



# Identification and phylogentic analysis of sheep pox during an outbreak of sheep in Sharkia Governorate, Egypt

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**ABSTRACT.** Sheeppox virus (SPPV) is one of the listed and notifiable disease affect sheep production with major effect on the trade of new breed of sheep. This study was conducted to identify sheep pox by using cell culture, electron microscope (EM) and open reading frame (ORF) 103 gene during an outbreak of local breed of sheep occurred in Sharkia Governorate, Egypt in April 2017. Affected adult sheep showed typical skin pox lesion on face, the inner side of lips, inner aspect of the thigh and under the tail. The incidence rate of infection was 23.5% and the mortality rate in young lambs aged 3 to 6 months old was 8.2%. Forty-three scabs and tissue samples from clinically diseased adult sheep and dead lambs were collected and subjected to culture on Vero cell. The cytopathic effect (CPE) was observed within 3 to 7 days in 40 samples. A typical Poxvirus was a brick-like shape with round ends by negative staining of EM and ovoid like structure with dumb-bell shaped DNA core with concave bodies sides by positive staining of EM. By conventional PCR utilizing *ORF103* gene and obtained bands of about 570 bp referred to SPPV. The sequence amplicon was analyzed by NCBI-BLAST and register in GeneBank under accession N. MG873537 and phylogentic tree was designed which revealed that the isolated strain of SPPV was resembled with other strains of SPPV isolated in Egypt, India, China, and USA. Finally, both EM and PCR are considered as sensitives, rapids, and powerful methods to identify SPPV from tissue and scab's samples without the need of further culture in addition to the useful and easily use of *ORF103* gene to differentiate SPPV from other Capripoxvirus.

**Key words:** Sheeppox virus; Electron microscope; PCR; *ORF 103* gene; GeneBank; Phylogentic analysis.

## INTRODUCTION

Sheep is considered one of the most important economic sources due to its high-quality meat and wool production. Sheep are raised either by small-scale farmers or in village flocks managed by shepherds. Sheep pox disease is considered as one of an economically important disease in sheep producing area all over the world. Sheep pox disease is caused by *Sheeppox virus* (SPPV), DNA virus, recorded as one of the largest viruses (170-260 nm by 300- 450 nm), belonged to genus *Capripoxvirus*; (CaPVs), subfamily; *Chrodopoxvirinae*, family *Poxviridae* (Matthews, 1982; Kitching and Taylor, 1985). The other members of the genus are *Goatpox virus* (GTPV) and *Lumpy skin disease virus* (LSDV) of cattle (Murphy et al., 1995). *Sheeppox virus* (SPPV) and *Goatpox virus* (GTPV) were antigenically and genetically closely related to each other (Fenner et al., 1996). The current criterion used for classifying CaPVs. The genus was based on animal species from which the viruses were isolated, SPPV from sheep, GTPV from goats and LSDV from cattle (Babiuk et al., 2009).

Sheep pox disease is endemic in the Middle East, including Egypt, Iran, Afghanistan, North Africa, Turkey, Iraq and the Indian subcontinent. In South-Eastern Europe, sporadic outbreaks occur (OIE, 2017). SPPV is one of the 15 animal pathogens listed by Animal World Health Organization (OIE) and 23 by (Animal and Plant Health Inspection Agency USDA) which can be used as an animal biological warfare agent (USDA, 2002).

SPPV is a highly contagious disease, spread through aerosols and/or close contact with infected animals, indirect means such as contamination of cuts and abrasions (Kitching and Carn, 2004). Poor conditioned animals, overcrowding, poor feeding, general mismanagement, and abnormal uses of vaccination considered the main causes for distribution of sheep pox disease (Sheikh-Ali et al., 2004; Zangana and Abdullah, 2013). SPPV characterized by fever, appearance of pox lesions as papular, pustular, scab stages on areas devoid of wool such as checks, lips, nostrils, inner aspect of the thigh and under the tail in which the skin lesions usually heal within 5-6 weeks (Davies and Otema, 1981).

Laboratory confirmation of *Poxvirus* infection by electron microscopy (EM) based on the visualization and morphological identification of virus particles in diseased tissue samples. EM is considered as one of the methods used for differential diagnosis of skin lesions induced by other viruses (Curry et al., 2006 and Yadav et al., 2010). Negative staining of EM had the advantages of easily sample preparation, rapid diagnosis (same day result) and the undirected (open view) of EM allowed rapid morphological identification and differential diagnosis of agents which present in the isolate supernatant (Hazelton and Gelderblom, 2003). Positive staining of EM detects the inner structure of SPPV which helps in confirm diagnosis of disease. Polymerase chain reaction (PCR) is considered a rapid, sensitive and good specific technique on detection and differentiation of SPPV based on the open reading frame (ORF) 103 genes from other similar diseases affecting sheep. *ORF103* gene was used for genotyping and phylogenetic analysis of SPPV (Zhu et al., 2013). The disease agent was confirmed as SSPV by clinical signs, post-mortem examination, detection and identification of isolated *Poxvirus* by isolation of the causing agent, using electron microscope (Negative and Positive staining) and polymerase chain reaction (PCR) technique.

## MATERIAL AND METHODS

### Outbreak of the disease

A natural outbreak of typically clinically diseased non-vaccinated flock of sheep with SPPV was recorded in Kafr Shalshamoun, Menya Al Qamh, Sharkia, Egypt in April 2017. Eighty-five local sheep flock with history of non-vaccination for 7 years which consisted from 56 adult sheep and 29 lambs in which the incidence of the infection was 23.5% (20 infected) and 8.2% mortality rate of young infected lambs aged between the 3-6 month of age. The owner was quickly separate clinically infected lambs from non-infected ones. Thirty skin samples of crusted scabs lesions were collected from 20 affected adult sheep in addition to 13 postmortem lesions were collected from dead lambs; the collected samples were stored at -70°C until further examination.

### Clinical examination of sheep

Sheep were clinically observed for the manifestation of general clinical signs related to skin lesions as papules, nodules and scab's formation on an area free from wool and hair that lead to suspect infection with pox disease in addition to post-mortem examination of dead lambs (Constable et al., 2017).

## **Preparation of suspected tissue samples**

According to (OIE, 2017) 10% suspension of suspect tissue samples (papules and scabs) prepared in phosphate buffer saline (PBS) containing antibiotic [penicillin (100 U/ml), streptomycin (100 µg/ml), neomycin (2.5mg/ml) and nystatin (50 U/ml)]. The samples were ground with sterile sand in a mortar. The homogenized suspension was frozen–thawed three times and then partially clarified by centrifugation at 5000 rpm for 10 minutes to remove tissue depress and then stored at -70°C till used.

## **Isolation of virus from suspected tissue samples on cell culture**

Viral antigens grown in Vero cell culture African green monkey kidney cell line (Vero) was used for inoculation of the tissue sample in trials for isolation and propagation of virus. It was supplied from Egyptian organization for Biological Products and Vaccines, Agouza, EL- Giza, procedure was performed according to (OIE, 2017). A 200 µl of clarified tissue preparation supernatant is inoculated to a 25 cm<sup>2</sup> Vero cell-culture flask of 90% confluent cell, and supernatant is allowed to absorb for 1 hour at 37°C after washing with warm PBS and 10 ml of a suitable medium, such as 199E media, containing antibiotics and 2% fetal calf serum was added. The culture is examined daily for 7–14 days for appearance 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 and margination of the nuclear chromatin. At first only small areas of CPE can be seen within 4 days after infection then the following 4–6 days the growth expands to involve the whole cell sheet. If no CPE is apparent by day 7, the culture should be exposed to freeze–thaw three times and clear supernatant inoculated again into fresh tissue culture with further incubation for 4 to 7 days but if still negative, third passage is done but if the negative result is recorded again, the sample is considered negative.

## **Detection and identification of the virus by EM**

### **A-Negative staining**

The 10 skin tissue samples (papules and scabs) were ground in a sterile mortar with a small volume of distilled water and centrifuged for 15 min at 5000 rpm. The supernatant was collected and centrifuged again for 45 min at 13000 rpm, then the pellet was rinsed with distilled water, then a droplet of 1% phosphotungstic acid mixed with a droplet of tissue suspension on a copper grid coated with carbon formvar, The grid was drained using filter paper, air dried, washed by Paraformaldehyde buffered with phosphate buffered saline (PBS) after drying when the sample is too thick on the grid then grid dried again before examined under EM. The electron beams penetrate the virion but surface structures visible by the contrast of the embracing electron-dense tungsten that appeared black (Ohi et al., 2004).

### **B-Positive staining**

The detection of virus in suspected tissue samples (5 scabs and tissue samples) using positive staining technique of electron microscope according to (Kay, 1965): The samples were cut into small pieces and then embedded in glutaraldehyde 5% for 24 hours with further washing and embedded and finally the samples embedded inside molds with resin in order to be in the plastic caps, the embedded tissue samples were ready to be cut into thin and ultra-thin sections by ultra-microtone apparatus. Ultra-thin sections of the samples stained with heavy metals (uranyl acetate and lead citrate) for preparing sections to be inspected by an electron microscope.

## **Extraction of DNA**

The QIAamp DNA Mini Kit provides silica-membrane-based nucleic acid purification is used for purification of DNA from scabs and tissue samples according to manufacture instructions and the resulted DNA used as PCR template for amplification.

## **Amplification of PCR product**

Sheep pox open reading frame 103 gene (ORF 103) was designed as published data of Zhu et al. (2013) and obtained from Metabion (Germany) as the following (Forward primer, 5´ ATGTCTGATAAAAAATTATCTCG 3´) and (Reverse primer, 3´ ATCCATACCATCGTCGATAG 5´). The PCR was carried in 25 µl total mixture volume as illustrated in Table 1.

**Table 1.** Preparation of PCR master mix according to Emerald Amp GT PCR master mix (Takara)

Component	Volume/Reaction
Emerald Amp GT PCR master mix (2x premix)	12.5 µl
PCR grade water	4.5 µl
Forward primer (20 pmol)	1 µl
Reverse primer (20 pmol)	1 µl
Template DNA	6 µl
Total	25 µl

### Cycling conditions of the primers during PCR

Temperature and time conditions of the two primers during PCR are shown in the table (2) according to Zhu et al. (2013).

**Table 2.** Cycling conditions of the ORF 103 primers during PCR

Gene	Primary denaturation					Final extension
		Secondary denaturation	Annealing	Extension	No. of cycles	
Sheep pox	94°C	94°C	52°C	72°C	35	72°C
ORF 103	5 min.	30 sec.	45 sec.	45 sec.		10 min.

### Agarose gel electrophoreses (Sambrook et al., 1989) with modification

Mix 10 µl of each PCR product samples, negative control and a positive control with loading dye solution and load in 1.5% agarose gel in TAE (Tris/ Acetate/ EDTA) buffer containing ethidium bromide Load a parallel lane with a 50 bp DNA-marker ladder. Separate the products at 100 volts for 30–40 minutes and visualize using a UV Transilluminator. The gel was photographed by a gel documentation system and the data was analyzed through computer software and confirm the positive reactions according to the size (Sambrook et al., 1989).

### Sequencing and phylogenetic analysis

570 bp PCR band was exposed to sequence in an automated ABI DNA sequencer (Germany) and the obtained sequence was exposed to analysis using NCBI-BAST and compared with other *Capripoxvirus* sequences available in GeneBank as shown in table 3. The phylogenetic tree was obtained by the using of neighbor-joining method using MEGA program version 6.0 software.

**Table 3.** *Poxvirus* strains used to phylogentic analysis

Virus	Strain	Country	Accession number
<i>Sheeppox virus</i>	SPPVE1 Menya Al Qamh <i>ORF103</i> gene*	Egypt	MG873537
<i>Sheeppox virus</i>	El-Minufiya <i>ORF 103</i> gene	Egypt	MF443334
<i>Sheeppox virus</i>	SPPV/57-2823/Pune/2007	India	KX398522
<i>Sheeppox virus</i>	SPPV/32-01/Jalandhar/2011	India	KX398521
<i>Sheeppox virus</i>	SPPPV/30-02/Ahmedabad/2008	India	KX398520
<i>Sheeppox virus</i>	SPPV/3174/Pune/2007	India	KX398518
<i>Sheeppox virus</i>	SPPV/Pune/2008/P4	India	KX398505
<i>Sheeppox virus</i>	SPPV/Srinagar/2000/P5	India	KX398503
<i>Sheeppox virus</i>	SPPV/30-01/Ahmedabad/2008	India	KX398519
<i>Sheeppox virus</i>	SPPV/Jaipur/P3	India	KX398516
<i>Sheeppox virus</i>	SPPV/Roumanian Fanar/P37	India	KX398500
<i>Sheeppox virus</i>	SPPV/Makdhoom/2007/P6	India	KX398504
<i>Goatpox virus</i>	GTPV 143/Mukteswar/2012	India	KX398510
<i>Goatpox virus</i>	GTPV/Sambalpur/2001/P6	India	KX398512
<i>Goatpox virus</i>	GTPV/Uttarkashi/1978/P101	India	KX398499
<i>Goatpox virus</i>	GTPV/Akola/2008/P4	India	KX398506

Note: \*Our local isolate of SPPV.

## GeneBank accession number

The obtained *ORF103* gene sequence was aligned by CLUSTAL W version 1.81 software then submitted to GeneBank database under the accession number (MG873537) with the name of SPPVE1 Menya Al Qamh *ORF103* gene.

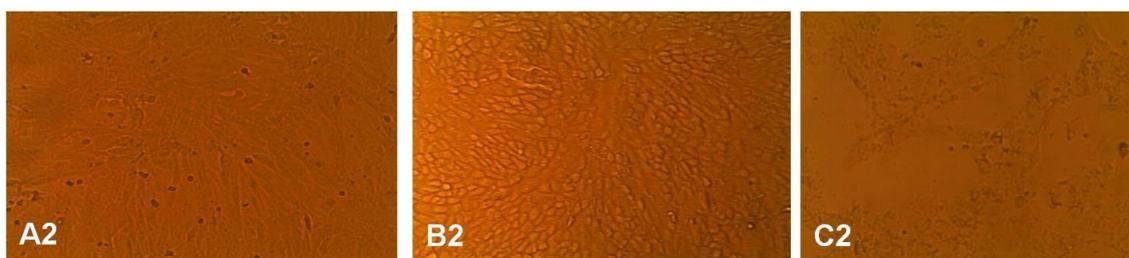
## RESULTS

A natural outbreak of typically clinically diseased non-vaccinated flock of sheep with SPPV was recorded in Kafr Shalshamoun, Menya Al Qamh, Sharkia, Egypt in April 2017. The local flock of sheep (85) was consisted from 56 adult sheep and 29 lambs with history of non-vaccination for 7 years, in which the incidence of the infection was 23.5% (20 infected from 56 adult sheep) and 8.2% mortality of young infected lambs of age range between 3 to 6 months. The clinical examination of the diseased sheep revealed that rise of body temperature (range between 40°C to 40.8°C), decrease food intake, nasal discharge and skin lesions vary from redness to papules and nodules moreover, ulcer formation and scabs in different sites include all face, inside lips, eyes, nostrils, innerside of the thigh and under the tails as illustrated in Figure 1. Postmortem examination of dead lambs revealed the presence of oedema, papules, nodules and less observed the formation of an ulcer on tongue, trachea and lung in addition to congestion of abomasal mucosa.



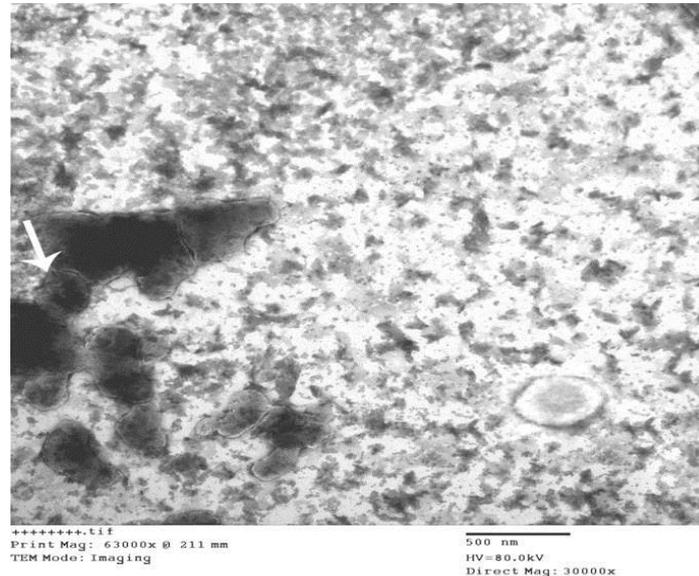
**Figure 1.** Photographs of sheep with typical skin lesions of sheep pox virus: A1: Scattered nodules in all face of sheep, B1 & C1: Typical nodules of pox lesion on inner side of lips, D1, E1 & F1: Forms of sheep pox disease under the tail (small erythematous papules, nodules, and scab formation).

Thirty skin samples of crusted scabs lesions were collected from 20 affected adult sheep in addition to 13 postmortem lesions were collected from dead lambs. Forty-three homogenated samples were subjected to isolation of SPPV by inoculation 200 µl of 10% suspension on Vero cell culture, the inoculated cells were examined microscopically daily for detection the development of cytopathic effect (CPE). The positive samples showed the characteristic granulation of cells followed by cell rounding and aggregated separately; this occurred after 3 days post inoculation and gradually increased till 70-80% of the sheet that was completely detached in some samples after 5-7 days (Figure 2 B&C). The control Vero cell culture and the 3 negative cells were characterized by roughly or slightly spindle shape cell in monolayer confluent sheet (Figure 2 A). Forty samples were success to culture and showed CPE on Vero cell but only 3 samples were failed to develop on cell culture.



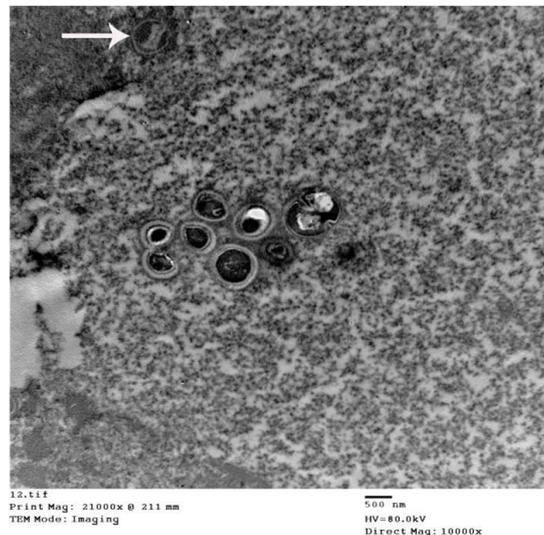
**Figure 2.** Vero cell-culture (Mag. 40x): A2: Control culture, B2: CPE of pox virus on Vero cell as a retraction of cell membrane, C2: CPE of pox virus on Vero cell as cell rounding and aggregated together.

Negative staining of EM used to visualize SPPV morphology in 10 original papules and scab suspensions. SPPV virion in examined samples revealed that electron micrograph of a typical *Poxvirus* was brick-like shape with round ends as shown in Figure 3.



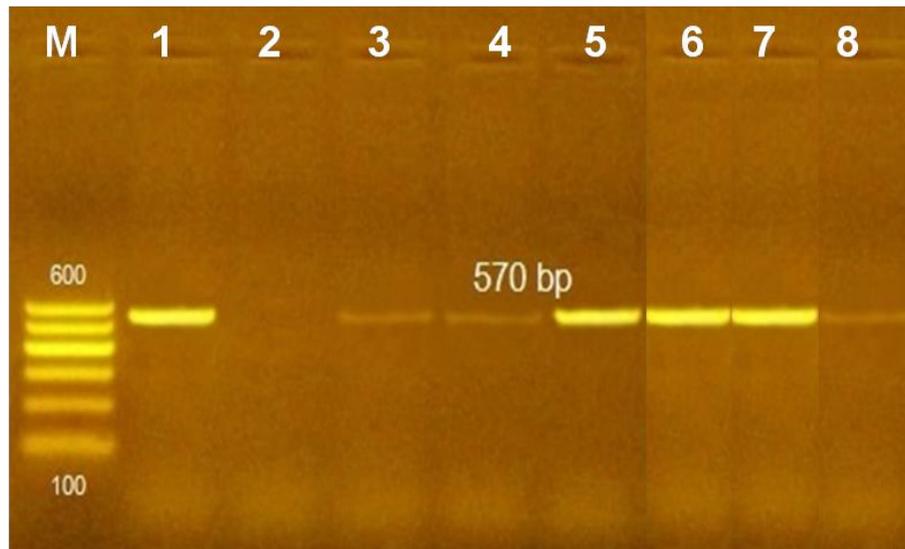
**Figure 3.** Virion of SPV by negative staining of EM (brick-like round end virion) (Mag. 30000x).

Positive staining of EM used to visualize the inner structure of SPPV in 5 scabs and tissue samples were revealed that electron micrograph of a typical SPPV was ovoid like structure with dumb-bell shaped DNA core with concave bodies side as illustrated in Figure 4.



**Figure 4.** Ovoid-like structure with dumb-bell shaped DNA core with concave side bodies of virion of SPV (positive staining of EM).

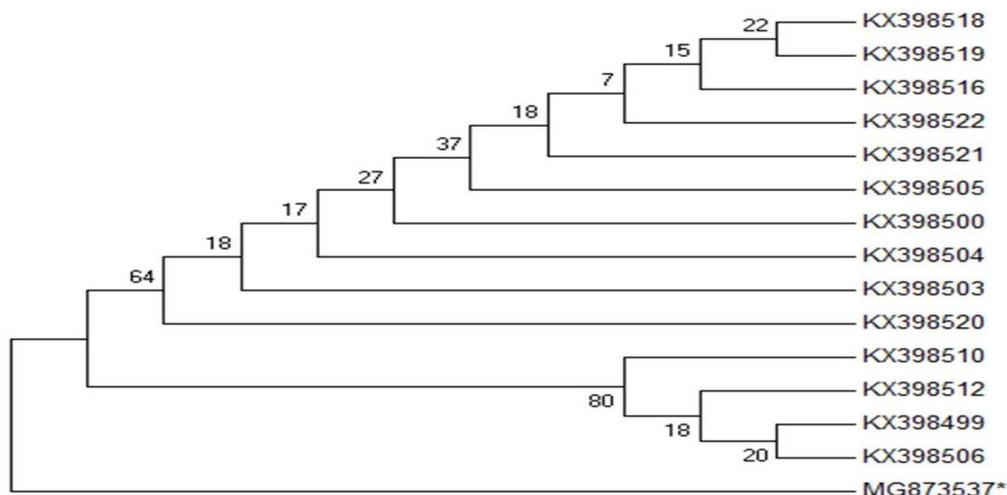
Based on *ORF 103* gene, 10 infected tissues and scabs were used to detect of sheep pox virus by PCR technique. Amplicon of expected size (570 bp) was observed on agar gel electrophoresis as seen in Figure. 5. Amplification of specific DNA of SPPV was evident in all the samples. One of amplified amplicon was cut, purified and sequenced. The obtained sequence was aligned by CLUSTAL W 1.81 and admitted to GeneBank under the accession number MG873537.



**Figure 5.** Conventional PCR by *ORF 103* gene showing SPV (amplicon of about 570 bp): M: DNA ladder of 600bp, Lane 1: positive control, Lane 2: negative control, Lane (3,4,5,6,7,8): positive SPV amplicons.

*ORF103* sequenced gene was analyzed using NCBI-BLAST. The result of NCBI-BLAST analysis revealed that the strain of SPPV obtained was related to other *Capripoxvirus* worldwide distributed with percentage of identity range from 100% with other strains of SPPV include (*Sheeppox virus* isolate SPPV-GL, complete genome and *Sheeppox virus* NISKHI, complete genome ...etc) to 99% with other example of *Sheeppox virus* as (*Sheeppox virus* isolate SPPV/Roumanian Fanar/P37 virion core protein gene, complete cds) to 97% with other GTPV as (*Goatpox virus* isolate GTPV/Sambalpur/2001/P6 virion core protein gene, complete cds and *Goatpox virus* isolate GTPV 143/Mukteswar/2012 virion core protein gene, complete cds) and with LSDV as (*Lumpy skin disease virus* isolate SERBIA/Bujanovac/2016, complete genome and *Lumpy skin disease virus* isolate Evros/GR/15, complete genome).

Phylogenetic tree was designed by the using of MEGA version 6.0 program as shown in Figure. 6. The analysis of tree was gave a good picture to illustrate that our strain was nearly similar to SPPV isolate El-Minufiya *ORF 103* gene, partial cds, Egypt (accession number MF443334) and closely related to other strains of SPPV isolated in India as SPPV/Roumanian Fanar/P37 (accession no. KX398500) and SPPV/30-01/Ahmedabad/2008 (accession no. KX398519) and SPPV isolated in USA as SPPV 10700-99 strain TU-V02127 (accession no. AY077832, data not included in phylogenetic tree) and other isolated in China as *Sheeppox virus* isolate SPPV-GL (accession no. KT438551, data not included in phylogenetic tree).



**Figure 6.** Phylogenetic tree of different Capripoxvirus depend on produced nucleotide sequence of *ORF 103* gene, The tree was constructed by the use of MEGA version 6.0. \* Our local isolate of *Sheeppox virus*.

## DISCUSSION

Sheep pox is considered as one of notifiable disease as recorded by WHO with worldwide distribution as widely found in India, China, Egypt, Saudi Arabia, Greece, Iran, Iraq, Bangladesh and Pakistan (Rao and bandyopadhyau, 2000; Maksyutov et al., 2013; Hani et al., 2015; Constable et al., 2017; OIE, 2017). Presence of sheep pox disease in any country limits the trade of new breeds and development of intensive sheep production. The *Sheeppox virus* infection is one of the common diseases among those causing economic losses occur in the form of high mortality rate in young, productivity reduction and poor wool and leather quality (Parthiban et al., 2005). Diagnosis of SPPV was based on clinical signs and postmortem findings followed by virus isolation on cell cultures with further confirmation by EM and PCR.

An outbreak of sheep pox occurred in April 2017 on a local breed of sheep in Kafr Shalshamoun, Menya Al Qamh, Sharkia, Egypt include 85 sheep (56 adults one and 29 young lambs aged 3 to 6 months old). The total percentage of diseased animals was 23.5% and the total percentage of dead animals was 8.2%. The incidence was higher than recorded by Mondal et al. (2004) reported that the incidence of infection was 18.4% during outbreak of SPPV occurred in December 2001 in Jammu, India and also higher than outbreak of SPPV that occurred in Greece 2007 with incidence of 8.45% (Mangana et al., 2008) and other detected in Sudan (18.9%) and in India (7.2%) by Nour et al. (2012) and Selvaraju (2014), respectively. But less than other reported during an outbreak of SPPV among sheep occurred in Ningxia Hui, China among sheep during November 2011, the incidence of the disease was 29.2% (Zhu et al., 2013).

In this investigation the mortality rate was 8.2% which higher than the percentage recorded by Mondal et al. (2004) in which the percentage of death was 6.3% and less than recorded by Ammar et al. (1999) detected that large number of sheep at El-Karada, Sakha and El-Gemiza in Kafr El- Sheikh Province showed skin eruptions on wool less areas of the skin with mortality rate 68.4%, diagnosis referring to SPPV infection, Zhu et al. (2013) 14.6%, and by El-Sabagh et al. (2014) during outbreak of sheep pox disease occurred in Al-Hassa province of Saudi Arabia during the period between the winter and the spring 2013, mortality was 15%. This variation in both incidence rate and mortality rate may be attributed to difference in the study time, total number of examined animals, study localities and method of rearing.

In the current study, the observed clinical signs in suspected infected sheep were increase of body temperature, nasal discharge, lacrimation, and scabs on head, face, ears, nostrils, oral commissure, ulcer formation inside the lips, multiple cutaneous papules and nodules in inner aspect of thigh and under the tail were observed and considered as first indicator for *Poxvirus* infection. Similar clinical signs were observed by Daoud (1997) in Jordan, Davis and Otema (1981) in Kenya, Achour et al. (2000) in Algeria, Singh et al. (2007) in India, Sharawi et al. (2011) in Egypt, Zangana and Abdullah (2013) in Iraq and Hamouda et al. (2017) in Saudi Arabia. Post-mortem findings of dead lambs revealed the presence of oedema, papules, nodules and less observed the formation of an ulcer on tongue, trachea and lung in addition to congestion on the abomasal mucosa. Equally postmortem examination of dead young lambs affected with SPPV was recorded by Diallo and Viljoen (2007) and by Zhu et al. (2013) of examined 3 provinces in China during the period between 2009 to 2011 and also by Hamouda et al. (2017) during outbreak of SPPV in Saudi Arabia.

Further identification of SPPV infection by isolation was necessary to avoid miss-diagnosis and differentiate it from other similar diseases Chana et al. (2007). Skin tissue samples (papules and scabs) were selected for virus isolation as it was scientifically known that it has high survived of *Poxvirus* making it the best for isolation on cell culture Nawal et al. (2006); El- Sabagh et al. (2014); Amal et al. (2008) and Sharawi et al. (2011). Regarding isolation of *Poxvirus* on Vero cell culture revealed that appearance of cytopathic effects on 40 samples (93%). The cytopathic effects appeared as granulation of cells, cell rounding and aggregation separately without any changes in control cells. SPPV gave clear CPE on Vero cell similar to those obtained by Rhizhallah (1994); Maity et al. (1997) and Amal et al. (2008). In contrast, some strains as ranipet strain of ovine *Poxvirus* resist growing on Vero cells (Davis, 1976; Jassim and Keshavamurthy, 1982). In this study sheep pox was adapted on cell culture on the 3<sup>rd</sup> day post inoculation and the CPE appeared as granulation of cells followed by cell rounding and aggregated together separately within 4-5 days post inoculation. The same result was reported by (Rizkallah, 1994; Olfat, 2000 and Amal et al., 2008). Three samples were failed to grow which may be attributed to easily contamination of culture during isolation of virus especially from field samples, may be isolated virus was previously found bind to neutralizing antibodies or cross-reacted with other viral strains as ORF as mentioned by Mangana-Vougiouka et al. (2000).

Recently, confirmation of *Poxvirus* infection by EM and/or PCR is more useful and prominent than conventional isolation which is more time-consuming, at least 10 days in routine cultural isolation, and needs compulsory cell culture passages. EM and PCR overcame this disadvantage as they can identify virus within 24 hr after completing the set-up procedures (Plowright and Ferris, 1958; Oguzoglu et al., 2006).

Electron microscope negative staining was used for confirmation of SPPV infection in 10 original tissue suspensions. The obtained results were mature virus particles appeared in 10 samples as brick-like shape with rounded ends. The obtained viruses characteristic brick shape was similar to those obtained by Buller and Palumbo (1991); Moss, (2001); Babiuk et al. (2009) and Bhanu Prakash et al. (2010). Negative staining of EM had the advantages of easy sample preparation, rapid analysis and the undirected that allowed rapid morphological identification of different agents (Hazelton and Gelderblom, 2003).

On the other hand, EM positive staining was used for detection of inner structure of *Poxvirus* from 5 scabs and tissue lesions. The obtained results were mature oval viruses particles with dumb-bell shape DNA core in all samples. The obtained results were agreed with Murray et al. (1973) and Wen et al. (2012). In conclusion, EM considered as one of a method for *Poxvirus* confirmation but the disadvantages facing it and limit it is used as diagnostic laboratory test, is the need for training microscopists, need for a suitable room for its operation and the inexactness in differentiating between many similar viruses (Biel and Gelderblom, 1999). PCR has been considering as perfect tool for good identifying and differentiation of *Capripoxvirus* strains for as the uses of *E10R* gene, *RPO 132* gene, *P32* gene, *ORF 095* gene, *RPO 30* gene and *ORF 103* gene have expressed as powerful tools for identifying of SPPV as mentioned by (Zhou et al., 2012; Zhu et al., 2013; Hamouda et al., 2017 and Zhao et al., 2017).

*ORF 103* gene was allowed to amplify of an amplicon of about 570 bp in agar gel electrophoresis. Amplification of specific DNA of SPPV was evident in all 10 samples. One of the amplified amplicon was cut, purified and sequenced. The obtained sequence was aligned by CLUSTAL W 1.81 and admitted to GeneBank under the accession number MG873537. *ORF103* sequenced gene was analyzed using NCBI-BLAST then subjected to analysis by Phylogentic tree. We concluded from the analysis of NCBI result and phylogenetic tree that our isolated strain of SPPV has identity similar to other SPPV with % of identity range from 100% as El-Minufiya *ORF 103* gene (MF443334) to 99% as SPPV/3174/Pune/2007 (KX398518) and also the isolated strain was closely related to GTPV and LSD with percentage vary from 99% to 97% as (*Goatpox virus* isolate GTPV 143/Mukteswar/2012 virion core protein gene, complete cds) and with LSDV as (*Lumpy skin disease virus* isolate SERBIA/Bujanovac/2016, complete genome). The analysis also gave a good image to illustrate that our strain was nearly similar to local strain isolated in Egypt (accession number MF443334) and closely related to other strains of SPPV isolated in India as SPPV/Roumanian Fanar/P37 (accession no. KX398500) and SPPV isolated in USA (accession no. AY077832 and other isolated in China (accession no. KT438551). From the present result may give predication for identify and the epidemiology of *Sheeppox virus* in Egypt and with the possibility of isolation from different provinces inside Egypt.

## CONCLUSION

EM and PCR have used to identify *Sheeppox virus* from collected tissue and scab samples. Both techniques have been considered as highly sensitive and moreover rapid and powerful to identify and differentiate *Capripoxvirus*. In addition to PCR method can help to give information about local strains of SPPV in our country without the need of further culture. Further study by use of *ORF 103* gene is needed to confirm the presence of the same strain in different localities in Egypt and to confirm the degree of homology with the already used Romanian strain of SPPV as vaccine for near future the possibility of use the local strain as a vaccine.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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