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Prevalence and molecular characterization of Lumpy Skin Disease in cattle during period 2016-2017

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ABSTRACT

The present study was carried out to determine the prevalence of Lumpy skin disease (LSD) in different governorates in Egypt in relation to different factors. A total of 875 blood samples and 10 specimens (skin nodules) were collected from apparently health and clinically infected balady cattle of different ages and two sexes in different seasons during period 2016 – 2017. The prevalence of LSD in cattle in different governorates was 35.4%, 29.6%, 25.3%, 24.7%, 15.6% and 13.8% in Beni Suef, El-Faiyum, EL-Dakahlia, El-Qalyubia, El-Beheira and El-Gharbia, respectively. The prevalence of the disease was higher in females (29.2%) than males (21.2%). Also, the prevalence rate was higher in age group (> 12-36 m) 33 % and (> 36-50 m) 23.5% in comparison with age groups (3-6 m) 15.6% and (> 6-12 m) 16%. The disease was highly occurred during summer and autumn (27.6% and 26%, respectively). PCR reaction was carried out on skin nodules samples to confirm and characterize LSDV. selective positive samples undergo sequencing and compared with other LSDV strains and also other Capri-poxviruses in database in GenBank. The local LSDV samples reveled high identity in phylogenetic analysis to each other and high identity and clustered with other world LSDV strains and Capri-poxvirust.

1. INTRODUCTION

Lumpy skin disease (LSD) is a highly infectious disease of cattle caused by Neethling pox virus of genus Capri-poxvirus belongs to family poxviridae (Al-Salihi, 2014). LSD was first reported in Central and southern Africa then continues to circulate through the Middle East region and spread also in non-African countries in Asia and Europe (Abutarbush et al., 2015; Tageldin et al., 2014). The first LSD case had been recorded In Egypt in 1988 (House et al1990). At least two outbreaks occurred between 1989 and 2006 (El-Kholy et al., 2008; and Salib and Osman, 2011). Specific vector of LSDV was not absolutely confirmed, but there are strong evidences supporting that the LSDV was mechanically transmitted by Aedes mosquitoes (Chihota et al., 2001) and by ticks (Tuppurainen et al., 2011). LSD affecting cattle of all ages and breeds but the young calves and cows in the peak of lactation are more severely affected (Tageldin et al., 2014). The disease is characterized by sudden eruption of nodules on the skin, which may cover the whole body of the animal accompanied by fiver& systemic effects including pyrexia, anorexia, pneumonia, and generalized lymphadenopathy (Abutarbush et al., 2015). The disease cause considerable economic losses due to fertility problems, reduced milk production and damage to the hide in addition to high morbidity rate with some mortalities (Molla et al., 2017). The diagnosis usually based on serological analysis like ELISA (Alkhamis and VanderWaal, 2016) or molecular technique like PCR assay, which is specific, rapid, sensitive and accurate assay for detection of LSDV (Pestova et al., 2018).

The study aimed to determine the prevalence of LSD in some governorates in Egypt. In addition, molecular detection and characterization of Egyptian LSD strain.

2. MATERIAL AND METHODS

2.1. Sampling

2.1.1. Serum samples

A total of 875 serum samples were collected from clinically infected (300) and apparent healthy cattle (575) during the period from February 2016 to December 2017 from different governorates (El-Faiyum- El-Beheira- El-Qalyubia- El-Gharbia- EL-Dakahlia - Beni Suef).

2.2.2. Tissue specimens

Ten specimens (skin nodules) including whole skin thickness and skin crusts were collected for detection and typing of local virus strain.

2.2 Serological analysis

All collected serum samples were examined using ID Screen Capri-pox Double Antigen Multi-species (CPVDA-5P, ID vet innovative Diagnostics, France). The serological test

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(indirect ELISA) was carried according to manufacturer's instruction.

2.3 Molecular typing

The viral DNA was examined with specific pairs of primer LSDVF (5'actagtggatccATGGACAGAGCTTTATCA 3) and LSDVR (5'gctgcaggaattcTCATAGTGTTGTACTTCG 3) to amplify 472 bp fragment. PCR product was analyzed on 1.5% agarose gel electrophoresis.

The product of two positive samples was partially sequenced targeting fusion gene. The purified PCR products were sequenced directly using the ABI PRISM® Big Dye TM Terminators v 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences were edited and alignment was done with BioEdit software. Also, the phylogenetic analysis for the obtained sequence were performed by Mega7 software using the neighbor-joining tree method with 1000 bootstrap replicates.

3. RESULTS

The results of clinical investigation of animals under study revealed that the clinical signs began with fever, anorexia, skin lesions in the form of nodules all over the body. It was complicated with respiratory manifestation and corneal opacity. Necrosis and ulceration occurred and slough off leaving large ulcerative wound.

The prevalence rate of LSD varied between different localities under study. It was 35.4%, 29.6%, 25.3%, 24.7%, 15.6% and 13.8% in Beni Suef, El-Faiyum, EL-Dakahlia, El-Qalyubia, El-Beheira and El-Gharbia, respectively, as shown in table 1.

The prevalence was higher in female 29.2% than in male 21.2% as in table 3. The prevalence was higher (33% and 23.5%) in age groups (>12-36 m) and (> 36-50 m) in comparison with other age groups as shown in table 2. The prevalence of the disease was higher during summer 27.6% and autumn 26%, table 3.

Table 1 The prevalence of LSD in examined cattle in relation to areas

Govern	Total examined cattle	No. of infected animals	Percentage of infection
Beni Suef	164	58	35.4%
El-Faiyum	152	45	29.6%
EL-Dakahlia	150	38	25.3%
El-Qalyubia	97	24	24.7%
El-Gharbia	203	28	15.6%
El-Beheira	109	17	13.8%
total	875	210	24.0%

Table 2 The prevalence of LSD in examined cattle in relation to sex

Sex	Total examined cattle	Infected	Percentage of infection
Female	308	90	29.2%
Male	567	120	21.2%

P value 0.039

Table 3 The prevalence of LSD in examined cattle in relation to age

Age	Total examined cattle	Infected	Percentage of infection
3-6 months	96	15	15.6%
>6- 12 months	374	60	16.0%
>12-36 months	269	89	33.0%
>36-50 months	136	32	23.5%

The results revealed that ten skin samples were positive by PCR assay with amplified a DNA fragment of 472 bp as shown in fig 1. Two selective samples undergo sequencing and compared with other LSDV and other Capri-poxviruses in GenBank. The local LSDV samples reveled high identity to each other and high identity and clustered with other world LSDV strains and Capri-poxviruses. The isolated strains genetically characterized as lumpy skin disease virus by partial sequence of fusion gen (LSDV117) with high similarity to Herbivac LS strain, Neethling vaccine LW1959 strain, Sheep pox virus isolate Srinagar 38/00 fusion protein gen and Lumpy skin disease virus isolate Evros/GR/15 strain by Phylogenetic analysis figure (3) and (4).

Table 4 The prevalence of LSD in examined cattle in relation to season

Season	Total examined cattle	Infected	Percentage of infection
Summer	398	110	27.6%
spring	122	25	20.5%
Autumn	153	40	26%
Winter	202	35	17.3%

P value<0.0001

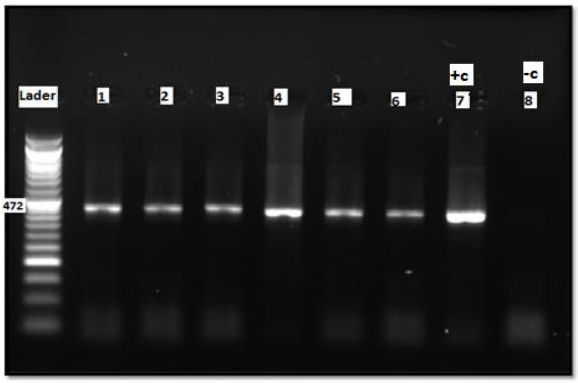

Figure 1the PCR amplicons (472 bp) of genomic DNA from lane 1 till lane 6 the tasted samples (all tested samples are +v) give specific band at 472pb. Lane 7 and 8 are +v control &-v control.

4. DISCUSSION

LSD is considered as enzootic disease in Egypt and causes severe cyclical outbreaks continue to occur in Egypt (Salib and Osman, 2011). Control of the disease to decrease the economic losses is depending on rapid and accurate diagnosis (Aspden et al., 2002). In the present study, clinical findings in suspected lumpy skin diseased cows were reported in Governorates under consideration began with fever, anorexia, skin lesions in a form of nodules all over the body. It was complicated with respiratory manifestation, corneal opacity. Necrosis and ulceration were occurred and slough off leaving large ulcerative wound. This agreement with signs recorded by (Tuppurinen and Oura 2012; Abutarbush et al., 2015). The results showed that the prevalence of the disease in cattle was higher in Beni Suef and El-Faiyum in comparison with other studied areas. This may be due to high insect populations and defects in vaccination programs in these governorates. Regarding to sex, the prevalence of the disease in females is more than males by significance difference which come in agreement with Ayelet et al. (2014) and Elhaig et al. (2017).

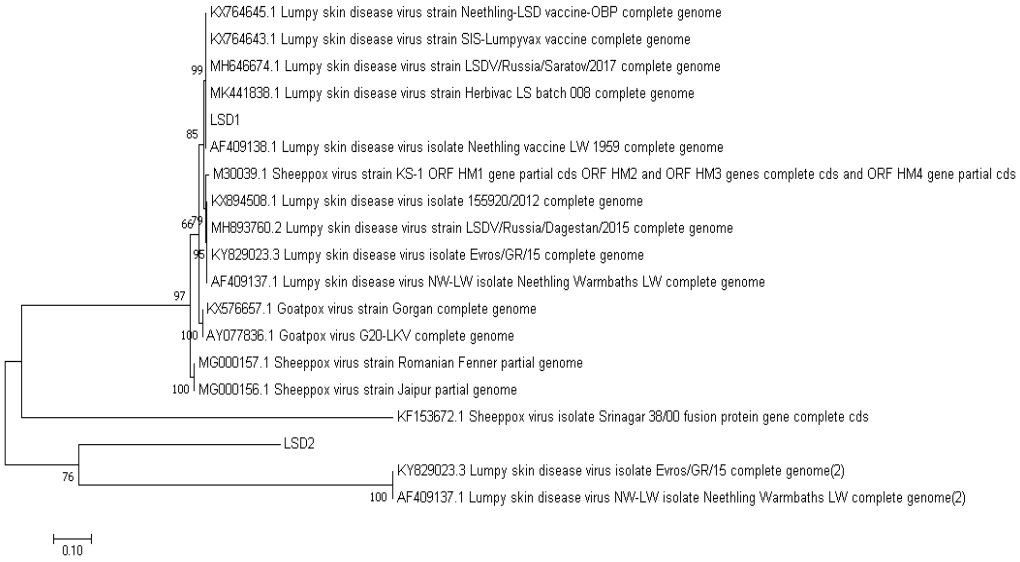

Figure 2 Phylogenetic analysis of fusion gene of LSDV

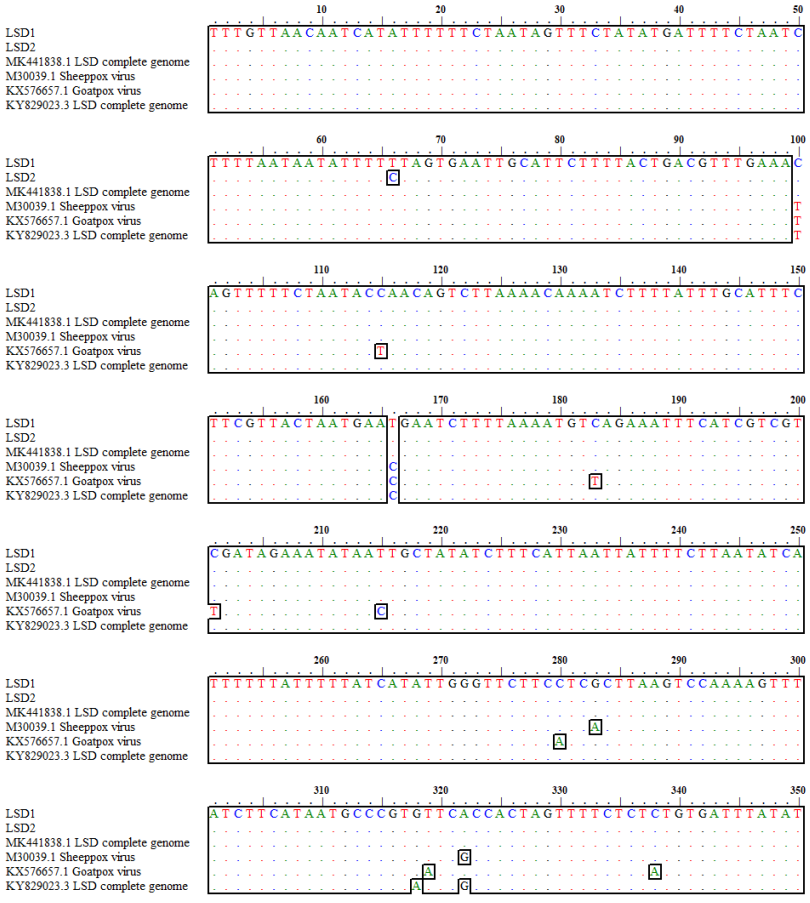

Figure 3 Alignment report of partially Nucleotide sequence of fusion gene of LSDV

This result may be due to stress factors as pregnancy, lactation and overcrowding (Tuppurainen et al., 2011). The obtained results revealed that the disease more occurred in older animals than young ones, this evidence come in agreement with that recorded by Sameea Yousefi et al. (2017). This may be due stress factors as pregnancy, lactation and fattening. However, other studies proved that the cattle of young ages were more susceptible than the oldest (Ayelet et al., 2014; Tuppurainen et al., 2011). In this study, the low prevalence of the disease in young ages may be due to passive maternal immunity (Adedeji et al., 2017).

Also, the results showed that LSD was more common during summer and autumn. This may be due to warm, wet weather that motivated the growth and distribution of insects which considered the main vector in transmission of LSDV (Chihota et al., 2001; Gari et al., 2015). The results come in accordance with previous studies (Al-Salihi and Hassan, 2015; Şevik et al., 2016). The phylogenetic analyses and sequence alignments of local LSDV isolates showed that the LSD viral isolates were highly identical to each other and closed related to other LSDV strains and other Capri-poxviruses (goat and sheep pox) strains. These results agreed with the theory which proved that all capri-poxviruses are genetically related and originated from the same lineage (Tulman et al., 2001). So cattle have been vaccinated with SPV will develop neutralizing antibodies to LSDV (Chihota et al., 2001). These results support the justified use of sheep pox virus vaccine for control of LSD and agree with the previous work of many authors (El-Kholy et al., 2008; El-Tholoth and El-Kenawy, 2016).

5. CONCULSION

From the previous results, it can be concluded that, the LSD is endemic disease in Egypt and causes severe economic losses in cattle industry. The obtained results explain that the LSD is widespread in different governorate in Egypt in all age and both sexes of the cattle all over the year by different percentage. Direct sequencing of PCR amplicons and comparative genetic analyses were useful for epidemiological studies and development of new efficient LSDV vaccines

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