Short Comunication

Role of Ixodid (Hard) Tick in the Transmission of Lumpy Skin Disease

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Abstract | The aim of this study is to investigate the potential role of ixodid (hard) ticks in the transmission of lumpy skin disease (LSD), which is an economically important disease of cattle and is caused by the LSD virus (LSDV). LSD is endemic in most countries of Africa and Middle East and can be transmitted either by mechanical as well as intrastadial and transstadial routes. Since capripoxviruses are serologically identical, their specific identification relies exclusively on the use of molecular tools. In this study, we analysed the G-protein-coupled chemokine receptor (GPCR) genes of two LSDV isolates from Ixodid (hard) ticks (Amblyomma hebraeum) in Egypt. Multiple alignments of the nucleotide sequences revealed that both isolates had nine nucleotide mutations in comparison with the local reference strain, LSDV-Egypt/89 Ismalia. Compared with the GPCR sequences of SPV and GPV strains, 21 nucleotide insertion and 12 nucleotides deletions were identified in the GPCR genes of our isolates and other LSDVs. The amino acid sequences of GPCR genes of our isolates contained the unique signature of LSDV (A11, T12, T34, S99 and P199). Phylogenetic analyses showed that the GPCR genes of LSDVs identified from ticks were closest genetically to the previously detected LSDVs from infected ruminants, indicating a potential role of Ixodid ticks for transmission of LSDV. This study showed the role of A. hebraeum ticks for transmission of LSDV. So, tick control is a crucial part, which should be included as a part of LSDV control measures in endemic countries.

Editor | Muhammad Munir, The Pirbright Institute, UK.
Received | February 15, 2017; **Accepted** | May 10, 2017; **Published** | June 25, 2017
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DOI | http://dx.doi.org/10.17582/journal.hv/2017/4.3.46.53
Citation | Hussein, H.A., O.M. Khattab, S.M. Aly and M.A. Rohaim. Role of ixodid (Hard) tick in the transmission of lumpy skin disease. *Hosts and Viruses*, 4(3): 46-53.
Keywords: Lumpy skin disease, Transmission, Ixodid, GPCR

Lumpy skin disease virus (LSDV) is a *Capripoxvirus* (CaPV) that belongs to the subfamily *Chordopoxvirinae* of *Poxviridae*, the largest of animal viruses (Murphy, 1999). The average size of LSDV is length 294±20 nm and width 262±22 nm (Kitching and Smale, 1986). LSDV genome is double-stranded DNA of 151 kbp. LSD is considered an Office International des Epizooties (OIE) - listed disease, has the potential for rapid spread and ability to cause severe economic losses (OIE, 2015). The disease is endemic in central, southern Africa and different Middle East countries while absent in Asia (Diallo and Viljoen 2007; Babiuk et al., 2008). LSD was first reported in Egypt in 1988 via cattle importation from Somalia (House et al., 1990; Ali et al., 1990). Recent LSD outbreaks were reported in 2006 after an apparent absence of 17 years most probably due to importation of infected cattle from the African Horn countries (El Kholy et al., 2008).

The possible introduction of new strains of LSDV by the uninterrupted movement of animals across bor-

ders is a major constant threat. Yet, in case of there is no history for introduction into the infected herds, the assumption of infection will be related to blood-feeding arthropods (flies and ticks) (Yeruham et al., 1994). Although many insect species are likely to be mechanical vectors of LSDV, no other clinical transmission trials on possible insect vectors of LSDV have been carried out. So, it is necessary to fully understand the role of different arthropod species in transmission of LSDV in order to effectively control the spread of the disease. Therefore, an important question still remain: does the virus replicate in tick cells? The vector capacity of hard ticks has recently been under intense investigation. Mechanical transmission of LSDV by male ticks was demonstrated (Tuppurainen et al., 2013b). Arthropod vectors are the main route of transmission of LSDV either by direct or indirect contact between infected animals (Carn and Kitching, 1995). Tick species identified as vectors for transmission of LSDV. Till now the method of lumpy skin disease virus transmission – a growing problem in herds in Africa and the Near East – has not been fully understood and mostly been associated with flying insects (Lubinga et al., 2014).

Rapid and specific diagnosis of the disease as well as rapid implementation of control measures is very important to control the transboundary transmission and spread of the disease (Carn, 1993). PCR-based diagnosis is superior to other techniques in terms of sensitivity and speed (Mercer et al., 2007). The key objective of this study is to detect LSDV from ticks collected over the skin of clinically infected cattle and water buffalo based on molecular basis for the G-protein coupled chemokine receptor (GPCR) gene, for host range phylogeny of CaPVs that will support the host-range discrimination. The complete nucleotide sequences of the CaPV genomes are 97% identical to each other. The CaPV homologue of G-protein-coupled chemokine receptor (GPCR) gene may play a role in the cell proliferative lesions and immunosuppression induced by CaPV infections. It was previously shown to be one of the most variable genes within the CaPVs (Tulman et al., 2002).

In the present study, two pools (3 ticks/ each) adult male Ixodid (hard) tick (A. hebraeum) samples were collected over the skin of infected cattle and water buffalo belonging to a herd in a village in the Nile delta (Sharkia governorate) during the 2014 LSD outbreak in Egypt. Ticks were washed three times to reduce possible surface contamination by the virus, thereby increasing the confidence that the virus detected passed through the larval tissues. Madin Darby Bovine Kidney (MDBK) cell line was used for virus isolation (OIE, 2015). Extraction of DNA from tick homogenates was based on the protocol used by Tuppurainen et al. (2005). Proteins were digested by adding 25ul of proteinase K (Vivantis, Malaysia) to samples followed by incubation at 56° C for overnight (tick homogenate). Genomic DNA was extracted and purified by GF1- tissue DNA extraction kit (Vivantis, Malaysia). The entire GPCR gene was amplified using the designed primers to amplify nucleotide 6961–8119 of the genome, (Le Goff et al. 2009). Two additional primers were positioned internally for sequencing (Le Goff et al., 2009). All primers were synthesized by Metabion International AG (Germany). PCR was carried out using the DreamTaq Green PCR Master Mix (2X) (Thermo Scientific, USA) according to the manufacturing instructions.

PCR products were directly sequenced in both orientations by the dideoxy chain-termination method using the amplification primers described above. The nucleotide and amino acid sequences of this gene were aligned using Bioedit (Hall, 1999) and BLAST 2.0 search program (National center for Biotechnology Information (NCBI) (Altschul et al., 1997). Phylogenetic analysis was carried out by means of the neighbour-joining method (Saitou and Nei, 1987). Dissimilarities and edge length of dissimilarities between the sequences were first determined with Bioedit software (Hall, 1999). Tree construction was based on the unweighted neighbour-joining method proposed by Gascuel (1997). Trees were generated with the MEGA 5 program (Tamura et al., 2013).

The culturing of the tick homogenates in MDBK cell culture for three passages was not enough to induce observed CPE in this study. Conventional PCR were used for confirmative the potential role of Ixodid ticks for transmission of LSDVs. Viral DNAs were detected in the two isolates by PCR that indicates that PCR could serve as a rapid, effective and specific method for laboratory confirmation of CaPVs.

The obtained nucleotide sequences of the full length GPCR gene of LSDVs reported in this study revealed an open reading frame (ORF) of 1135 bp. The sequences were aligned with the GPCR gene sequences of CaPVs available in the GeneBank using Clus tal-

Figure 1: *The multiple alignments of first 115 nucleotides of the obtained nucleotide sequences of LSDV G-protein-coupled chemokine receptor genes of LSDVs detected from ticks along with sequences of published CaPVs on GeneBank. The figure shows addition of a 21 nucleotide fragment (from position 31 to 51) and deletion of a 12 nucleotide fragment (from position 87 to 98) in LSDVs when compared with sequences of sheep poxvirus and goat poxvirus.*

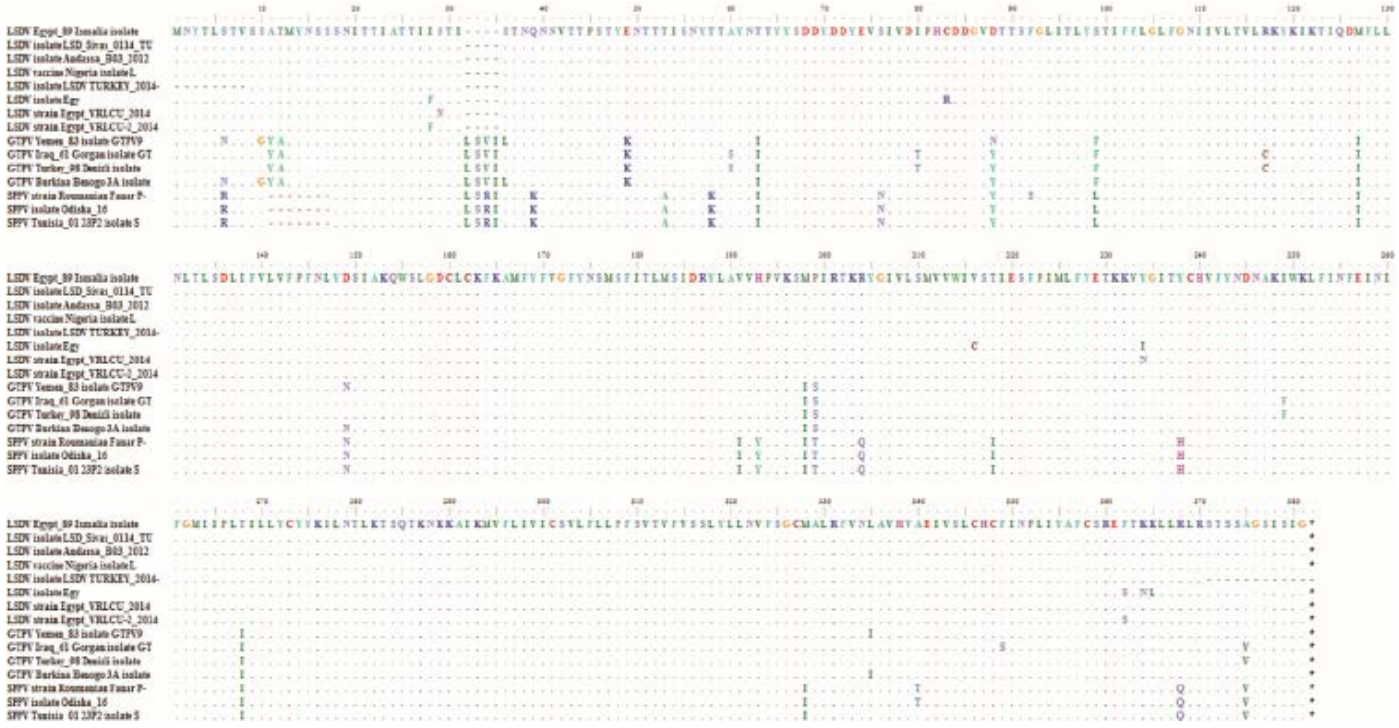

Figure 2: *The multiple alignments of the deduced amino acids of G-protein-coupled chemokine receptor gene of LSDV isolates from ticks along with sequences of reference LSDVs retrieved from GeneBank show the unique signature of LSDV (A11, T12, T34, S99 and P199).*

W2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The multiple alignments of the nucleotides sequencesrevealed that MF156212 Egypt_VRLCU-2_2014 differed from MF156211 Egypt_VRLCU_2014 isolate at four positions: T82A, C241T, C543A and C1085T, while tissue culture-adapted Egypt_89 Ismalia strain differed from MF156212 Egypt_VRLCU-2_2014 isolate at four positions; T82A, T111A, C241T and C1085T and three position difference; A86G, T111A, A700T with MF156211 Egypt_VRLCU_2014 isolate (Figure 2). The multiple alignments of the deduced amino acids of G-protein-coupled chemokine receptor gene of LSDV isolates in this study along with sequences of reference revealed that the unique signature of LSDV (A11, T12, T34, S99 and P199) (Figure 2).

The phylogenetic tree from the alignment of the sequenced viruses and references CaPVs available in GeneBank was constructed. Phylogeny of CaPVs

based on the alignment of the nucleotide sequences of the GPCR genes revealed that three closely related genetic clusters consisting of LSDVs, GPVs and SPVs lineages (Figure 3). Our two LSDV isolates were segregated into LSDV lineage and were closest genetically. It appeared that LSDV and GPV were more genetically related to each other than to SPV. The phylogenetic analysis did not reveal distinct distance in the diversity between the vaccine and virulent strains of LSDV (Egypt_89 Ismalia strain) (Figure 3).

The natural hosts for capripoxviruses (CaPVs) are ruminants, including cattle, sheep and goats. CaPVs are subdivided into three virus species according to their host origins: sheep poxvirus (SPPV), goat poxvirus (GTPV) and lumpy skin disease virus (LSDV) of cattle. CaPVs are generally considered to be host-specific, leading to outbreaks in one preferred host. This is partially true since some SPPV and GTPV isolates are capable of causing severe diseases in both sheep and goats (Kitching et al., 1989). Although, CaPVs are antigenically closely related; restriction enzyme pattern analysis, cross-hybridization studies and, more recently, nucleic acid sequencing have shown that nearly all CaPVs can be grouped according to their host origins (Kitching et al., 1989, Tulman et al., 2002).

Transmission of LSDV may occur either mechanically by mouth parts or intrastadially – if the virus survives in the salivary gland. Nuttal et al. (1994) showed that the main route of virus transmitted by infected ticks via saliva secreted during feeding of ticks. Blood meal of ticks occur in the last 24 hours before detached from host , therefore, the virus move from digestion of blood in the midgut. This allow the virus to be transmitted easily in the tick cell (Sonenshine, 1991). Therefore, the aim of the present study was to detect and molecular characterize LSDV in Ticks collected from cattle and water buffalo in Sharkia governorate demonstrating typical lesions of LSDV based on PCR, sequencing and phylogeny.

The ticks in this study were collected over infected animals with LSDV typical lesions, it is not surprising to detect the virus in ticks as in previous studies they verified the survival of LSDV in ticks even in the absence of disease symptoms (Tuppranine et al., 2011). Also, Ticks feed on skin lesion in viremic cattle were found positive for the presence of viral DNA of LSD when tested by PCR (Tuppranine et al., 2005). Taken together, it was expected to detect LSDV in ticks and this could represents the main source of mechanical transmission of LSDV. As we did homogenation of the ticks' tissue, we are not able to determine the origin of the virus from either the outer surface of ticks or the damaged tissue and or blood of ticks. However, multiplication of the virus in tick tissue is not confirmed, DNA extracted from the ticks may be of live or dead virus particles. Although, the number of samples used in this study was limited, the study indicates the important role of ticks in transmission of LSDV.

The significant of the present study in detection of the virus in ticks from field samples indicating the transmission of LSDV in ticks. The culturing of the tick homogenates in MDBK cell culture for three passages was not enough to induce observed CPE in this study. Several reason for such observation: first, the samples may need further cell culture passages. Second the physiological stage in ticks where discrepancies in virus titers (Lubinga et al., 2014). Likewise, the survival of LSDV in ticks depends on susceptibility of tick organs to infection that not undergo histolysis (Labuda and Nuttall, 2004). Hence, it could be possible that the virus detected in the ticks was dead. Previous studies have shown variations in genetic determinants important for virus replication between ticks and mammalian hosts (Mitzel et al., 2008). The infection rate of LSDV in ticks was 100% (Kaufman and Nuttal, 2003; Lubinga et al., 2014). Therefore, we expect that ticks is the source of virus infection in the present study. Still, the geographical distribution of LSD differs markedly from that of sheep and goat pox, which tend to coexist over most of their distribution range. Likewise, the exact pattern of circulation of CaPVs between cattle, sheep and goats remains still need more studies and clarifications that has long been hampered by the lack of differential identification tools.

Recent studies have shown that the three CaPVs can be distinguished genetically (Le Goff et al., 2005; Tulman et al., 2002). The Q2/3L gene, which encodes a homologue of a GPCR (Glycoprotein Chemokine Receptor) (Tulman et al., 2001), known to be a single copy gene located in the left terminus of the genome,is likely to affect the virus virulence (Tulman et al., 2002; Kara et al., 2003). The GPCR gene is one of the most variable genes within CaPVs that originally acquired from their host and adapted them for their viral benefits for control of the host antiviral responses

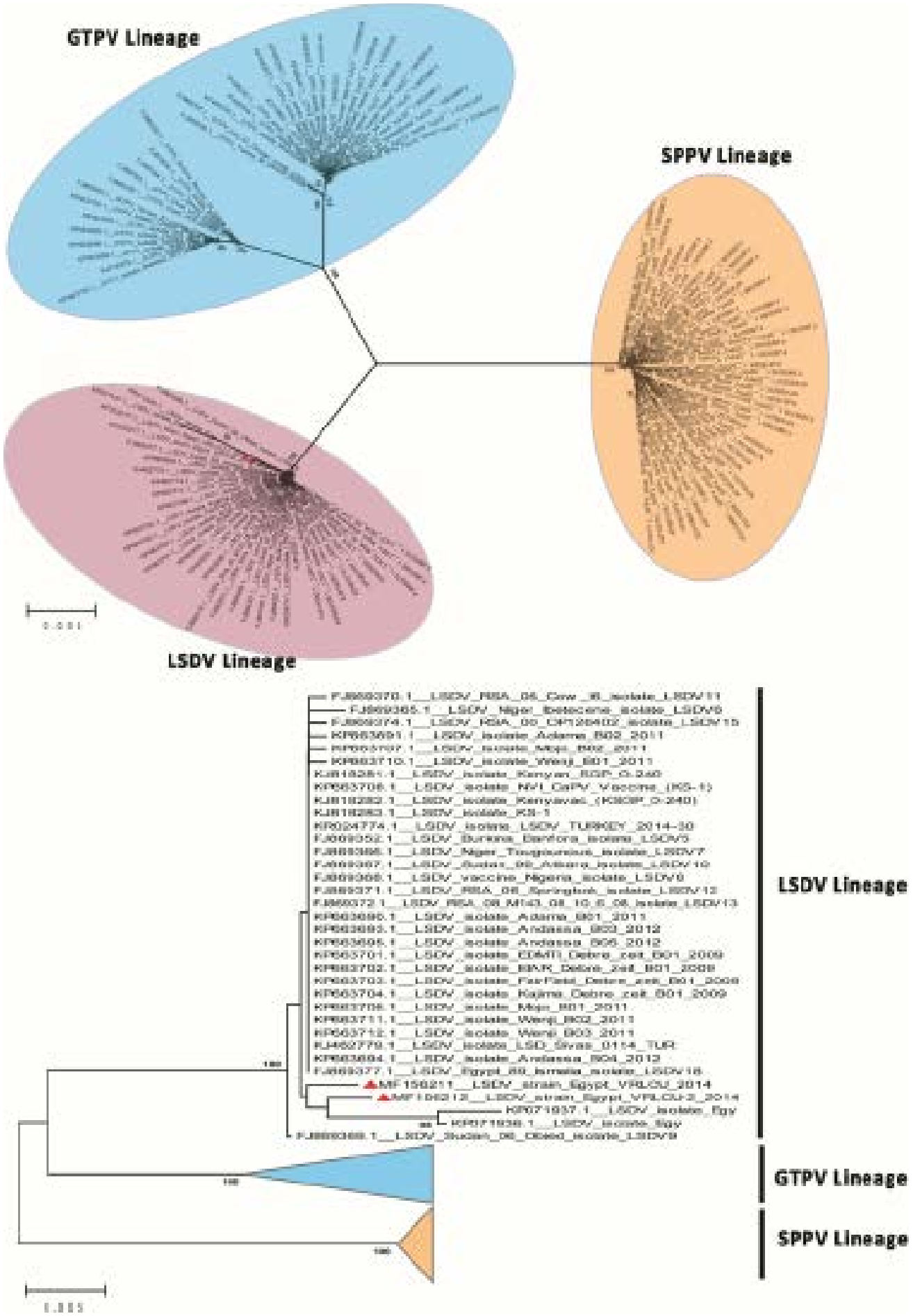

Figure 3: *The phylogenetic tree based on G-protein-coupled chemokine receptor (GPCR) nucleotides sequences of LSDV isolates from ticks with other CaPVs which retrieved from the GeneBank database.*

and may play a role in the cell proliferation lesions and immunosuppression induced by CaPVs (Tulman et al., 2002; Kara et al., 2003; Le Goff et al., 2009). The GPCR gene LSDV isolates collected from ticks over infected cattle and water buffalo as a host discriminative gene were amplified by PCR followed by sequencing and phylogeny.

During the course of GPCRs evolution, herpes- and poxviruses have probably acquired their chemokine receptor genes from their hosts. Although still largely unknown, the pathogenic effects of such virally encoded GPCRs may include increased cell trafficking and proliferation, cell lysis, and cytokine down regulation (Rosenkilde et al., 2008). Because it was previously shown to be one of the most variable genes within the CaPVs (Tulman et al., 2002), we supposed that this gene would be a suitable target for genetic discrimination between ruminant poxviruses. Phylogeny on this gene reported here confirms that the CaPVs can be divided into three distinct lineages; GTPV, SPPV and LSDV (Tulman et al., 2002) where our isolates are related to LSDV lineage.

In conclusion, this study highlights the significant of hard ticks in the transmission of LSDV. Ticks act as reservoirs for LSDV, as the virus can persist in these external parasites during periods between epidemics. This disease is of economic importance due to the damage it can cause to the skin, the reduced milk and meat production and lowered fertility of cattle. Ticks consider an important component of lumpy skin disease control to ensure that these parasites do not contribute to the spread of the virus to other parts of the world. The change of climate due to global warming is making it possible for ticks to successfully survive and may be able to transmit the virus, and this may require a series of approaches to control, such as the aerial and ground application of insecticides; and treatment of cattle with either a systemic insecticide, or a topical insecticide that will repel insects and or reduce the population of target insects as well as housing for animals might also be considered.

Acknowledgements

The authors would like to acknowledge the support and cooperation provided by all staff of the Virology Department, Faculty of Veterinary Medicine, Cairo University whenever it is needed.

Competing Interests

The authors declare that they have no conflict of interest.

Authors' Contributions

Conceptualization: H.A. Hussein, O.M.Khattab, S.M. Aly and M.A. Rohaim.Data curation: H.A. Hussein and M.A. Rohaim. Formal analysis: H.A. Hussein and M.A. Rohaim. Investigation: H.A.Hussein, O.M.Khattab and S.M. Aly. Methodology: O.M.Khattab, S.M.Aly and M.A.Rohaim. Software: M.A. Rohaim. Supervision: H.A.Hussein and M.A.Rohaim. Validation H.A.Hussein. Writing – original draft: O.M.Khattab, S.M. Aly, and M.A.Rohaim. Writing – review& editing: H.A.Hussein and M.A.Rohaim.

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