic junction (Fig. 2).

Histo-pathological Findings

Histomorphological figures of hard plate showing pseudo diphtheritic membrane, mucous and necrosis of air-tree mixed with inflammatory cells. In the lung, necrosis of bronchus and alveoli filled with inflammatory exudate.

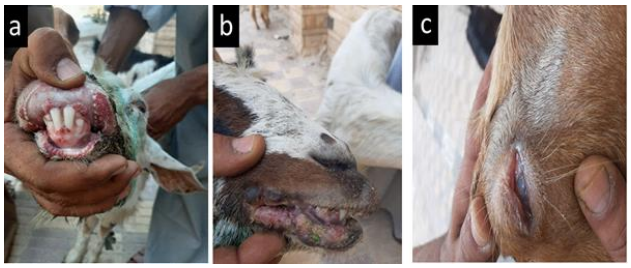

Fig. 1: Clinical finding of suspected PPRV infection, (a, b) erosion and necrotic stomatitis, (c) eye opacity.

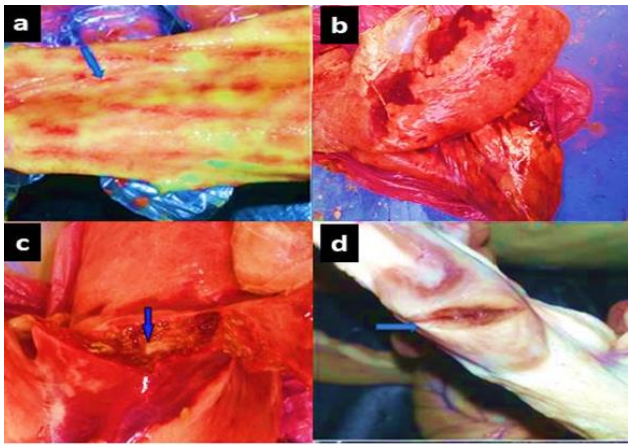

Fig. 2: Post-mortem findings showing Zebra strips of rectum and recto-colon junction mucosa (a). Congestion and consolidation of the lungs (b, c). Enlarged mesenteric lymph node (d).

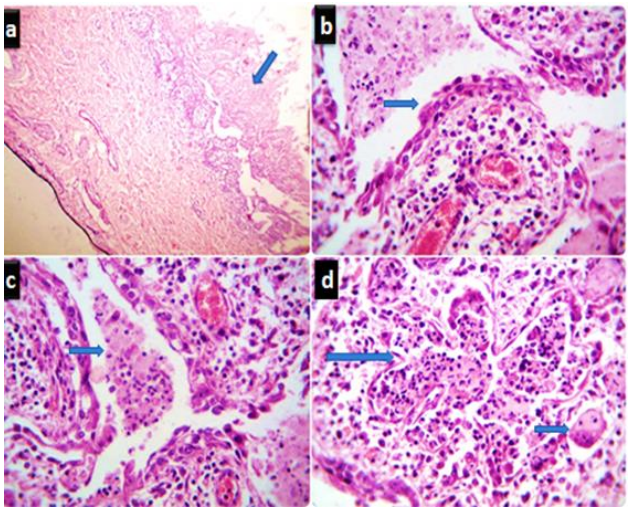

Fig. 3: Histomorphological figures at post-mortem goats infected with PPR virus showing pseudo diphtheritic membrane in hard plate (arrow) (a) H&E X200. Severe necrosis of air-tree (arrow) which contain mucous admixed with inflammatory cells in lumen (arrow) (b,c). Remained alveoli engorged by inflammatory exudates (d). H&E X400.

Congestion, necrosis in the lining epithelium of intestine with infiltration of inflammatory cells in the lamina propria. Depletion of lymphocytes and infiltrated macrophages were shown in lymph nodes (Fig. 3, 4).

Diagnostic Assays

RT- PCR was conducted to amplify parts of NP gene of PPRV on 24 different samples collected from diseased and dead animals. The test successfully detected 13

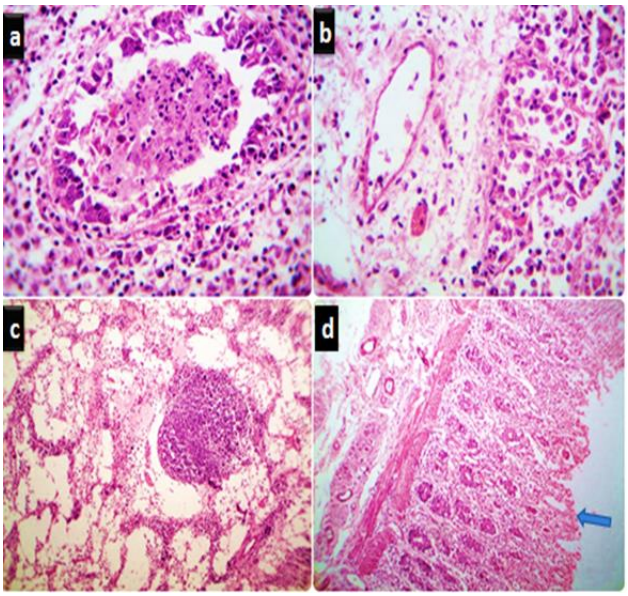

Fig. 4: Histomorphological figures showing necrotic bronchus filled with inflammatory exudate (a). Severe edematous of inter lobular septa adjacent necrotic alveoli. Severe depletion of mesenteric lymph node. Erosion of intestinal mucosa admixed with infiltrated by inflammatory cells. H&E X200.

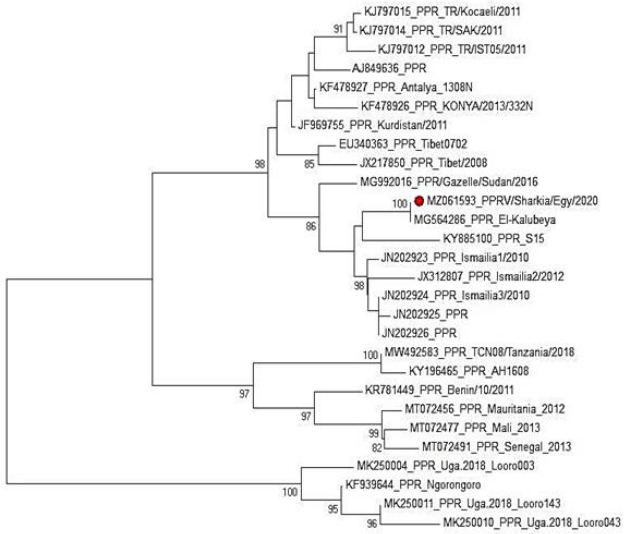

Fig. 5: Phylogenetic tree of different nucleotide sequences of PPRV N protein gene.

samples (five oculonasal swabs, three buffy coats, two mesenteric LNs, two lungs, one spleen). The results of diagnostic assays indicated that PPRV antigen could be isolated from either oculo-nasal swabs, buffy coats and tissue samples (Table 3).

Sequence and Phylogenetic Analysis

The N sequenced gene of Egyptian PPRV PCR amplicon submitted to GenBank and provided with accession number MZ061593. The analysis of phylogenetic tree as shown in (Fig. 5) illustrated that the isolated strain in this study was related to others PPRV strains isolated in Egypt as (MG564286 PPR El-Kalubeya, JN202924 PPR Ismailia3/2010, JN202923 PPR Ismailia1/2010 and JX312807 PPR Ismailia2/2012) with a percentage of identity range from 100, 99.4, 99.1 and 95.5%, respectively.

Table 1: The thermal profile cycle of RT-PCR.

Stage	Temp.& duration	No. of cycles
Reverse transcription	50 °C for 30 min.	One
Initial PCR activation step (Inactivates RT and activates polymerase)	95 °C for 30 min.	One
PCR amplification of cDNA		
Denaturation	94 °C for 30 sec.	
Annealing	69 °C for 30 sec.	40 cycle
Extension	72 °C for 1 min.	
Final extension	72 °C for 10 min.	

Table 2: Epidemiological feature of suspected PPRV outbreak

Species	No. of animals	Morbidity rate	Mortality rate	Fatality rate
Goats	34	88.2% (30/34)	23.5% (8/34)	26.7% (8/30)
Sheep	11	63.6% (7/11)	------	------
Total	45	82.2% (37/45)	17.8% (8/45)	21.6% (8/37)

Table 3: Detection of PPRV by RT-PCR assay

Species	No. of examined samples	Positive samples	
		No	Percentage
Goats	15	9	60
Sheep	9	4	44.4
Total	24	13	54.2

Isolated PPRV strain also closely related to others worldwide distributed isolates as (MG992016 PPR/Gazelle/Sudan/2016, JF969755 PPR Kurdistan/2011, MW492583 PPR TCN08/Tanzania/2018, MT072456 PPR Mauritania_2012…etc).

Statistical Analysis

Although goats showed higher morbidity rate (88.2 %) than sheep (63.6%), statistical significance did not exist with P value (0.085)**.** Also, Positivity of PPRV in samples by using RT-PCR was not significantly related to the species type (P>0.68).

DISCUSSION

In Egypt, small ruminants are kept mainly in small farms or cared by Bedouins populations or rural villagers with trading in-between. This situation makes it more likely of contagious diseases as is observed in PPRV recurrent outbreaks. PPRV can be misdiagnosed with other respiratory diseases that caused high mortality of sheep and goats and livestock owners were not familiar with its clinical and pathological findings (Tibbo et al. 2001).

In this study, the case history showed that the infected flock was fixed (non-migratory) and geographically located in Abou-Kabeir in Al-Sharkia governorate. Introduction of new animals to the flock without quarantine was largely practiced in the current study which made the flock at higher risk for introduction of PPR by incubatory carriers after developing clinical signs as previously reported by Couacy-Hymann et al. (2009). Newly purchased animals from live animal market have been considered a source of PPRV infection in many previous studies (Muhammad et al. 2009; ElAshmawy et al. 2018).

The present study reported an outbreak of PPRV disease between goats and sheep admitted to Zagazig Veterinary clinic. The morbidity rate of the disease in this study was higher in goats (88.2%) than in sheep (63.6%), In addition, PPRV positivity in examined samples by RT-PCR from goats (60%) was higher than in sheep (44.4%). However, statistical significance did not exist, this could be due to the fact that sheep and goats are equally exposed to the PPR risk factors such as climatic conditions and lack of quarantine, or it could be that the circulating field strain has overcomed the natural resistance of sheep (Abd El-Rahim et al. 2010; Delil et al. 2012). In contrast, previous studies have reported significantly higher PPR infection rate in sheep as compared to goats (Khan et al. 2008; Saeed et al. 2010).

Clinically, infected goats and sheep suffered from fever, nasal and ocular discharges, ulceration in buccal mucosa and diarrhea which are considered a first indicator of PPRV infection (Rahman et al. 2011; ElAshmawy et al. 2018; Saeed et al. 2021). During this outbreak, necropsy findings showed emaciation of the carcass, erosion of buccal mucosa, consolidation of the lung, hemorrhage along the lining folds of rectum (Zebra strips) as well as enlarged lymph nodes of lung and intestine. These findings were recorded previously by (Abd El-Rahim et al. 2005; Khan et al. 2018).

Histologically, pseudo diphtheritic membrane as well as necrosis of air-tree in the lining epithelium of hard plate. Necrosis with inflammatory exudate in bronchus and alveoli of the lung. Lining epithelium of intestine infiltrated with large mononuclear cells with congestion and necrosis in the lamina propria. In lymph nodes, infiltrated macrophages and depletion of lymphocytes. Similar histopathological features were previously described elsewhere (Islam et al. 2001; Kul et al. 2007).

In this study, PPRV detection was carried out through targeting of NP gene at 351 bp fragment using 24 samples including oculonasal swabs, buffy coats and tissue samples. Our results revealed that 60 and 44.4% of samples of goats and sheep were positive, respectively with a total infection rate of 54.2% in examined samples. In viremia, buffy coat samples are best for increasing the sensitivity of detecting PPRV using molecular diagnostic techniques which may be due to the fact that PPRV is propagated in the lymphoid tissues, leading to severe leucopoenia and immuno-suppression (Rahman et al. 2011). Also, the PPRV can be detected in oculo-nasal swabs which are easy to obtain by non-invasive method, and it is recommended to collect lymph nodes, portions of the spleen and the lungs during postmortem examination from dead animals (Shahriari et al. 2019; Khaliq et al., 2020).

Regarding the choice of gene directed primers, recent studies have proved that NP gene sequence were highly polymorphic in discriminating of different strains in comparison to F and H genes (Kwiatek et al. 2007). As a result, previous studies recommended NP gene as an inclusive view of PPRV molecular epidemiology (Munir et al. 2012; Kumar et al. 2014; ElAshmawy et al. 2018; Alhussain et al. 2020). Taken together, it seems that the choice of sample type and gene directed primers is vital for optimizing the efficiency of diagnosing PPR in sheep and goats.

In this study, PPRV strain was highly identical to isolates previously identified in Egypt (MG564286 PPR El-Kalubeya, JN202924 PPR Ismailia3/2010, JN202923 PPR Ismailia1/2010 and JX312807 PPR Ismailia2/2012) with a percentage of identity (100, 99.4, 99.1 and 95.5%), respectively. Consequent, this strain belonged to lineage IV as In the previous studies from different localities of Egypt (Sharawi and Abd-El-Rahim 2011; Soltan and Abd-Eldaim 2014; Ahmed et al. 2021). This similarity between the Egyptian isolates may be associated with uncontrolled movement of sheep and goats within and between the governorates.

In Egypt, NP gene phylogenetic analysis of PPRV strains showed a high percent of homology with the African PPRV isolates. While a previous study showed a high identity between Egyptian and Ethiopian PPRV strains in the period from 2014 to 2016 (Elhaig et al. 2018), PPRV strain of this study was closely to an Algerian strain (KY885101) as previously diagnosed by (Baazizi et al. 2017) in Cheraga in 2015. Interestingly, the phylogenetic analysis of Algerian strain detected by (Baazizi et al. 2017) suggested a strong historical and geographic connection between the strains isolated in Maghreb region (Morocco/2008, Algeria/Cheraga/2015) and viruses identified in the region of East Africa (Ethiopia/2010). Since Egypt imports small ruminants from various African countries including Sudan and Ethiopia to overcome the deficiency in meat production, hence, it is more likely that the lineage IV PPRV in Egypt appears to have sprung from East Africa.

Conclusion

In the present study, PPRV is detected from an outbreak in sheep and goats flock and still circulating in Egypt. The high percent of identity between the strain of this study and the previous PPRV strains in Egypt demonstrates the presence of the same circulating virus throughout Egypt. Movements of sheep and goat between flocks should be limited to prevent the spread of the virus. Vaccination program against PPRV infection should be applied for sheep and goats flocks before the winter season as its prevalence was mostly associated with winter season. Further disease surveillance by veterinary authorities to apply an effective control program.

Author's Contribution

All authors contributed equally in this work to study design, methodology, interpretation of results, and writing of the manuscript.

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