

## DIAGNOSIS AND PHYLOGENETIC ANALYSIS OF THE CIRCULATING PESTE DES PETITS RUMINANTS VIRUS IN AI-SHARQIA GOVERNATE

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**Abstract:** Peste des petits ruminants (PPR) is a Morbillivirus within the Paramyxoviridae family which characterized by highly contagious nature with high morbidity and mortality rates in domestic small ruminants. The aim of this study was to investigate an outbreak of P PRV in a flock of sheep and goats in Belbes city, AL-Sharqia governorate in 2018 by virus isolation and conventional RT-PCR. Phylogenetic analysis of N gene sequence of PPRV isolate. Also, compare it with other isolate from previous outbreak in Zagazig city, Al-Sharqia governorate in 2017 as a measurement of the infection status in Egypt. The current study applied on a flock of 55 small ruminants (42 goats and 13 sheep) in Belbes city which suspected to be infected by PPRV. All infected animals were not vaccinated against PPRV and randomly move from place to place. The morbidity rate was 100% and mortality rate was (23.8% in goats and 7.69% in sheep). Diseased animals suffered from fever, mucopurulent ocular discharge, nasal discharge, dyspnea, diarrhea, necrotic tissue and diphtheritic membrane in oral cavity. The ten samples (6 tissue scraping from oral lesions and 4 oculo-nasal swabs) were tested by conventional reverse transcription PCR which revealed 100% sensitivity compared to VI (virus isolation) 70%. Comparative of N gene sequence of both PPRV isolates revealed that homogenous population of PPR virus isolates up to 99%. Also, the PPRV isolate of the current study is related to Ethiopian strain and the previous Egyptian strains (Ismailia 3/ Egy/2010 isolate, El-Kalubeya isolate and Ismailia 1-2014 isolate). The PPRV isolate of present study and all previous Egyptian isolates belong to lineage IV in phylogenetic analysis. The results emphasize the importance of molecular methods for a broader understanding of the epidemiology and development of the virus in the country.

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**Key words:** PPRV; Conventional RT- PCR; phylogenetic analysis; Al-Sharqia

### Introduction

Sheep and goats contribute significantly to the economy of farmers in African and Asian nations. Sheep and goats are a source of meat,

milk, and wool similarly to their speedy growth and reproduction. Poor man considers goats as cows in developing international locations (1).

The primary isolation of PPRV became recorded in Egypt in 1987 (2). The causative agent

of PPRV is a member of the genus morbillivirus underneath the circle of relatives of the paramyxoviridae (3). The genome of PPRV is a linear, single stranded, non-segmented, negative sense RNA encoding six structural proteins: nucleoprotein (N), phosphoprotein (P), matrix (M), fusion (F), haemagglutinin (H) and a large polymerase (L) and two nonstructural (C and V) proteins (4, 5).

The incubation period of PPRV is ranged from 2–7 days (6) and the disease is manifested clinically by fever, presence of vesicles, ulcers on tongue and inside oral cavity, ocular discharge, leukopenia, profuse watery diarrhea, and respiratory manifestation (7, 8). PPRV cause high morbidity up to 100% and mortality up to 90% (6, 9). The most extensively used cell culture for virus isolation are Vero cells (10). The cytopathic effects (CPE) produced by PPRV in Vero cells are giant cells, cell rounding, formation of grape-like clusters, and small syncytia (10, 11). Reverse transcription polymerase chain reaction (RT-PCR) accompanied by nucleotide sequencing is the maximum diagnostic method used for PPRV identity (12, 13). Although PPRV is consider as one strain or serotype(14) , genetic type, based at the fusion (F) protein gene and the nucleoprotein (N) gene (15), has diagnosed four wonderful lineages (lineages I, II, III and IV) and is considered a powerful tool for the worldwide spread of virus. Lineage I is disbursed in West African strains in the 1970s and in Central African strains in those years; Lineage II in west Africa in Guinea, Ivory coast, and Burkina Faso strains; Lineage III in Sudan, east African, Oman and Yemen strains and lineage IV in The Arabian Peninsula, south east Asian, middle east, northern and central African strains (16) .

Thus, the aim of this study was to investigate the outbreak of PPRV in a flock of sheep and goats in Belbes city, AL-Sharqia governorate in 2018 by virus isolation and conventional RT-PCR. Phylogenetic analysis of N gene sequence of PPRV isolate and compare it with o ther isolate from previous outbreak in Zagazig city, Al-Sharqia governorate in 2017 as a measu rement of the infection status in Egypt..

## Materials and methods

The experimental design was approved by Zagazig University's Animal Care and Use Committee (ZU-IACUC/2/F/19/2019).

### *Field examination*

A flock of 55 small ruminants (42 goats and 13 sheep) in Belbes city In Al-Sharqia governorate in August 2018 was suspected to be infected by PPRV. All infected animals were not vaccinated against PPRV. The case history showed that these infected animals were randomly move from place to place.

Ten samples of clinically diseased animals (6 tissue scraping from oral lesions and 4 oculo-nasal swabs) were collected. Until laboratory testing, the samples were kept at-20 ° C.

### *Samples preparation*

Preparation of oculo-nasal swabs (four samples)

Oculo Nasal swabs were obtained on saline containing 10% pen-strep- amphotericin B to avoid bacterial and fungal contamination. Mixing well by pulse vortexing. The mixture was pipetted into a plain tube. Centrifugation at 2000 rpm for 10 minutes. The supernatant was pipetted into sterilized epindorf tube and stored at - 20°C till laboratory examination.

Preparation of tissue scraping from oral lesions (six samples)

Tissue samples were homogenized using tissue homogenizer. Nine milliliters of phosphate buffer saline was added to make 10 % tissue suspension. Centrifugation at 2000 rpm for 10 minutes was carried out and supernatant was taken into sterilized tube and stored at - 80°C till laboratory examination.

### *Isolation of PPRV on tissue culture*

PPRV grown in Vero cell-culture supplied by Tissue Culture Unit in Animal Health Research Institute according to (17). The cells should be examined daily for 7–14 days for evidence of cytopathic effect (CPE). Infected cells developed a characteristic CPE consisting of retraction of the cell membrane from surrounding cells, and eventually rounding of cells. The cells with typical CPE were freeze thawed three

times to take the virus. If cytopathic effect were not evident after 4-7 days of the third passage, the samples were declared negative.

#### *Purification of PPRV RNA*

It was carried out using QIAamp viral RNA minikit (Qiagen, Germany) according to manufacturer instructions.

#### *Conventional RT-PCR*

It was carried out for confirmation of PPRV in suspected diseased 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 as described by (12) and according to instructions of the manufacturer.

#### *PPRV isolates sequence*

PPRV, isolate of the current study was compared with PPRV isolate from mesenteric lymph node of dead carcass of goat within a flock infected by PPRV in Zagazig city, Al-Sharkia governorate in 2017 (18). The N gene specific PCR amplicons were sequenced and assessed for sequence variations.

A purified RT-PCR product was sequenced in the forward direction on the automated DNA sequencer Applied Biosystems 3130 (ABI, 3130, USA). Use the BigDye Terminator V3.1 cycle sequencing kit ready for reaction. (Perkin-Elmer / Applied Biosystems, Foster City, CA), with Cat. No. 4336817. A BLAST® (Basic Local Alignment Search Tool) (19) was initially performed to determine the identity of the sequence for GenBank accessions. The sequence reaction was done according to the manufacturer's instructions.

#### *Phylogenetic analysis*

A comparative sequence analysis was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of MegAlign of the Lasergene DNASTar software module Pairwise, which was designed by (20) and phylogenetic analyses were performed using maximum likelihood, neighboring joining and maximum parsimony in MEGA6 (21).

## **Results**

### *Field examination*

Clinical examination of diseased animals showed fever, congested mucus membrane, normal lymph nodes, increase heart and respiratory rates.

The clinical signs observed were mucopurulent ocular and nasal discharges, conjunctivitis, stomatitis, ulcers and diphtheritic membrane inside the oral cavity, dyspnea and diarrhea (Fig. 1).

The morbidity rate was 100% and mortality was (23.8% in goats and 7.69% in sheep) (Table 1).

### *Isolation of virus on Vero cell culture*

Out of 10 clinical samples, 7 (70%) samples showed CPE after three successive passage of virus on Vero cell culture as cells exhibited cell detachment, vacuolation and cell rounding (Fig. 2).

### *Conventional RT-PCR*

The result of Conventional RT-PCR revealed all tested 10 samples (6 tissue scraping from oral lesions and 4 oculo-nasal swabs) were positive. (Table 2).

### *PPRV Sequence and phylogenetic analysis*

Sequence analysis of the N gene of the current study (PPRV isolate from infected animals in Belbes City 2018) and isolate from the mesenteric lymph node of dead goat carcass in a flock infected with PPRV in Zagazig City, Al-Sharkia governorate in 2017 showed that the two isolates shared a 99 percent homology. The percentage of our isolate identity to Ethiopian strain was 98% applied from gene bank. Furthermore, the nucleotide isolate identity to the previous Egyptian isolates (Ismailia 3/Egy/2010 isolate, El-Kalubeya isolate and Ismailia 1-2014 isolate) were 99%, 98% and 98% respectively (Fig. 3). The PPRV isolate of the present study belong to lineage IV in phylogenetic analysis.

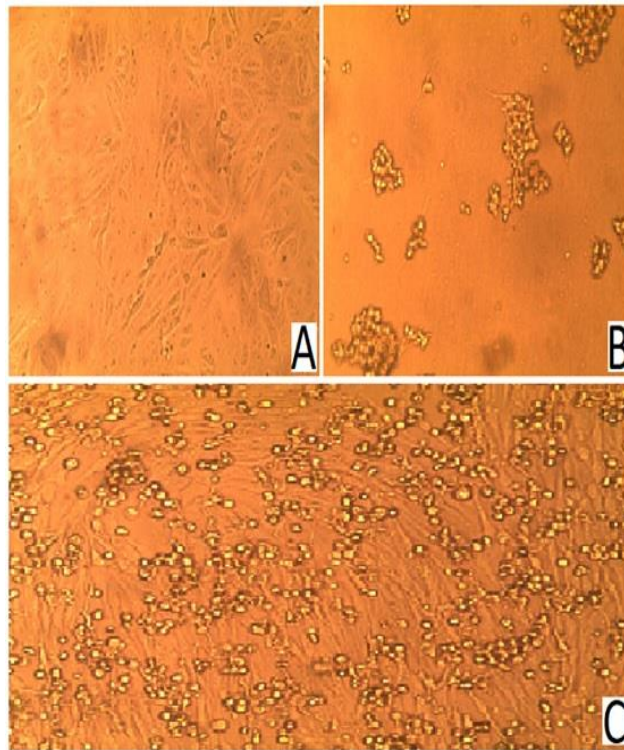
**Table 1:** The morbidity and mortality rates of infected animals with PPRV

Speaches	Total number of animals	Number of diseased animals	Number of dead animals	Morbidity rate	Mortality rate
Goats	42	42	10	100%	23.81
Sheep	13	13	1	100%	7.69
Total	55	55	11	100%	20%

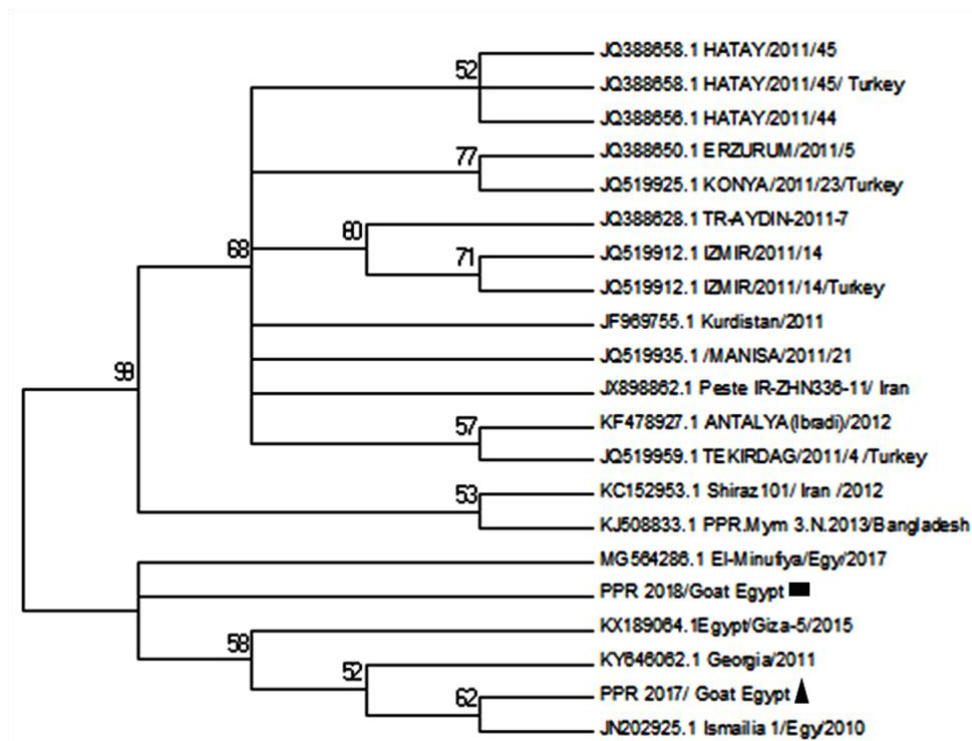
**Table 2:** Percentage of positive samples in virus isolation and conventional RT-PCR

Samples	Number of Samples	Viral isolation		Conventional RT-PCR	
		Number of +ve samples	Percentages %	Number of +ve samples	Percentages %
tissue scraping from oral lesions	6	4	66.66%	6	100%
Oculo-nasal swabs	4	3	75%	4	100%
Total	10	7	70%	10	100%

**Figure 1:** Diseased animals showed (A) Mucopurulent ocular discharge. (B) Erosion and diphtheritic membrane of hard palate. (C) Necrotic tissue and ulceration of tongue. (D) Soiling of hind quarters revealing profuse watery diarrhea



**Figure 2:** (A) Control Vero cell culture, spindle shape of cells. (B) Cytopathic effect of PPRV on Vero cell culture showed cell detachment. (C) Cytopathic effect of PPRV on Vero cell culture showed cell rounding



**Figure 3:** Phylogenetic relationship and comparative of PPR virus isolate of the current study (PPR 2018/goat Egypt ■) with other virus isolate, from previous study in Zagazig city 2017 (PPR 2017/goat Egypt ▲) based on partial sequences of N gene. Also, Neighbor-joining tree of PPRV N gene showing phylogenetic relationships of the PPRV isolates of present study with ather closely related Egyptian isolates available from GenBank

## Discussion

Peste des petits ruminants (PPR) is a Morbillivirus within the Paramyxoviridae family which characterized by highly contagious nature with high mortality rates of domestic small ruminants (22). The disease is manifested by sudden onset of depression, fever, mucopurulent ocular and nasal discharges, oral lesions, dyspnea, diarrhea and death (23).

The Food and Agriculture Organization (FAO) reported that approximately 63 percent of small ruminants are highly susceptible to PPRV infection specially in South Africa, Central Asia and Southern Europe (24). In Egypt, PPR was first recorded in 1987(2). PPR has been diagnosed with polymerase chain reactions in various locations in Egypt over the past 10 years (13, 25, 26 and 27). Therefore, PPRV considered one of the most seriously contagious viral disease of small ruminant causing huge economic losses in Egypt.

The present study applied on a flock of 55 small ruminants (42 goats and 13 sheep) in Belbes city in Al-Sharkia governorate in 2018, suspected to be infected with PPRV as infected animals showed fever, depression, off food, mucopurulent ocular discharge, mucopurulent nasal discharge, dyspnea, diarrhea, necrotic tissue and diphtheritic membrane in oral cavity. These symptoms agree with those described by (7, 8).

The present study revealed that the morbidity rate was 100% in both goats and sheep and mortality rate was (23.8% in goats and 7.69% in sheep). The high mortality rate percentage in goats may be due to severe immunosuppression of infected goats (28) as PPRV downregulate CD46 (29), inhibit the leukocyte proliferation (30), and/or apoptosis of mononuclear cells in peripheral blood (31). These finding were supported by the previous studies of (32) and (33) who observed that the PPRV symptoms in sheep were less severe than goats. Furthermore, mild infection in sheep has been reported in previous studies of (25, 27, 34, and 35). On the contrary, (36) observed significantly higher incidence rate of PPRV in sheep 39.1% than in goats 23%.

The ten samples (6 tissue scraping from oral lesions and 4 oculo-nasal swabs) were tested by conventional reverse transcription PCR which revealed 100% sensitivity compared to VI (virus isolation) 70%. The CPE produced after three successive passage were cells detachment, cell rounding, and formation of grape-like clusters. Our result supported by (10, 11) who noted that (CPE) produced in Vero cells by PPRV consist of giant cells, cell rounding, formation of typical grape-like clusters, and formation of small syncytia.

Despite low sensitivity, virus isolation is the most valuable method for detecting PPRV (37). Low-quality samples, poor transport, poor storage, aging cells and virus isolation in suboptimal cell culture (without the lymphocyte activation molecule (SLAM) that helps to isolate them) are predisposing factors for low VI sensitivity (38). Viral isolation also takes long time and requires further confirmation (39).

This study reported that all tested 10 samples 100% were positive by conventional RT-PCR. These findings endorsed those obtained by (13) who found that the positive samples tested by conventional RT-PCR and real-time PCR were 90% and 100% respectively. (37, 40) observed that conventional RT-PCR could detect virus in eye swabs in experimentally infected goats four days after infection.

PCR is the most favorable and highly sensitive tool for virus identification and molecular epidemiological studies among the various techniques used for PPRV detection (39).

Phylogenetic analysis of N gene sequence is the key method for differentiation between circulating PPRV lineages and help in molecular epidemiology of the disease (41).

The comparative of N gene sequence of the current study (PPRV isolate from Belbes City 2018) and the isolate from the previous study in Zagazig City 2017 showed that the two isolates had a 99 percent homology suggesting that there are no rapid genetic changes to circulating viruses. Nucleotide sequences were aligned with other PPRV sequences available in GenBank and phylogenetic tree were used to determine the genetic lineage of the circulating virus.

Circulating strains have been classified as lineage IV.

The high percent homology between the two isolates may be due to uncontrolled movement of sheep and goats within Al-Sharqia governorate. Also PPRV outbreaks are more common around festivals periods.

In addition, the PPRV isolate of the current study related to Ethiopian strain and the previous Egyptian strains (Ismailia 3/Egy/2010 isolate, El-Kalubeya isolate and Ismailia 1-2014 isolate).

The origin of the circulating viruses may be from Ethiopia. Egypt imports small ruminants from different African countries including Ethiopia because it has meat production deficit.

The similar result demonstrated by (13) who reported that, the cause of outbreak in Ismailia and Suez in period 2014 and 2016 was Ethiopian origin. Another study revealed that the Sudanese strain caused PPRV outbreaks in Ismailia, Egypt during the 2010-2012 (27). Also, this study confirmed that all Egyptian isolates classified as PPRV lineage IV strains circulate extensively from governorate to other without controlled measures.

## Conclusion

The study confirms that PPRV has been detected and circulated in a flock of goats and sheep in the city of Belbes, Al-Sharqia. The N gene sequence and phylogenetic analysis confirm that the PPR virus circulating in this region of the country is of lineage IV and closer to PPRV isolated in Zagazig in 2017. Also PPRV isolate of present study revealed high level of homology with majority of the previous Egyptian isolates and Ethiopian strain. The movement of unbarred animals and their trade could be one of the reasons for the transmission of this virus. The results emphasize the importance of molecular methods for a broader understanding of the epidemiology and development of the virus in the country. The information from these studies will help to achieve the goal of the disease control and eradication.

## Conflict of interest

There is no conflict of interest.

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