Molecular differentiation between field lumpy skin disease isolate and *Capripoxvirus* vaccinal strains in Egypt 2018

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Abstract

Lumpy skin disease virus is a member of the *Capripoxvirus* genus of the *Poxviridae* family, which affects cattle and causes a notifiable disease with significant economic losses. It is controlled by vaccination with capripox live attenuated vaccines. The aim of the study was the isolation and identification of the lumpy skin virus field virus strain during 2018. Nodular skin lesions were collected from clinically infected lumpy skin disease cattle that were used for the virus isolation on the chorioallantoic membrane of specific pathogen free embryonated chicken eggs and Madin Darby Bovine Kidney tissue culture. Polymerase chain reaction targeting the *Capripoxvirus* CaPV ORF103 gene was applied on the isolated virus and three *Capripoxvirus* vaccinal strains (Kenyan sheep pox virus, Held goat pox virus and Ismailia lumpy skin disease virus). The amplicons of the four strains of *Capripoxvirus* (one isolated and three vaccinal strains) were used for sequencing. Reference capripox viruses were obtained from GenBank to create the phylogenetic tree. The virus isolated from the collected nodular skin samples on chicken eggs showed clear typical pock lesions on the chorioallantoic membrane and on tissue cultures and showed a characteristic cytopathic effect. Positive samples of the isolated strain were identified by PCR for the CaPV ORF103 gene that yielded expected amplicon sizes of 570 bp. This was confirmed through gene sequence and analysed by BLAST, and submitted to GenBank under accession number MW 546997_LSD_Aziz_LSD. The phylogenetic tree revealed that the field isolate strain of LSDV had different identity percentages ranging from 98.2–99.8% with the tested vaccinal *Capripoxvirus* strains in Egypt. The amino acid sequence showed only different amino acid found in the field isolate strain and not in other tested vaccinal strains, and a maximum homology (100%) of the isolated strain nucleotide sequence was with two GenBank recorded strains. We recommend maintaining

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the routine lumpy skin disease vaccination programme in Egypt, frequent eradication of the insect population, and further genetic studies on the genomes of this virus strain and the *Capripoxvirus* vaccinal strains to reach the most related and homologous vaccinal strain given the massive genome of this disease.

Key words: *molecular differentiation; phylogenetic analysis; lumpy skin disease; Capripoxvirus; orf103 gene*

Introduction

Lumpy skin disease (LSD) is a list A disease of the World Organisation for Animal Health (OIE). It is a viral skin disease in cattle that is also known as pseudo-urticaria, bovine nodular exanthema, Neethling and knopvelsiek. LSD inflicts high morbidity, leading to substantial economic losses resulting from decreased milk yield, weak body weight gain, abortion, infertility, decline in hide quality, and death of young cattle in Africa, Asia, the Middle East, Russia, and Europe (Tageldin et al., 2014; Abutarbush et al., 2015, 2016; Tasioudi et al., 2016; Sprygin et al., 2018 a.b).

Lumpy skin disease virus (LSDV) is a *Capripoxvirus* belonging to the *Poxviridae* family. It is one of the largest viruses, with a double-stranded DNA genome approximately 150,000 base pairs (bp) long (Tulman et al., 2001). The Capripox virus is one of eight genera within the *Choradopox virus* subfamily of the *Poxviridae* (Kitching and Taylor, 1985).

Moreover, LSDV shares over 97% sequence identity with viruses of the Capripoxvirus genus, including the sheep and goat pox viruses (Gershon and Black, 1988; Gershon et al., 1989; McLysaght et al., 2003; Hughes and Friedman, 2005).

LSDV diagnosis is reached by visual observation of clinical signs, and performance of virus isolation, electron microscopy, histopathological examination, serological, and molecular identification techniques (OIE, 2019).

The characteristic clinical signs have been described in detail (Weiss, 1968; Prozesky and Barnard, 1982; Babiuk et al., 2008; Salib and Osman, 2011). In the majority, evidence of infection starts with lacrimation and fever (40–41°C), though cases may be non-febrile. Subscapular and precrural lymph nodes become noticeably enlarged. After fever, skin nodules (1–5 cm in diameter) appear in numbers varying from few to multiple covering the animal, with systemic affects included pyrexia, anorexia and pneumonia (Davies, 1991). In severe cases, ulcerative lesions in the mucous membranes cause excessive salivation, lacrimation and nasal discharges. Pox lesions may also be present in the pharynx, larynx, trachea, lungs and throughout the alimentary tract. In post-mortem examination of severe cases, pox lesions may be present on the surface of almost all organs (Babiuk et al., 2008). Oedematous swelling of one or more legs may develop in some infected animals that is associated with lameness. The incubation period in nature is estimated to be between 1 and 4 weeks (Coetzer, 2004).

In LSD outbreaks, the morbidity rate varies from 3% to 85% depending on the immunological status of the hosts and the abundance of mechanical arthropod vectors (Prozesky and Barnard, 1982; Hamoda et al., 2002; Buller et al., 2005).

Vaccination against LSD is an important process for maintaining animal health and farm economy. Either homologous vaccines consisting of live attenuated LSD virus or heterologous vaccines consisting of live attenuated sheep pox or goat pox virus can be used for the control of LSDV. While heterologous vaccines exhibit lower

efficacy than homologous vaccines, they do not cause post-vaccinal symptoms of the disease following vaccination, which can be caused by the replication capacity of live attenuated LSDVs (Christine et al., 2014; Tuppurainen et al., 2017; Sprygin et al., 2020). The EU and Balkan countries have opted for the attenuated Neethling vaccines, whereas Turkey, Georgia, and Russia resorted to SPPV vaccines that establish cross-protection against LSDV (Tuppurainen et al., 2018). The choice of vaccine is complicated by conflicting findings of their safety, efficacy, and transmission capacity (Bedeković et al., 2018; Calistri et al., 2019).

The LSD virus is genetically and antigenically related to the sheep pox virus, so that sheep pox vaccines were recommended for LSD control (Kitching, 2003; Bhanuprakash et al., 2006). In Egypt, the control of LSD among cattle depends on the vaccination programmes by using a heterologous cross-reacting sheep pox virus vaccine which is antigenically related to LSD virus, and which induces reasonable immunity against LSD in cattle (Michael et al., 1994).

Seasonal occurrences of LSD outbreak have been recorded, and were thought to be caused by the introduction of more virulent strain of LSD virus, sheep pox vaccination failure in cattle and dense insect populations (Chihota et al., 2003). Insects play a very important role in the transmission of LSD virus (Davies, 1981).

Bull et al. (2018) studied the replication competence of live vaccines and found that the risk of reinfection and subsequent recombination are low for these poxvirus vaccines, making live vaccines the preferred choice in most countries. Recombination in DNA viruses is not rare (Lee et al., 2012).

However, since detection of Neethling vaccine isolates is exempted from the World Organization for Animal Health notification (OIE, 2019), the actual situation of LSDV circulation worldwide remains heavily underrepresented. Sprygin et al. (2020) reported that the policy of using certain live vaccines requires revision in the context of the biosafety threat of its occurrence, due to the appearance of a novel LSD virus strain which was not genetically linked to the previous field strain.

Diagnosis of LSD is of a crucial role in the implementation of prevention measures. LSD diagnosis can be based on clinical signs. However, unapparent and mild forms require available and rapid laboratory assay to confirm diagnosis. Laboratory detection of LSD could be carried out by using virus identification based on PCR tests developed for more accurate and rapid detection of LSDV in appropriate specimens (Khalefa et al., 2015). An accurate polyvalent molecular tool for LSDV detection is required and further investigations using the positive LSDV DNA samples can be supplemented by sequencing to clarify the genetic background of viral strains (Alexander et al., 2019).

Polymerase chain reaction (PCR) is a rapid, sensitive and specific technique for the detection and differentiation of capripox viruses based on the open reading frame (ORF) 103 gene. It has previously been used for genotyping and phylogenetic analysis (Zhu et al., 2013; Abd-El Fatah et al., 2018). PCR is considered the perfect tool for identifying and differentiation of *Capripoxvirus* strains by using several genes (E10R, RPO 132, P32, ORF 095, RPO 30 and ORF 103) that have been expressed as powerful tools for identifying of SPPV (Zhou et al., 2012; Zhu et al., 2013)

The aim of the study was to isolate and identify the field virus strain of LSD during 2018. Molecular characterization of the isolated field LSD virus strain by PCR, sequencing the PCR product of the field LSD virus strain and vaccinal strains of capripox viruses in Egypt, and determining the identity of the

field LSD virus strain in addition to the *Capripoxviruses* vaccine strains were tested by phylogenetic analysis and amino acid sequencing.

Materials and methods

Animals

Cattle clinically infected with LSD were examined in the Giza governorate, Egypt during 2018. Clinical examination was based on measuring body temperature and examining skin and superficial lymph nodes. Clinical examinations, observations of lesions and complications for all suspected animals were concentrated on physical stature, body temperature, superficial lymph nodes and skin lesions (Salib and Osman, 2011).

Ethical approval

Sample collection was performed according to standard procedures without any stress or harm to animals (European Union, 2010). All laboratory works were conducted at the Agriculture Research Center (ARC), Central Laboratory for Evaluation of Veterinary Biologics (CLEVP), Cairo, Egypt.

Sample collection and preparation

During 2018, fifteen nodular skin lesions were collected from cattle showing LSD infection symptoms in the Giza governorate. Samples were transferred aseptically to the laboratory in a transport medium (PBS) containing antibiotics under chilled conditions and stored at -20°C until LSD virus isolation.

For virus isolation, the nodular skin lesion samples were minced separately using sterile scissors and forceps and then were ground under sterile condition with a pestle in a mortar containing sterile sand. Ten millilitres of PBS containing gentamycin (0.1 mg/mL), ampicillin (0.05 mg/mL) and fungistatine were added. The suspensions were left to stand overnight at 4°C. The samples were partially clarified by centrifugation at 2000 rpm for 3–5 min to remove gross particles and the supernatants were taken for the LSD virus isolation (OIE, 2019). The prepared sample supernatants were used for LSD virus isolation and identification.

Field LSD virus Isolation

1. SPF Embryonated chicken eggs (ECEs)

From Koum Oshiem SPF, Fayoum, Egypt specific pathogen free (SPF) embryonated chicken eggs (ECE), 9–11 days of age, were inoculated via the chorioallantoic membrane route (CAM) with the prepared sample supernatants (House et al., 1990). The ECEs were examined after 7 days and 10 days for the detection of the pock lesions specific to the LSD virus, and CAMs showing pock lesions were collected.

2. Madin Darby Bovine Kidney (MDBK) cell line

The MDBK cell line obtained from CLEVB was propagated with Eagle's minimum essential medium (EMEM) and supplemented with foetal bovine serum, were used for virus isolation from the prepared samples supernatants according to Irons (2009) and OIE (2019), where 0.5 ml of the supernatant was inoculated into the monolayer of MDBK cell culture. Cultures were observed daily until appearance of the cytopathic effect (CPE) that is specific to the LSD virus.

Capripox viruses vaccinal strains

Lumpy skin disease virus (LSDV)

The virus was supplied from the pox department, Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt. The Ismailia strain was isolated from Egypt during the 1988 outbreak (House et al., 1990). The virus was adapted in Madin-Darby bovine kidney cells (MDBK) (Daoud et al., 1998).

Kenyan sheep pox virus (KSPV)

The virus was supplied from the Foreign Animal Disease Diagnostic Laboratory (FADDL), Plum Island, New York, USA. It was passaged on foetal bovine lung for successive passage then adapted on the vero cell (African Green Monkey Kidney Cell) in Egypt (Rizkallah, 1994).

Held goat pox virus (HGP)

The virus was also supplied from FADDL (Plum Island, New York, USA). It was passaged two times on lamb testicle cells and for one passage on sheep choroid plexus cells. In Egypt, virus adaptation was completed for another sixteen passages on lamb testicle cells and for fourteen passages in Vero cells (Olfat, 2000).

Nucleic acid extraction

Whole nucleic acid extractions (Sambrook et al., 1989) from field isolated LSD virus and the three capripox viruses vaccinal strains (infected tissue culture samples) were performed using the QIAamp minielute virus spin kit (Qiagen, Germany, GmbH). Briefly, 200 µl sample suspensions was incubated with 25 µl Qiagen protease and 200 µl AL lysis buffer at 56°C for 15 min. After incubation, 250 µL 100% ethanol was added to the lysate. The samples were then washed and centrifuged following the manufacturer's recommendations. Nucleic acids were eluted with 100 µl elution buffer (Yousif et al., 2010).

Oligonucleotide Primers and Thermocycling

The Capripox virus ORF 103 gene specific primer pair was supplied from Metabion (Germany) and is listed in Table 1 (Zhu et al., 2013).

Polymerase chain reaction
PCR amplification

The extracted nucleic acid samples were amplified by separate PCR reactions using the primer pair of capripox ORF 103 in a 25 µL reaction containing 12.5 µL EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µL each primer at a concentration of 20 pmol, 4.5 µL water, and 6 µL DNA template. The reactions were performed in an Applied biosystem 2720 thermal cycler (Zhu et al., 2013).

Analysis of PCR Products

The PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µL product was loaded in each gel slot. A gene ruler 100 bp DNA ladder (Fermentas, Thermo, Germany) was used to determine fragment sizes. The gel was

Table 1. Primer sequences, target genes, amplicon sizes and cycling conditions

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension
				Secondary denaturation	Annealing	Extension	
Capripox ORF 103	5`-ATGTCTGATAAAAAATTATCTCG-3` 5`-ATCCATACCATCGTCGATAG-3`	570	94°C 5 min.	94°C 30 sec.	52°C 40 sec.	72°C 45 sec.	72°C 10 min.

photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analysed using computer software.

DNA sequencing

PCR products were purified using the QIAquick PCR Product extraction kit (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by the Applied Biosystems 3130 genetic analyser (HITACHI, Japan). The obtained sequences were analysed using the online Basic Local Alignment Search Tool analysis (BLAST®) (Altschul et al., 1990) that was initially performed to establish sequence identity to GenBank accessions and compared with those of capripox viruses available in the GenBank database. Nucleotide sequencesobtained in this study designated as Is-LSD (Ismalia strain LSDV), Aziz_LSD, KSV (Kenyan sheep poxvirus) and HGV (Held goat poxvirus) were deposited in GenBank database under accession numbers MW546996-MW546999.

Phylogenetic analysis

The phylogenetic tree derived from the nucleotide sequences deduced was constructed for the capripox viruses and was created by the MegAlign module of Lasergene DNA Star version 12.1 (Thompson et al., 1994). Phylogenetic analyses were performed using maximum likelihood, neighbour joining and maximum parsimony in MEGA6 (Tamura et al., 2013).

Results

Clinical findings

The LSD virus infected cattle (Figure 1) suffered from skin lumps, enlarged lymph nodes nodular skin lesions and emaciation.

Virus isolation

The isolated field LSD virus grown on CAM of ECE at passage 4 showing pock lesions with CAM thickening and haemorrhage (Figure-2a) and on MDBK cell line at passage 3 showing cytopathic effects characterized by cells coalesced together, forming clusters (Figure 2b).

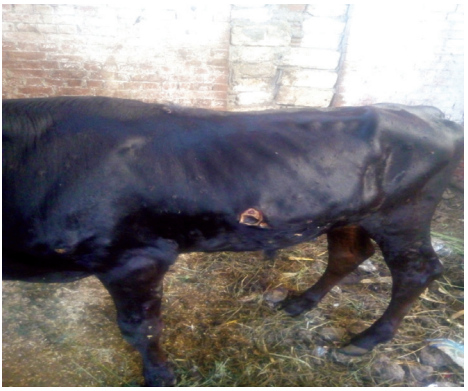

Figure 1. LSD virus infected cattle suffered from skin lumps, enlarged pre-femoral lymph node, emaciation and sit-fast lesions

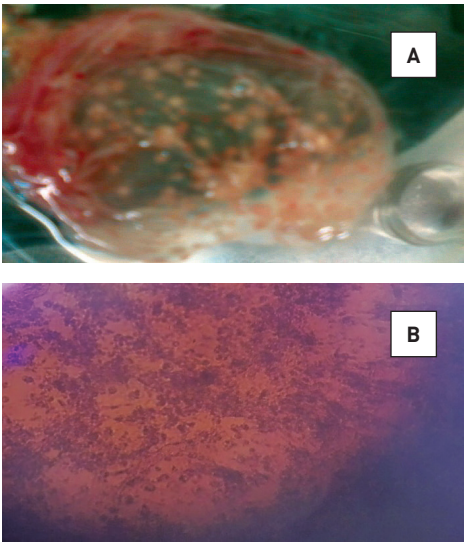

Figure 2. A. Pock lesions on CAM of ECE on day 7 post-inoculation for the field LSD isolate. **B.** Cytopathic effect of field LSD isolate on MDBK cell line on day 10 post-inoculation

Polymerase chain reaction

Identification of nucleic acids in harvested virus solution from the MDBK tissue culture was conducted using conventional PCR of all samples. Characteristic 570 bp fragments of capripox virus strains ORF103 gene from the field LSD virus isolate and three selected *Capripoxvirus* vaccinal strains were amplified and run. The strong running bands were produced and detected by Ethidium Bromide-stained Agarose Gel, as shown in Figure 3.

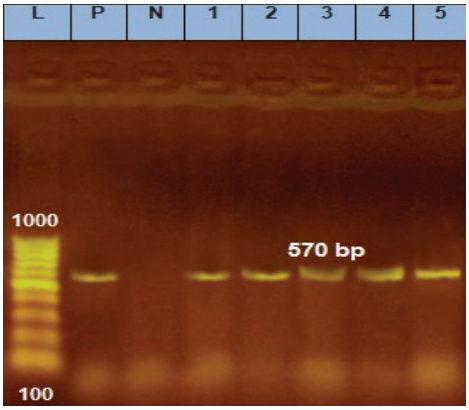

Figure 3. PCR products of the capripox ORF 103 gene at 570 bp length DNA band of capripox virus.
Lane (L): ladder (100-1000 bp)
Lane (P): positive control
Lane (N): negative control
Lane (1): LSD virus Ismailia strain
Lane (2): field LSD virus isolate
Lane (3): Kenyan sheep pox virus
Lane (4): Held goat pox virus

Sequencing and construction of the phylogenetic tree

For further confirmation, PCR products were purified and used for sequencing. The DNA sequence was obtained and analysed, and was initially performed to establish GenBank accessions, recording sequence identity and comparison of capripox viruses available in the GenBank database, as shown in Figure 4.

Deduced amino acid sequence distance of the ORF103 gene of the tested Capripox strains (generated by lasergene

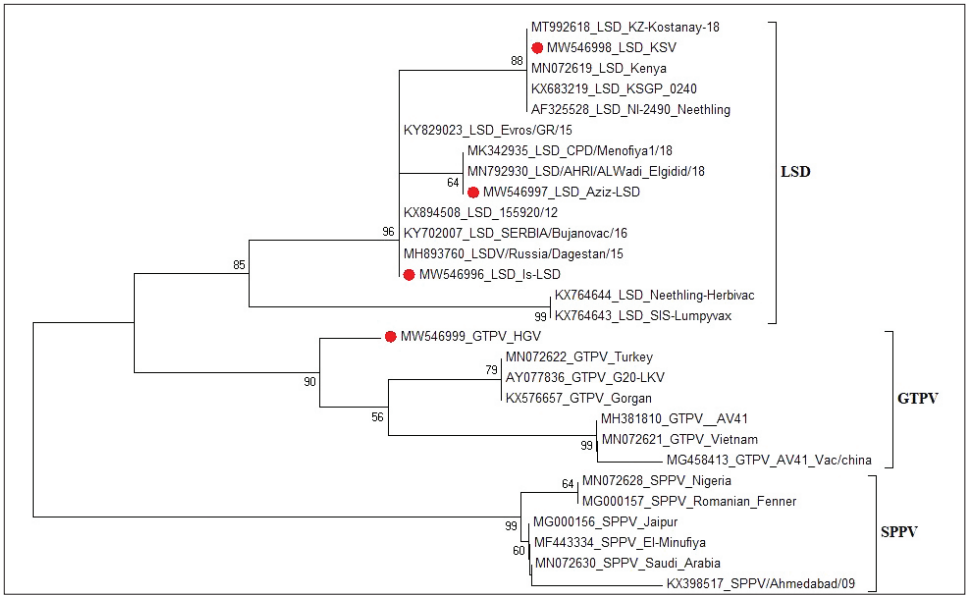

Figure 4. Phylogenetic relatedness of the ORF103 gene. Maximum-likelihood unrooted tree generated after 500 bootstraps indicated clustering of the tested strain with Capripox strains

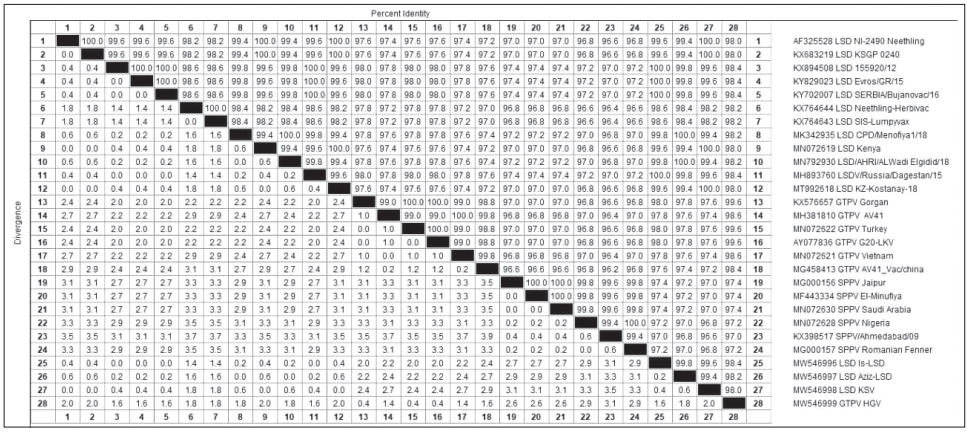

Figure 5. Deduced amino acid sequence distance of the ORF103 gene of the tested Capripox strains (generated by lasergene software) showing an identity range of 96.2–100% with the isolated LSD strain

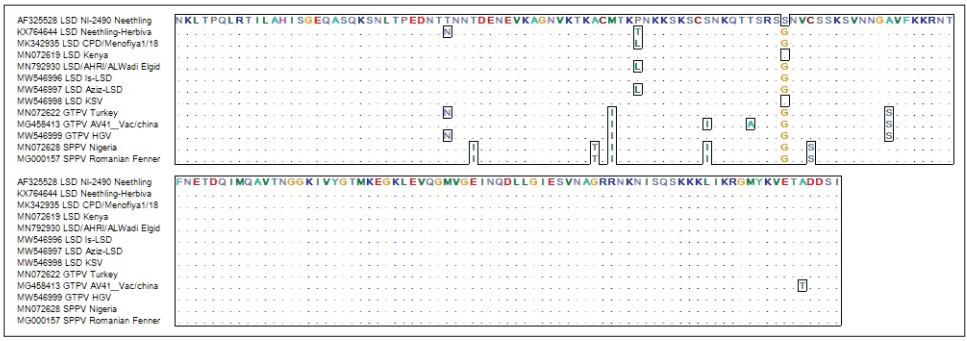

Figure 6. Deduced amino acid coloured alignment report for the 13 studied strains

software) showing an identity range of 96.6–100% with the isolated LSD virus strain, as presented in Figure 5.

Nucleotide alignment of the 13 analysed strains showed the relations between the current study strains and international Capripox viruses strains, as illustrated in Figure 6. The amino acid sequence showed that only one different amino acid was found in the field isolate strain and not in other tested vaccinal strains. The maximum homology (100%) of isolated LSD virus strain nucleotide sequence was with two GenBank recorded strains (MK342935_LSD-CPD/Menofiya1/18) and (MN792930_LSD/AHRI/_Wadi_Elgdid/18).

Nucleotide and deduced amino acid alignments of the analysed strains showing the relation between current study strains and international Capripox strains

Discussion

Control and eradication of Lumpy skin disease depend on accurate diagnosis and vaccination**.** The diagnosis of LSDV is based on the characteristic clinical signs, virus isolation, electron microscopy, histopathological examination, serological and molecular identification techniques (OIE, 2019).

Christine et al. (2014), reported that vaccines include homologous live

attenuated LSD vaccines containing Neethling strain, as used in South Africa and Namibia, and heterologous live attenuated Sheep pox vaccines, as used in Egypt, Jordan, certain Middle East and African countries.

As shown in Fig. 1, the recorded signs were skin nodules, nodular skin lesions, enlarged lymph nodes and emaciation, which agree with reports by Tuppurainen et al. (2005) that LSD is an acute to chronic viral disease of cattle characterized by skin nodules that may have inverted conical necrosis (nodular skin lesions) with lymphadenitis accompanied by persistent fever. Salib and Osman (2011) observed that the clinical forms of LSD were recorded among Egyptian cattle from mild to severe. Furthermore, skin nodules had congestion, haemorrhage, oedema, and vasculitis with consequent necrosis and involve all layers of the epidermis, dermis, subcutaneous tissue, and often adjacent musculature. Lymph nodes draining the affected areas were enlarged up to 10 times the normal size, with extensive lymphoid proliferation, oedema, congestion, and haemorrhage (Prozesky and Barnard, 1982).

The field LSD virus strain was isolated from the nodular skin lesions of infected cattle on CAM of SPF-ECE 9-11 days old which passaged till the characteristic pock lesions that were observed with mild inflammatory signs in the form of thickening, oedema and haemorrhage of the membrane after the 2nd passage, becoming clear at the 4th passage (Fig. 2A). These findings agree with the literature (House et al., 1990; Tamam, 2006; Gamil et al., 2019) reporting successful cultivation of LSDV on CAM of ECE.

Also, MDBK cell culture showed characteristic cytopathic effect (Fig. 2B). CPE was characterized by cell rounding, cell aggregation and coalesced together forming clusters at the 3rd passage. These findings agree with the literature reports of LSDV isolation from skin biopsies on MDBK cell culture (Davies, 1991; Ibrahim, 1999; Fahmy, 2000).

Virus isolation is considered the most important step for diagnosis, though this is time-consuming and requires several blind passages (Joshi et al., 1996; Oguzoglu et al., 2006).

In this study, we identified the field LSD virus and three capripox virus vaccinal strains in positive cell cultures using PCR by primers that bind and amplify the partial ORF 103 gene. The results (Fig. 3) showed the presence of fragments (570 bp) on gel electrophoresis for the four strains. These results corroborate the literature reports (Tulman et al., 2001; El-Nahas et al., 2011; Sharawi and Abd El-Rahim, 2011; Zhu et al., 2013; El-Khabaz, 2014; Sohair and Gaafar, 2016; Khameis et al., 2018; Hala et al., 2021).

For further identification and confirmation of the identity of the detected Capripox virus strains, PCR amplified products were purified and used for sequence reaction. The entire sequence of the ORF 103 gene was analysed by a BLAST analysis that was initially performed to establish sequence identity to the GenBank accessions. The locally isolated field LSDV was submitted to GenBank under accession number MW 546997_LSD_Aziz_LSD.

The phylogenetic tree of the different Capripoxvirus strains was created based on the deduced nucleotide sequence of the ORF 103 gene of different Capripoxvirus strains. The phylogenetic relationship (Fig. 4) and Sequence distance of the ORF103 gene showed a high identity overlap of the field isolated LSD virus (Aziz_LSD) with the tested vaccinal strains: 99.8% with the Ismalia strain, 99.4% with the Kenyan sheep pox virus, and 98.2% with the goat pox virus. The overlap with other reference strains from the GenBank ranged from 96.2% to 100%, revealing that local isolates of LSDV are highly related not only to other LSDV strains, but also to other *Capripoxviruses*.

Maximum homology (100%) of isolated LSD strain nucleotide sequence was found with MK342935_LSD-CPD/Menofiya1/18 and MN792930_LSD/AHRI/_Wadi_Elgdid/18, (Figures 4 and 5). Field isolated LSD virus (Aziz_LSD) has only one nucleotide that differs from the Ismalia strain and two nucleotides from the Kenyan sheep pox strain, while it differs from the Held goat pox virus strain by eight nucleotides, where six are present in the HGP and two in the field LSD isolate. The field LSD isolate has only one amino acid (L) not present in the other strains, except Menofiya1/18 and Alwadi-elgidid/18 which showed 100% overlap with the field isolate. This may be related to the virulency or location as shown in Fig. 6. This result agreed with Chibssa et al. (2018) who recorded that the field isolates of SPPV could not be clearly differentiated from LSDVS and GTPVS; and also with Mansour (2017), who found that the field LSDV that caused disease in Iraq was highly related with other *Capriopoxviruses* and had high nucleotide sequence identity and a close genetic relationship in phylogenetic analysis tree (Fulzele et al., 2006; Stram et al., 2008).

These results suggest that all *Capripoxviruses* are genetically related and originated from a single ancestral lineage (Tulman et al., 2001; El-Bagoury et al., 2009) and that the neutralizing antibodies to LSDV develop in cattle vaccinated with any Capripoxvirus vaccine (Capstick and Coackley, 1961). This also corroborates Christine et al. (2014), who studied the acquired humoral and cellular acquired immunity and the results proved that the LSD vaccine vaccinated group acquired a slightly more prominent immunity than RSP vaccine vaccinated group, concluding that both the Romanian SP and Ismailia LS vaccines can protect cattle against lumpy skin disease. Christine et al. (2017) showed that the LSD and dual vaccine (Romanian sheep pox and Held goat pox vaccine) was more immunogenic than the RSP and HGP vaccines alone.

More effective vaccines and diagnostic reagents is important in controlling disease, so the molecular characterization of viruses is important in understanding the mechanisms of viral pathogenesis and epidemiology.

Conclusions

The lumpy skin disease virus strain from locally infected cattle in Giza, Egypt (2018) was isolated on ECE and MDBK. It was identified by PCR then confirmed by gene and amino acids sequences. The recent LSD virus isolate showed maximum homology with other isolates from other areas in Egypt, and showed high homology with the tested capripox virus vaccinal strains (98.2–99.8%). The routine LSD vaccination programme of LSD should be retained in Egypt, and further recommendations include frequent eradication of the insect population and application of genetic studies on the genomes of LSD virus strain and the capripox virus vaccinal strains to determine the most related and homologous vaccinal strains, due to the large genome of the LSD (150,000 base pairs).

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Molekularna diferencijacija izolata bolesti kvrgave kože na terenu i cjepnih sojeva virusa *Capripox* u Egiptu 2018.

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Virus bolesti kvrgave kože pripada rodu *Capripox* virusa porodice *Poxviridae*, koji pogađa stoku i uzrokuje bolest sa značajnim ekonomskim gubitcima, a koja se kontrolira cijepljenjem živim oslabljenim *capripox* cjepivima. Cilj studije bio je izolirati i identificirati soj virusa BKK na terenu tijekom 2018. godine. Prikupljene kvrge na koži u stoke klinički inficirane bolešću kvrgave kože (BKK) rabljene su za izoliranje virusa na korioalantoičnoj membrani (CAM) embrioniranog kokošjeg jaja (ECE) bez specifičnog patogena (SPF) i staničnoj kulturi bubrega goveda Madin Darby Bovine Kidney (MDBK stanična linija). Lančane reakcije polimerazom (PCR) usmjerene na ORF103

gen virusa *Capripox* (CaPV) primijenjene su na izolirani BKK virus i tri cjepna soja virusa *Capripox* (kenijski virus ovčjih boginja, Held virus kozjih boginja i Ismailia virus bolesti kvrgave kože). PCR amplikoni četiriju sojeva CaPV (jednog izoliranog i tri cjepna soja) rabljeni su za sekvenciranje i dobivanje pristupnog broja u banci gena te ilustriranje filogenetskog stabla u usporedbi s drugim referentnim virusima *Capripox* dobivenima iz banke gena. BKK virus izoliran iz prikupljenih uzoraka kvrga na koži na embrioniranom kokošjem jaju pokazao je jasne tipične pustula lezije na korioalantoičnoj membrani, a na staničnim kulturama (MDBK stanična linija) pokazao je karakterističan citopatski učinak. Pozitivni uzorci izoliranog soja BKK identificirani su PCR-om za CaPV ORF103 gen koji je dao očekivane veličine amplikona od 570 bp te potvrđeni sekvenciranjem gena uz analizu putem BLAST-a i su dostavljeni banci gena pod pristupnim brojem MW 546997_ LSD_Aziz_LSD. Dizajnirano je filogenetsko stablo i otkriveno je da je soj izolata virusa BKK na terenu imao postotke različitog identiteta koji su se kretali od 98,2-99,8 % s testiranim cjepnim sojevima virusa *Capripox* u Egiptu. Sekvenca aminokiselina pokazala je samo jednu posebnu aminokiselinu koja je pronađena u soju izolata s terena, ali ne u ostalim testiranim cjepnim sojevima. Maksimalna podudarnost (100%) nukleotidne sekvence izoliranog BKK soja bila je s dva soja zabilježena u banci gena (MK342935_ LSD-CPD/Menofiya1/18) i (MN792930_LSD/ AHRI/_Wadi Elgdid/18). Zaključeno je da je izolirani soj virusa BKK imao velike postotke identiteta (98,2-99,8 %) s testiranim cjepnim sojevima virusa *Capripox* u Egiptu. Da bi se postigao najpovezaniji i homologni cjepni soj, jer je genom BKK virusa velik preporučujemo nastavak programa rutinskog cijepljenja za BKK u Egiptu, često uništavanje populacije insekata i provedbu dodatnih genetskih studija na genomima soja BKK virusa i cjepnih sojeva virusa *Capripox* da bi se postigao najpovezaniji i homologni cjepni soj, jer je genom BKK virusa golem.

Ključne riječi: *molekularna diferencijacija, filogenetska analiza, bolest kvrgave kože, virus Capripox, orf103 gen*