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Research article

Incidence of lumpy skin disease virus with its characterization in vaccinated pregnant Holstein cows in Dakahlia governorate, Egypt

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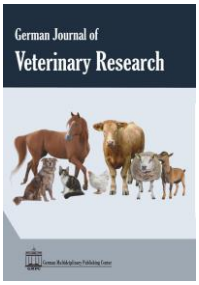

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Abstract

Lumpy skin disease (LSD) is an infectious, economically important viral disease of cattle. Lumpy skin disease virus (LSDV) is still circulating in Egypt, despite the annual mass vaccination with the sheep pox virus vaccine recommended by the Egyptian authorities. This study was carried out on two farms of pregnant Holstein cows vaccinated with Bovivax LSD-N® vaccine (Neethling strain) (farm I) and Servac Capri-C® vaccine (live attenuated sheep pox) (farm II). After 40-60 days post-vaccination, mild clinical signs were detected in 3% of cows on farm I, whereas a more severe clinical infection was developed in 40% of cows on farm II. LSDV was isolated on the chorioallantoic membrane (CAM) of 11-day-old embryonated chicken eggs (ECEs) and Madin Darby bovine kidney (MDBK) cell culture. LSDV was identified in collected skin tissues by transmission electron microscopy (TEM) and histopathological examination. Finally, LSDV was confirmed by amplification of 192 base pairs (bp) of the P32 gene using polymerase chain reaction (PCR), and two samples were selected for DNA sequencing. LSDV developed characteristic pock lesions on the CAM of the inoculated ECEs. MDBK cell culture developed a prominent LSDV cytopathic effect at the 3rd passage. Viral particles were detected in the cytoplasm of both epidermal cells and dermal macrophages by TEM. Histopathological examination revealed different lesions correlated with LSDV infection age. LSDV was confirmed in all tested samples by PCR. Our strains (Dakahlia-2020-1 and Dakahlia-2020-2) were closely related to other Egyptian LSDV strains in the GenBank with 98.2-100% identity. The present study proved conclusive evidence that the live attenuated sheep poxvirus vaccine poorly protects Egyptian cattle against LSDV, while the LSDV Neethling strain vaccine gave a promising and sufficient protection rate.

Keywords: LSDV, Neethling, Sheep poxvirus vaccine, RT-PCR, Phylogenetic analysis

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Introduction

Lumpy skin disease (LSD) is a highly contagious viral disease of cattle produced by LSDV, which belongs to the genus *Capripoxvirus* (together with sheep poxvirus and goat pox virus), subfamily *Chordopoxvirinae*, of the family *Poxviridae* (ICTV, 2021). LSDV has a DNA genome of about 151 kbp in length, which consists of a central coding region flanked by identical inverted terminal repeats (2.4 kbp each) and also contains 156 putative genes (Tulman et al., 2001). Great economic losses are incurred due to severe skin damage caused by circumscribed skin nodules that cover all the body parts, reduced milk production, mastitis, decreased fertility, abortion, and sometimes death. The cost-effectiveness of the disease contributed to its high morbidity rate (up to 90%) rather than mortality (less than 10%) (Babiuk et al., 2008; Tuppurainen and Oura, 2012).

The characteristic clinical signs are numerous raised painful cutaneous lesions that develop within 3-4 days from macules, then papules, to nodules (Sanz-Bernardo et al., 2020). These cutaneous lesions are accompanied by fever, oral, nasal, and ocular discharge (Kasem et al., 2018). Histopathological studies of skin samples showed vasculitis, necrotic epidermis, and intracytoplasmic inclusion bodies (El-Neweshy et al., 2013; Neamat-Allah, 2015).

LSD was reported for the first time in Zambia in 1929, then spread to other African countries, including Egypt (Maclachlan, 2010; Tuppurainen and Oura, 2012). According to the World Animal Health Information Database (https://www.oie.int/en/animal-health-in-the-world/wahis-portal-animal-healthdata/), LSD was first recognized in Egypt in 1988 in Suez Governorate at a quarantine station among cattle imported from Somalia (House et al., 1990; Davies, 1991). The spreading of the disease increased day by day with different degrees of clinical signs until it reached 22 out of the 26 Egyptian governorates by the summer of 1989. Approximately 1449 animals died in this epizootic; at the same time, the veterinary authorities applied a vaccination program using the sheep pox vaccine (SPV) to control the massive outbreak (Ali et al., 1990).

Although the annual mass vaccination is enforced by the Egyptian veterinary authorities with SPV, LSDV still circulates among livestock in each summer season. The disease reappeared as an extensive LSD outbreak hit Egypt, affecting 16 provinces in early 2006 after cattle importation from Ethiopia, then again in 2011 and 2014 as well as the disease was recorded in Upper Egypt during the summer of 2018 (Salib and Osman, 2011; Elhaig et al., 2017; Allam et al., 2020). The recurrent occurrence of LSD outbreaks is dependent mainly upon animal movements, immunological status, and the insect vectors involved in the transmission of the virus (Farah Gumbe, 2018).

LSDV is an arbovirus transmitted mechanically by *Aedes aegypti* mosquitoes, *Culicoides*. However, the hard ticks have a role in the transmission cycle (Alkhamis and VanderWaal, 2016; Hussein et al., 2017). Some previous studies suggested the possibility of biological transmission of LSDV by *Culicoides midges* and *Culicoides punctatus* (*Latreille*; *Diptera*: *Ceratopogonidae*) (Şevik and Doğan, 2017). The efficient control measures of LSDV in endemic areas as Egypt need a rapid and accurate vaccination strategy (Tuppurainen et al., 2017).

Live attenuated strains of Capripoxvirus are used in the vaccination of LSD because of the cross-protection among the genus Capripox, such as Romanian sheep pox virus strains and also Neethling LSDV strain (Tuppurainen et al., 2014). In Egypt, the Romanian sheep poxvirus vaccine strain was applied to immunize cattle and small ruminants against Capripoxviruses, although the re-appearance of new outbreaks in vaccinated animals has been recorded in the last few years (Mikhael et al., 2017). Reliance on LSD vaccination based on the Romanian SPV is not effective, as many outbreaks appeared at different time intervals (Ochwo et al., 2020; El-Khabaz et al., 2020).

In this regard, the present study was undertaken to assess the occurrence of LSDV in pregnant Holstein cows vaccinated with SPV (Romanian strain) in comparison to the LSDV vaccine (Neethling strain) in Dakahlia Governorate, Egypt. According to the authors' knowledge, this is the first study to address the efficacy of homologous LSDV vaccines compared with heterologous SPV in Egyptian livestock.

Materials and methods

Ethical statement

Sampling and examination procedures were performed according to the ethical standards of the Faculty of Veterinary Medicine, Mansoura University, Egypt, which fulfills all relevant Egyptian legislation. Cattle owners gave consent orally, which is in harmony with the nation's ethical regulations.

Vaccines

Bovivax LSD-N® Vaccine

A live attenuated tissue culture LSDV vaccine prepared from the Neethling strain was used. Each dose (2 mL) of vaccine contains about $10^{3.8}$ $TCID_{50}$ LSDV. The vaccine manufactured by M.C.I. Sant´e Animale, Lot 157, ZI Sud-Ouest P.O. Box 278, Mohammadia 28810, Morocco.

Servac Capri-C vaccine®

A live attenuated lyophilized tissue culture SPV prepared from a Romanian strain was used. Each dose (1mL) of vaccine contains about 106 TCID50 SPV. The vaccine was manufactured by the Veterinary Serum and Vaccine Research Institute (VSVRI), El-Sekka El-Beda St., Abbasia, Cairo, Egypt.

Animals

The study was carried out on two farms (farm I and farm II) of pregnant Holstein cows (100 cows/ farm) imported from Germany (LSDV free) at the same locality in Gamsa City, Dakahlia governorate, Egypt. Both farms applied the same hygiene and management systems for disease control and in-sects eradication.

Up on arrival to Egypt, all cows were examined clinically, and they were free from LSDV lesions with no previous history of LSDV vaccination or infection (they did not develop clinical signs of LSDV infection at any point in their lives). Cows in farm I (n=100) were vaccinated with Bovivax LSD-N® Vaccine, while cows in farm II (n=100) were vaccinated with Servac Capri-C vaccine®. At vaccination, the animals were 18-20 months of age and 5-7 months pregnant. After 40-60 days post-vaccination, the animals developed clinical signs consistent with LSDV infection. Clinical signs and mortality rates were recorded in both farms, as shown in Table 1.

Collection of clinical specimens

Skin nodules were collected from animals suspected to be infected with LSDV (n=43; three animals on farm I and 40 animals on farm II). These samples were collected aseptically after washing the area and removing the hair with a sterile scalpel blade. Then, a povidone-iodine (10% w/v) skin solution was applied to the wounds to evade secondary bacterial infection.

Each sample was divided into three parts: the first part was collected in sterile phosphate-buffered saline (PBS) for virus isolation and molecular identification of LSDV. The second part was collected in 4% buffered glutaraldehyde for electron microscopic examination, and the third part was collected in 10% neutral formalin for histopathological examination. Skin samples from three healthy cattle (with no skin lesions) were pooled and used as a negative control.

Table 1: Incidence of lumpy skin disease in cows vaccinated with Bovivax LSD-N® Vaccine in comparison to Servac Capri-C vaccine® and variance in clinical signs.

Item	Farm I- Vaccinated with LSD-N® Vaccine (n=100)	Farm II- Vaccinated with Servac Capri-C® Vaccine (n=100)
No. of Clinically diseased animals	3	40
Fever	0	35
Nodules % to the body	2-3%	20-60%
Edema	0	25
Abortion	0	8
Mortality	0	13

For virus isolation, the skin samples of different sizes were thawed at room temperature and then washed three times using sterile PBS with antibiotics and antifungals at a pH of 7.2. Tissue homogenates (10% W/V) were ready for centrifugation at 4000 rpm/10 minutes. The supernatant was collected and stored at -20°C until use in PCR and virus isolation (OIE, 2021).

Virus isolation

Specific Pathogen Free (SPF) Embryonated Chicken Eggs (ECEs)

SPF-ECEs (11-day-old), obtained from Koum Oshim farm, Fayoum-Egypt, were inoculated by the prepared supernatant of skin nodules samples (n=43) via chorioallantoic membrane (CAM) route according to Tuppurainen et al. (2005); Sharawi and Abd El-Rahim (2011). The collected CAMs were examined for pathological changes 7 days post-incubation at 33°C.

Madin Darby Bovine Kidney (MDBK) cell culture

MDBK tissue culture cells, which were propagated with Eagle's minimum essential medium (EMEM) and supplemented with 10% fetal bovine serum (FBS), were used for LSDV isolation from skin nodules samples (n=43). Cell cultures are observed daily for cytopathic effects (CPE) development. A sample was considered negative once no CPE was noticed after three blind passages according to the method described by Irons et al. (2005) and OIE (2021).

Transmission electron microscopy

The preparation and negative staining of skin specimens were done according to Johannessen (1978). Im- mediate fixation of cutaneous tissues taken from lumpy nodules (1mm cubes) was carried out by immersion in 4% buffered glutaraldehyde (4°C). Fixed tissue cubes were post-fixed in 1% osmium tetroxide, dehydrated in an up-graded ethanol series, and embedded in Epon 812. Semi-thin sections stained with 1% toluidine blue were examined for histopathological changes and also to localize the desired tissues. Consequently, ultra-thin sections were prepared, double-stained with uranyl acetate and lead citrate, and examined under TEM (JEOL 100 CXII) operated at 80 kv. TEM examination was done in the electron microscopy unit at Assuit University, Egypt.

Histopathological examination

Skin nodules from affected animals have removed after local anesthesia and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm thick and stained with H&E, and then examined microscopically for LSDV lesions as described by Suvarna et al. (2019).

Molecular identification of LSDV in collected samples

Viral DNA was extracted from the supernatant fluid of skin nodules homogenates with QIAamp® Mini Elute® Virus Spin Kit (QIAGEN, GmbH, Hilden, Germany) Cat. No. 51304, according to kit instructions. Bovivax LSD-N® Vaccine was used as a positive control sample, and a negative control skin sample was also involved in the PCR. The extracted DNA from skin nodules was used for screening of LSDV presence with PCR amplification of 192 bp of viral attachment protein-encoding gene (P32 gene) using a previously reported primer set by Ireland and Binepal (1998). The forward (5'- TCCGAGCTCTTTTCTT ACTAT - 3'), and reverse (5'- TATGGTACCTAAAT TATAT-ACGTAAATAAC -3') primers (Macrogen, South Korea) were used.

The 50 µL reaction mixture was composed of 25 µl of 2x Dream Green PCR Mix (Thermo Scientific, MA, USA), 1.0 µL of each primer (10 pmol/µL), 5 µL of DNA and nuclease-free water up to 50 µL. The thermal cycler (Bio-RAD, USA) was adjusted on the first denaturation cycle of 95°C/5 minutes, then 35 cycles of three steps: denaturation (95°C/45 seconds), annealing (50°C/50 seconds) and extension (72°C/1 minute) with a final extension cycle at 72°C for 10 minutes. The obtained PCR products were visualized by gel electrophoresis in 1% agarose gel (Sigma, USA) against Gene Ruler 100bp plus DNA Ladder (Thermo Scientific, MA, USA).

Sequencing and phylogenetic analysis of PCR products

The QIAquick Gel Extraction kit (Qiagen Inc., Valencia, CA, USA) was used to purify amplified PCR products of the two selected samples showing the sharpest bands in gel electrophoresis. Gel-purified DNAs from two selected samples were submitted to the Macrogen laboratory in South Korea for bidirectional DNA sequencing. The obtained DNA sequences were deposited to GenBank with accession numbers MZ102252 and MZ102253. Basic Local Alignment Search Tool (BLAST®) (Altschul et al., 1990) was used to establish sequence similarities to the sequences deposited in the GenBank database, and sequencing data were analyzed using ClustalW (http://www.ebi.ac.uk/Tools/MSA). Sequences were used for the generation of the phylogenetic tree, as well as divergence, and identity percent calculation was carried out via MEGA software (http://www.megasoftware.net/). Nucleotide and amino acid sequences were compared with other sequences from GenBank with Bioedit software-version 7.1 (Hall, 1999).

Results

Clinical signs

For diagnosis of LSDV and evaluation of its incidence in both farms, clinical signs and mortality rates were recorded in cows vaccinated with Bovivax LSD-N® Vaccine (farm I) in comparison to Servac Capri-C vaccine® (farm II). Only three cows (3%) in farm I exhibited mild clinical infection as circumscribed nodules covering 2-3% of their body area (Figure 1 A). Whereas 40% of cows in farm II developed more severe clinical infections with numerous circumscribed nodules covering 20-60% of their body surface (Figure 1 B and C). With the disease progression, the nodules became necrotic, and eventually, a deep scab formed (sit-fast), which detached, leaving porous skin (Figure 1 D). On-farm II, 35% of cows exhibited fever, and 25% of cows showed edema in the legs (Figure 1 C). Abortion was also recorded in 8% of cows in farm II, and the aborted fetuses showed skin lesions (Figure 1 E and F). The mortality rate was 0% on the farm I and 13% on farm II (Table 1).

Virus isolation

SPF-ECEs

Supernatant fluids of tissue homogenates were inoculated on the CAM of 11-day-old SPF-ECE for three successive passages, resulting in the characteristic pock lesions of LSDV (circular, opaque, white enlarged eas). The pock lesions were enlarged in size and numbers by consecutive serial passaging of the virus.

MDBK

The characteristic CPE does not appear up to the second passage level. The prominent CPE of inoculated LSDV appears in the third passage. MDBK cell culture showed cell aggregation, which progressed rapidly; also, rounding and degeneration of cells were observed (Figure 2 B). At the 5th day post-inoculation (pi), massive detachments of cells, vacuolation, and network formation were observed (Figure 2 C). The uninfected controls showed no such changes (Figure 2 A).

Transmission electron microscopy (TEM)

Viral particles were detected in the cytoplasm of both epidermal cells and dermal macrophages. Virions are large, mature, and ovoid-shaped with rounded ends. The viral particles appeared as ovoid electron-dense bodies with characteristic short mulberry appeared surface tubules (Figure 3).

Histopathological examination

The examined skin samples revealed different lesions correlated with the age of LSDV infection. Early epidermal hyperplasia with ballooning degeneration of the superficial layers, spongiosis, and necrosis with the presence of numerous eosinophilic intra-cytoplasmic inclusion bodies were found (Figure 4 A). Later on, vesicles formation and necrosis were noticed. Intense serofibrinous or fibrinopurulent exudate with or without bacterial colonies were seen in the dermis and extended deeper to the subcutaneous tissue beside necro- sis in the hair shaft (Figure 4 B). Intense necrosis of skeletal muscles with or without inflammatory cell infiltrations, congestion, and thrombosis of blood vessels was seen (Figure 4 C). Moreover, dystrophic calcification was evident in necrotizing muscles (Figure 4 D). Later on, ulceration of the skin and fibrous tissue proliferation replacing subcutaneous fats and muscles beside hemosiderin pigment were common (Figure 4 E). Finally, crust formation or necrotic skin replaced by granulation tissue covered by a few regenerated epidermis was encountered (Figure 4 F).

Molecular identification of LSDV in collected samples

All 43 tested samples (3 samples from farm I and 40 samples from farm II) were PCR positive, showing DNA bands at 192 bp (Figure 5). Phylogenetic analysis of the obtained DNA sequences from two selected samples with other LSDV field isolates and vaccine strains from GenBank showed that our strains (Dakahlia- 2020-1 and Dakahlia- 2020-2) were closely related to other Egyptian LSDV strains. The Egyptian strains LSDV-EGY-BUS1, LSDV-EGY-BUS2, and LSDV-EGY-BUS3 showed 100% and 99.4% identity with Dakahlia- 2020-1 and Dakahlia-2020-2 strains, respectively.

Furthermore, the Egy-2006 strain showed 98.2% and 98.8% identity with Dakahlia- 2020-1 and Dakahlia- 2020-2 strains, respectively. The strains Dakahlia - 2020-1 and Dakahlia- 2020-2 were also closely related to other LSDV isolates from China (Xin- jiang and GD01 strain), KSA (KSA3 strain), Turkey (Pendik strain), Kazakhstan (Kubash-KAZ-16 strain), Kenya (Kenya-001 strain) and Greece (Evros-GR-15 strain) (Figure 6). BioEdit software was used for comparison between nucleotide and amino acid sequences of our strains and other strains from GenBank. Dakahlia-2020-2 and Egy-2006 strains have a single nucleotide substitution (T43A), which leads to amino acid substitution (F15I). LSDV vaccines have two nucleotide substitutions (G22A and T117C), leading to a single amino acid substitution (V8I) when compared to our strains and other field isolates from GenBank (Figure 7 and Figure 8).

Discussion

LSD is one of the most prominent viral diseases affecting the cattle population in Egypt, causing a big loss among infected animals. The disease is still prevalent and spread throughout Egypt although the presence of extensive vaccination programs. The reoccurrence of LSD outbreaks is related mainly to unsuccessful vaccination and uncontrolled animal movement, together with the presence of insect vectors (Tuppurainen and Oura, 2012). In the current study, the common clinical LSD symptoms observed in cattle were fever and depression. The characteristic circumscribed nodules that cover the animal body with different sizes and edema in legs, nasal discharges, and abortion were prominent signs of the disease. These results were similar to LSD lesions recorded by (Tassew et al., 2018; Ochwo et al., 2020).

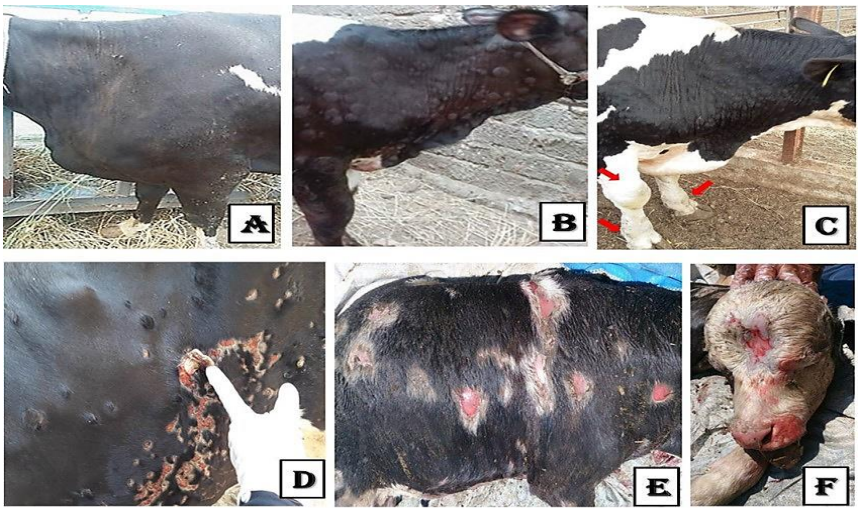

Figure 1: Typical cases of LSDV infection in cattle with cutaneous lesions. (A) A cow on the farm showed a few cutaneous lesions. (B and C) Cows in farm II show severe cutaneous lesions. (C) A cow in farm II showed swelling of legs and joints (red arrows). (D) Sloughed skin nodules with porous skin. (E and F) The aborted fetus in farm II appears with LSDV skin lesions.

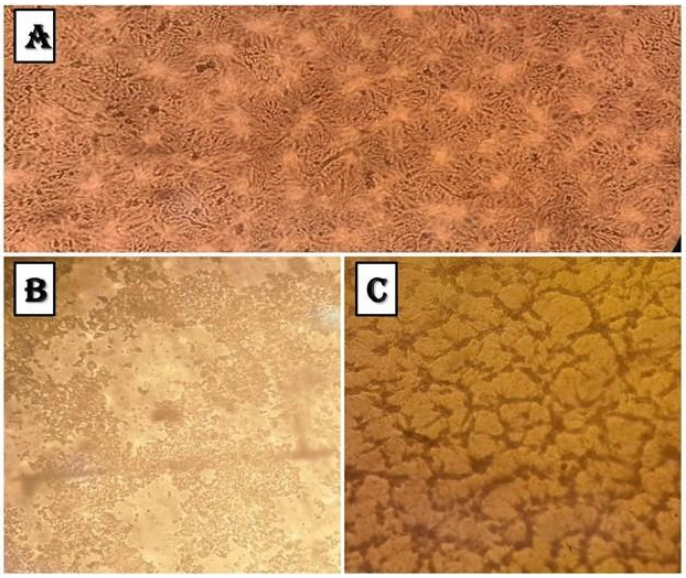

Figure 2: LSDV isolation on MDBK cells. (A) Normal uninoculated MDBK cells. (B) MDBK cells show aggregation, degeneration, and detachment of cells. (C) MDBK 5 days post-LSDV inoculation, showing severe detachment, vacuolation, and network formation (Magnificent power is 10 x).

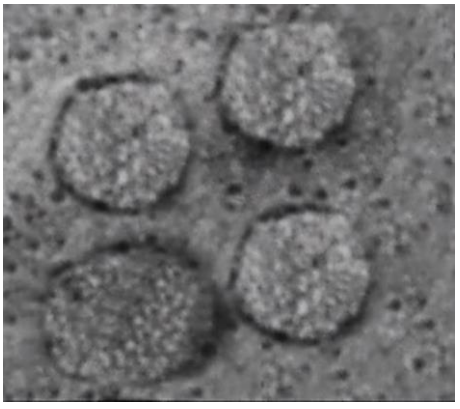

Figure 3: Transmission electron micrograph showing large LSDV particles in the cytoplasm of infected cells as a brick shape with rounded ends and characteristic short surface tubules (mulberry), (Magnificent power is 400000 x).

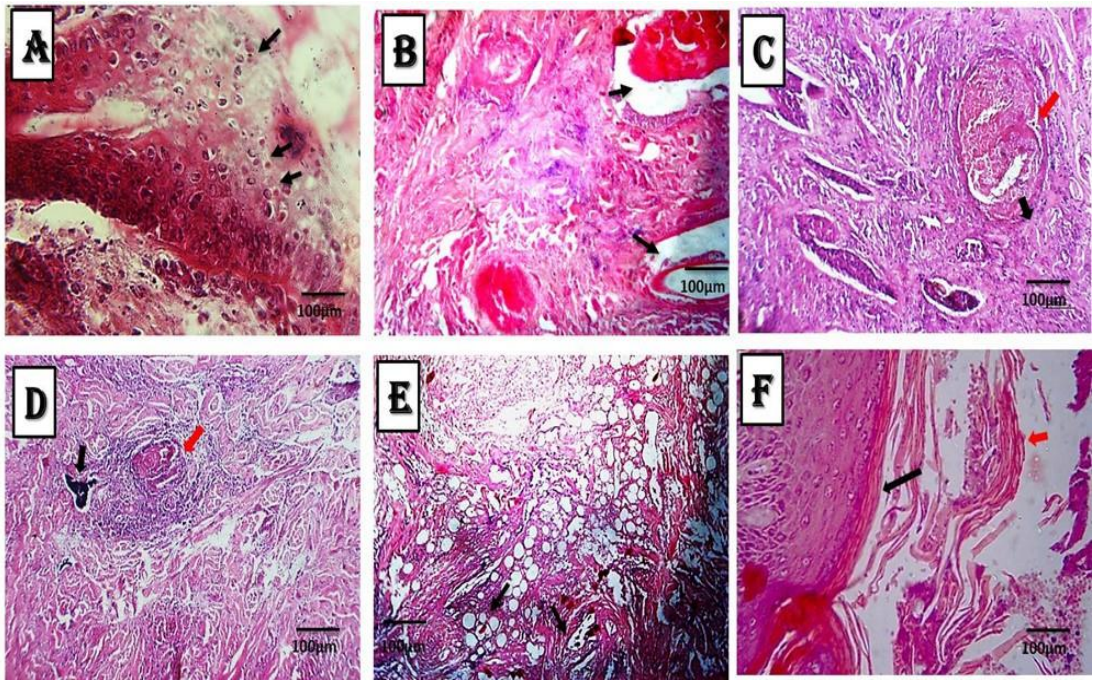

Figure 4: Histopathological changes in the skin of LSDV-infected cattle. (A) The skin of cattle shows numerous intra-cytoplasmic inclusion bodies in degenerated and necrotic epidermal cells (black arrows). (B) The skin of cattle shows necrosis in the hair shaft (black arrow) with bacterial colonies in the dermis and subcutaneous tissues. (C) Skeletal muscles of cattle show severely congested blood vessels (black arrow) and necrosis of muscles (Red arrow). (D) Skeletal muscles of cattle showing myositis (black arrow) and focal calcification (Red arrow). (E) Skeletal muscles of cattle show fibrous tissue replacing necrotic muscles and fat cells (black arrow). (F) Skin of cattle showing regenerated epidermis under surface crust (black arrow).

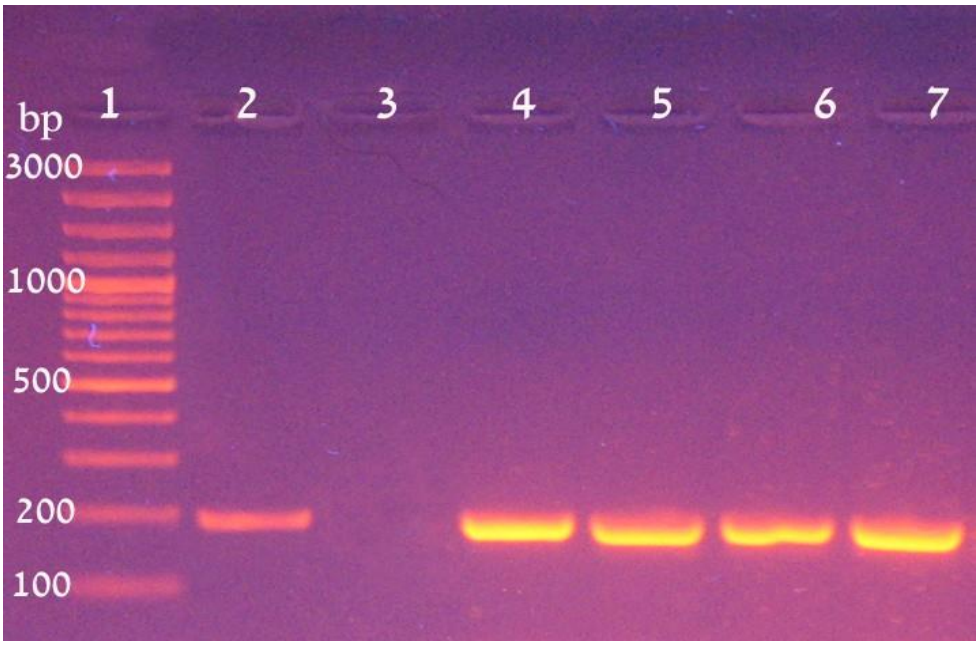

Figure 5: DNA PCR products, 1= 100 bp plus DNA ladder; 2= control positive sample; 3= control negative sample; 4, 5, 6, and 7= tested clinical samples showing DNA bands at 192 bp.

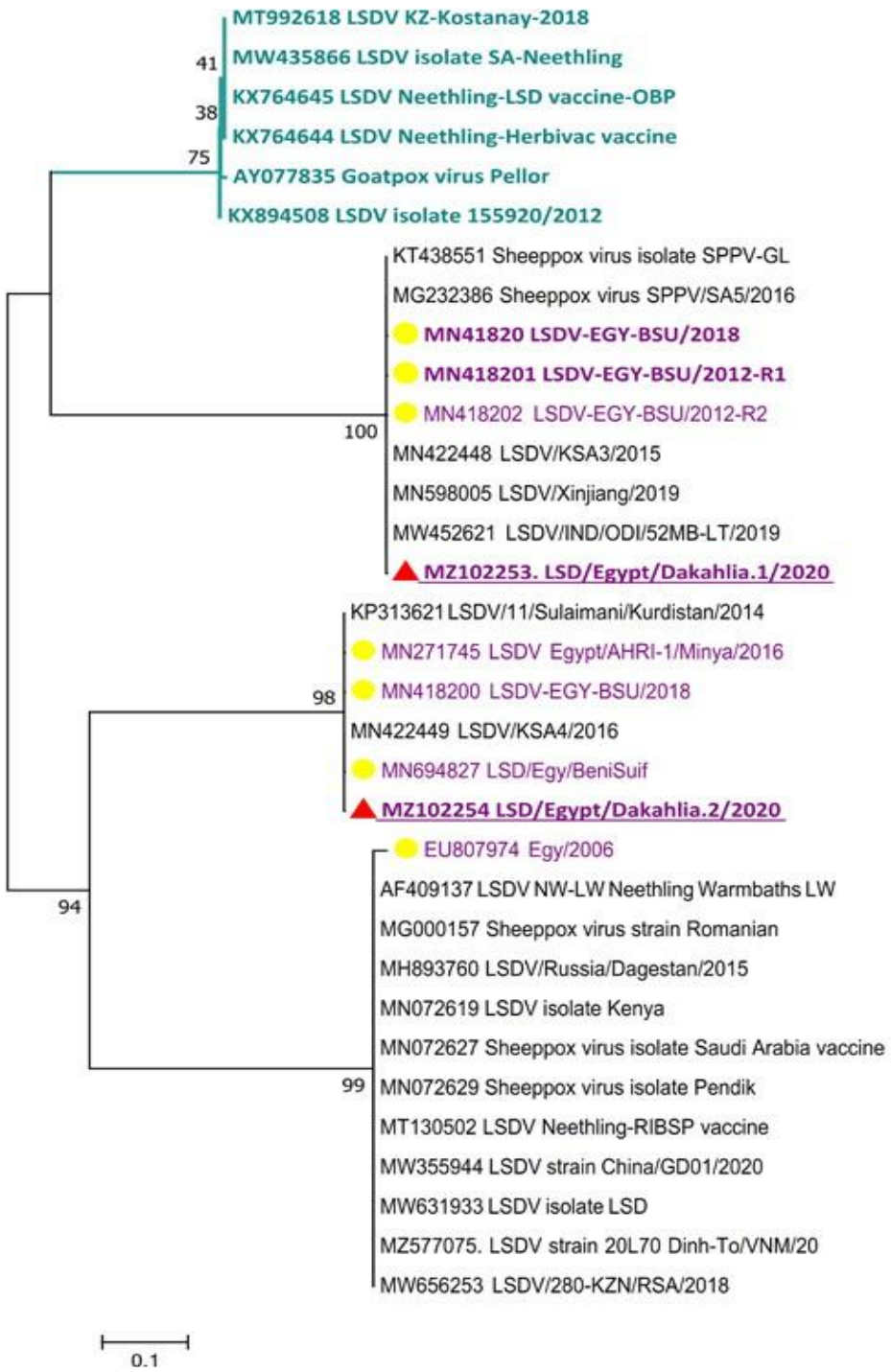

Figure 6: Neighbour-joining phylogenic tree constructed with MEGA software with 1000 bootstrap test for our LSDV strains (red triangles) with other Egyptian LSDV strains (yellow dots). The LSDV strains from other countries and LSDV vaccine strains were obtained from the GenBank database based on LSDV P32 gene sequences.

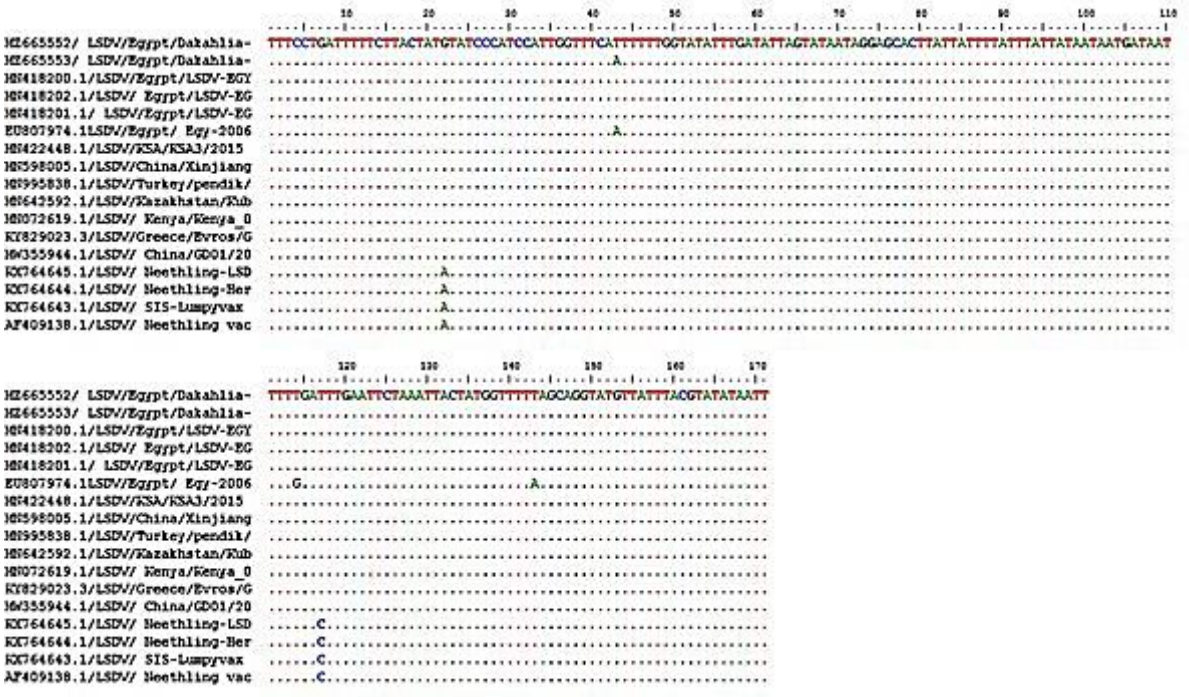

Figure 7: Comparison between nucleotide sequences of our strains (Dakahlia- 2020-1 and Dakahlia-2020-2) and other strains from GenBank using BioEdit software. LSDV identical nucleotides are represented with dots (.) and letters represent mismatches.

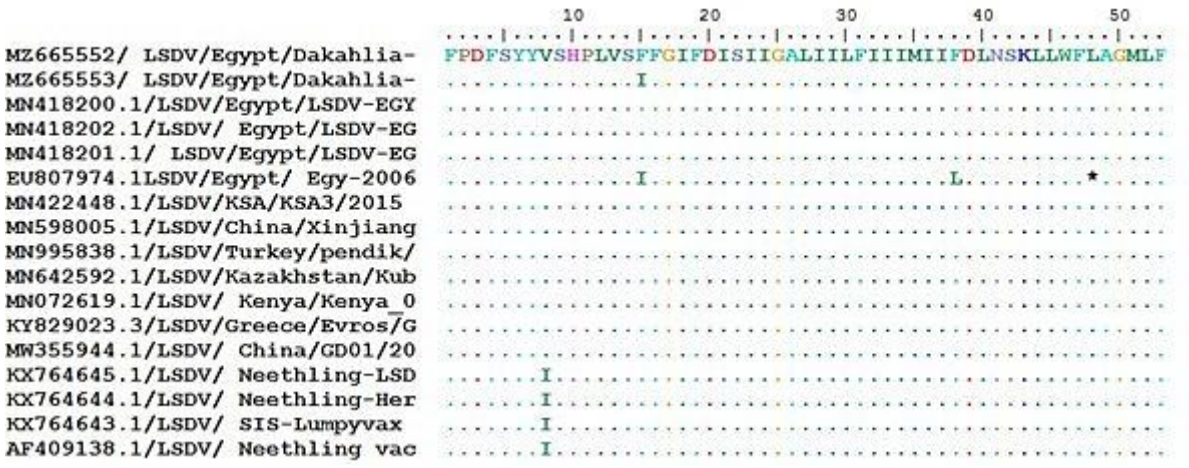

Figure 8: Comparison between deduced amino acid sequences of our strains (Dakahlia- 2020-1 and Dakahlia- 2020-2) and other strains from GenBank. LSDV identical amino acids are represented with dots (.), and letters represent mismatches.

The clinical signs and mortality rates were recorded in cows vaccinated with Bovivax LSD-N® Vaccine (farm I) compared to Servac Capri-C vaccine® (farm I). In farm I, only three cows (3%) exhibited mild clinical infection. Meanwhile, 40% of cows in farm II developed more severe clinical infections with numerous circumscribed nodules. Also, in farm II, 35% of cows exhibited fever, 25% of cows showed edema in the legs, and abortion was reported in 8% of cows. These results revealed that the clinical picture of the disease was more severe in farm II in comparison to farm I. This observation agrees with other studies on LSD vaccination with sheep pox virus vaccine, which is associated with incomplete protection and adverse post-vaccinal reactions against LSDV among cattle (Ayelet et al., 2013; Tuppurainen et al., 2014; Tassew et al., 2018). Also, the OIE reported in 2021 that the live attenuated sheep poxvirus vaccine could not protect Egyptian cattle against LSD (OIE, 2021).

These findings occurred previously in Ethiopia when Ayelet et al. (2013) and Tuppurainen et al. (2014) found that the Kenyan sheep pox vaccine strain used for LSD control cannot confer expected protection. Therefore, the inquisition of vaccine failure, including vaccine matching and alternative vaccine improvement, is necessary. These clinical results confirmed that the LSDV Neethling strain vaccine proved to be safe, more potent, and immunogenic than the Romanian sheep pox virus vaccine. The results also described by Ben-Gera et al. (2015) concluded that the Neethling vaccine is considerably more effective and stronger than the protection conferred by the X10 RM65 heterologous vaccine in controlling LSDV morbidity. Furthermore, Tuppurainen and Galon (2016) showed that homologous vaccinations, including locally prevalent strains, achieve more successive immunizations in protecting cattle against LSDV infection.

The morbidity rate of LSDV varies from 3-40%. These results were similar to Babiuk et al. (2008), Tuppurainen and Oura (2012), and Tassew et al. (2018), who reported that the morbidity of LSD was from 3 to 85%. On the contrary, Gelagay et al. (2012) recorded almost similar results of 22.5% and 25.9% for cross and local breeds, respectively. The mortality rate of the LSD was 0% on farm I and 13% on farm II out of the investigated 200 cattle in both farms. These results were not in accordance with Salib and Osman (2011), who mentioned that the mortality rate of LSD was 1-5%.

These morbidity and mortality rates may be enhanced due to immune suppression of all infected cattle, Romanian sheep pox vaccination failure, and dense insect populations. Tuppurainen et al. (2017); Badhy et al. (2021) also reported that the mortality rate is less than 10%, and the disease morbidity rate can reach up to 100%. With a more comprehensive view, in farm I, there was no mortality, while the morbidity was very low (3%) with mild clinical signs; these results proved the efficacy of the LSDV Neethling vaccine in controlling the morbidity of LSDV isolated from skin nodules samples collected from naturally infected animals through inoculation on CAM of SPF-ECEs. Characteristic pock lesions of LSDV were observed after the 1st passage and became clear after the 3rd passage; this finding agrees with Ateya et al. (2017) and Allam et al. (2020), who isolated LSDV through the CAM inoculation and detected pock lesions. Moreover, the MDBK cell line showed specific CPE, which appeared as cell aggregation and rounding, collected together to form clusters scattering all over the monolayer after the third-day pi. After the 5th day, massive detachments of cells were observed. These findings agree with those of ElHaig et al. (2013) and Mikhael et al. (2014), who mentioned that the highest LSDV titer was obtained from the cultures collected 5-6 days pi. LSDV was observed as large, mature, and brick-shaped virions with rounded ends. The viral particles appeared as ovoid electron-dense bodies under the electron microscope; similar results were detected by Ahmed and Zaher (2008). Mature and immature intracellular virions, as well as extracellular virion particles, were seen by TEM (Sanz-Bernardo et al., 2020).

In the present study, histopathological examination of the skin samples collected from LSD naturally infected cattle revealed different lesions correlated with age of infection. Characteristic skin lesions of LSD as epidermal hyperplasia with ballooning, degeneration of the superficial layers, spongiosis, and necrosis with the presence of numerous eosinophilic intracytoplasmic inclusion bodies, vesicles formation, and necrosis were noticed. These results are matched with Neamat-Allah (2015) and Sanz-Bernardo et al. (2020). Intense serofibrinous or fibrinopurulent exudate with or without bacterial colonies were seen in the dermis and extended deeper to subcutis beside the necrosed hair shaft; these results were previously reported by (Salib and Osman, 2011), who found serofibrinous Intense exudate in vesicles and necrosis of skeletal muscles with and without inflammatory cells infiltrations and congested thrombosed blood vessels were seen. Moreover, dystrophic calcification was evident in necrotizing muscles. These results were reported by El-Neweshy et al. (2013). Later on, ulceration of the skin and fibrous tissue proliferation replacing subcutaneous fats and muscles besides hemosiderin pigment were common. Finally, crust formation or necrotic skin replaced by granulation tissue covered by a few regenerated epidermises was encountered.

The variability of LSDV in the last few years makes it crucial to adjust the molecular differentiation between infected and vaccinated animals (DIVA) approaches based on the data of the circulating LSDV strain. This needs the persistent monitoring and characterization of LSDV field isolates. Molecular characterization of LSDV isolates using PCR with primers specific to the attachment gene revealed that all 43 tested skin nodules samples were positive in PCR, showing bands of 192 bp sizes.

PCR is highly sensitive (100%) to detect the LSDV DNA in its original skin samples, which is correlates very well with Elhaig et al. (2017), El-Khabaz (2014) and Zewdie et al. (2019). This may be due to its high persistence in the skin tissues. This result is in agreement with Fahmy and Gaafar (2016), Zeynalova et al. (2016) and Zeedan et al. (2019), who concluded that skin nodules are better samples for detection of LSDV using PCR.

Phylogenetic analysis of Dakahlia- 2020-1 and Dakahlia- 2020-2 with other LSDV field isolates and vaccine strains from GenBank showed 100% and 99.4% identity with the Egyptian strains (LSDV-EGY-BUS1, LSDV-EGY-BUS2, and LSDV-EGY-BUS3). These findings are in accordance with Ochwo et al. (2020). The phylogenetic tree was fully in agreement with the genotyping result of the classical PCR analysis. The sequence analysis showed no significant nucleotide variation among the field isolates included in this study. However, there is a single nucleotide substitution exists between Dakahlia-2020-2 and Egy-2006 strains (T43A) which leads to amino acid substitution (F15I). Similarly, LSDV vaccines have two nucleotide substitutions (G22A and T117C), causing a single amino acid variation (V8I) when compared to our strains and other field isolates. These results are in agreement with those reported by Tassew et al. (2018). These amino acid substitutions were not previously recorded, and their effect is still unknown.

Conclusions

The current study's results declare the importance of LSD as it is still one of the dangerous diseases threatening cattle in Egypt. Within the scope of the current study, the use of a homologous LSDV Neethling strain to immunize cattle against the epidermal layer without bacterial colonies. LSDV infection gave a higher protection rate compared to the heterologous Romanian SPV vaccine when used solely in the studied farms. More studies on circulating LSDV strains from different outbreaks are recommended to confirm these results.

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References

Ahmed, W.M., Zaher, K.S., 2008. Observations on lumpy skin disease in local Egyptian cows with emphasis on its impact on ovarian function. African Journal of Microbiology Research 2, 252–257.

Ali, A.A., Esmat, M., Attia, H., Selim, A., Abdel-Hamid, Y.M., 1990. Clinical and pathological studies on lumpy skin disease in Egypt. The Veterinary Record 127, 549–550. https://www.ncbi.nlm.nih.gov/pubmed/ 2281596.

Alkhamis, M.A., VanderWaal, K., 2016. Spatial and temporal epidemiology of lumpy skin disease in the Middle East, 2012-2015. Frontiers in Veterinary Science 3, 19. 10.3389/fvets.2016.00019.

Allam, A.M., Elbayoumy, M.K., Abdel-Rahman, E.H., Hegazi, A.G., Farag, T.K., 2020. Molecular characterization of the 2018 outbreak of lumpy skin disease in cattle in Upper Egypt. Veterinary World 13, 1262–1268. 10.14202/vetworld.2020.1262-1268.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. Journal of Molecular Biology 215, 403–410. 10.1016/S0022-2836(05)80360-2.

Ateya, L.A.F., Ahmed, S.A., Ayman, M.H., Ashraf, K.K., Abdel-Hady, H.A., 2017. Isolation and identification of lumpy skin disease virus in cattle in Kalubeya Governorate. Journal of Virological Science. https://researcherslinks.com/

ElHaig, M.M., Selim, A., Shahira, A., 2013. Polymerase chain reaction and dot blots hybridization techniques for diagnosis of lumpy skin disease in cattle in Egypt. Jokull Journal 63, 208–216.

Fahmy, S.R., Gaafar, K., 2016. Establishing the first institutional animal care and use committee in Egypt. Philosophy, Ethics, and Humanities in Medicine 11, 2. 10.1186/ s13010-016-0035-3.

Farah Gumbe, A.A., 2018. Review on lumpy skin disease and its economic impacts in Ethiopia. Journal of Dairy, Veterinary & Animal Research 7. 10.15406/jdvar.2018.07.00187.

Gelagay, A., Fasil, N., Jembere, S., Mekonen, G., Sori, T., Negussie, H., 2012. Study on immunogenicity of combined sheep and goat pox and peste des petits ruminants vaccines in small ruminants in Ethiopia. African Journal of Microbiology Research 6, 7212–7217.

Hall, T., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

House, J.A., Wilson, T.M., el Nakashly, S., Karim, I.A., Ismail, I., el Danaf, N., Moussa, A.M., Ayoub, N.N., 1990. The isolation of lumpy skin disease virus and bovine herpesvirus-4 from cattle in Egypt. Journal of Veterinary Diagnostic Investigation 2, 111–115. 10.1177/104063879000200205.

Hussein, H.A., Khattab, O.M., Aly, S.M., Rohaim, M.A.M., 2017. Role of Ixodid hard tick in the transmission of lumpy skin disease. Hosts and Viruses 4, 46–53.

ICTV, 2021. The international code of virus classification and nomenclature was ratified by the international committee on taxonomy of viruses. https://talk.ictvonline.org/.

Ireland, D.C., Binepal, Y.S., 1998. Improved detection of capripoxvirus in biopsy samples by PCR. Journal of Virological Methods 74, 1–7. 10.1016/S0166-0934(98)00035-4.

Irons, P.C., Tuppurainen, E.S.M., Venter, E.H., 2005. Excretion of lumpy skin disease virus in bull semen. Theriogenology 63, 1290–1297. 10.1016/j.theriogenology.2004.06.013.

Ayelet, G., Abate, Y., Sisay, T., Nigussie, H., Gelaye, E., Jem- berie, S., Asmare, K., 2013. Lumpy skin disease: preliminary vaccine efficacy assessment and overview on outbreak impact in dairy cattle at Debre Zeit, central Ethiopia. Antiviral Research 98, 261–265. 10.1016/j.antiviral.2013.02.008.

Babiuk, S., Bowden, T.R., Boyle, D.B., Wallace, D.B., Kitching, R.P., 2008. Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. Transboundary and Emerging Diseases 55, 263–272. 10.1111/j.1865-1682.2008.01043.x.

Badhy, S.C., Chowdhury, M.G.A., Settypalli, T.B.K., Cattoli, G., Lamien, C.E., Fakir, M.A.U., Akter, S., Osmani, M.G., Talukdar, F., Begum, N., Khan, I.A., Rashid, M.B., Sadekuz-zaman, M., 2021. Molecular characterization of lumpy skin disease virus (LSDV) emerged in Bangladesh reveals unique

genetic features compared to contemporary field strains. BMC Veterinary Research 17, 61. 10.1186/s12917-021-02751-x.

Ben-Gera, J., Klement, E., Khinich, E., Stram, Y., Shpigel, N.Y., 2015. Comparison of the efficacy of neethling lumpy skin disease virus and x10RM65 sheep-pox live attenuated vaccines for the prevention of lumpy skin disease- The results of a randomized controlled field study. Vaccine 33, 4837–4842. 10.1016/j.vaccine.2015.07.071.

Davies, F., 1991. Lumpy skin disease, an African capripox virus disease of cattle. British Veterinary Journal 147, 489–503. 10.1016/0007-1935(91)90019-J.

El-Khabaz, K.A.S., 2014. Rapid laboratory diagnosis of lumpy skin disease by using PCR technique. Assiut Veterinary Medical Journal 60, 37–41. 10.21608/avmj.2014.171046.

El-Khabaz, K.A.S., Shosha, E.A.M., Abdel-Raouf, A.M., 2020. New epizootic of lumpy skin disease in Assiut- Egypt: Molecular identification and characterization. Advances in Animal and Veterinary Sciences 9. 10.17582/journal.aavs/2021/9. 3.446.452.

El-Neweshy, M., El-Shemey, T., Youssef, S., 2013. Pathologic and immunohistochemical findings of natural lumpy skin disease in Egyptian cattle. Pakistan Veterinary Journal 3, 60–64. https://agris.fao.org/agris-search/search. do?{recordID}={PK2014000032}.

Elhaig, M.M., Selim, A., Mahmoud, M., 2017. Lumpy skin disease in cattle: Frequency of occurrence in a dairy farm and a preliminary assessment of its possible impact on Egyptian buffaloes. The Onderstepoort Journal of Veterinary Research 84, e1–e6. 10.4102/ojvr.v84i1.1393.

Johannessen, J., 1978. Instruction and techniques in electron microscopy in human medicine. volume I. Mcgraw Hill International Book Company.

Kasem, S., Saleh, M., Qasim, I., Hashim, O., Alkarar, A., Abu-Obeida, A., Gaafer, A., Hussien, R., Al-Sahaf, A., Al-Doweriej, A., Bayoumi, F., Hodhood, A., Abdelatif, M., 2018. Outbreak investigation and molecular diagnosis of lumpy skin disease among livestock in Saudi Arabia 2016. Transboundary and Emerging Diseases 65, e494–e500. 10.1111/tbed.12769.

Maclachlan, N.J., 2010. Fenner's Veterinary Virology. 5 ed., Academic Press.

Mikhael, C.A., Nakhla, O.E., Mohamed, N.A., 2017. Study on the capability of a dual capripox vaccine in protection of cattle against LSD infection. Journal of Veterinary Medical Research 24, 61–70. 10.21608/jvmr.2017.43265.

Mikhael, C., Ibrahim, M., Awad, M., Soliman, S., Michael, A., 2014. Comparative study on the efficiency of some capripox vaccines in protection of cattle against lumpy skin disease. Suez Canal Veterinary Medicine Journal 19, 177–192. 10.21608/scvmj.2014.65800.

Neamat-Allah, A.N.F., 2015. Immunological, hematological, biochemical, and histopathological studies on cows naturally infected with lumpy skin disease. Veterinary World 8, 1131– 1136. 10.14202/vetworld.2015.1131-1136.

Ochwo, S., VanderWaal, K., Ndekezi, C., Nkamwesiga, J., Mun- sey, A., Witto, S.G., Nantima, N., Mayanja, F., Okurut, A.R.A., Atuhaire, D.K., Mwiine, F.N., 2020. Molecular detection and phylogenetic analysis of lumpy skin disease virus from outbreaks in Uganda 2017-2018. BMC Veterinary Research 16, 66. http://dx.doi.org/10.1186/s12917-020-02288-5,10.1186/s12917-020-02288-5.

OIE, 2021. Chapter 2.4.12. lumpy skin disease, in: World Organization for Animal Health. Manual of Diagnostic Tests and vaccines for Terrestrial Animals, pp. 1–13. https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/.

Salib, F.A., Osman, A.H., 2011. Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate, Egypt. Veterinary World 4, 162–167. 10.5455/vetworld.2011.162-167.

Sanz-Bernardo, B., Haga, I.R., Wijesiriwardana, N., Hawes, P.C., Simpson, J., Morrison, L.R., MacIntyre, N., Brocchi, E., Atkinson, J., Haegeman, A., De Clercq, K., Darpel, K.E., Beard, P.M., 2020. Lumpy skin disease is characterized by severe multifocal dermatitis with necrotizing fibrinoid vasculitis following experimental infection. Veterinary Pathology 57, 388–396. 10.1177/0300985820913268.

Sevik, M., Do˘gan, M., 2017. Epidemiological and molecular studies on lumpy skin disease outbreaks in Turkey during 2014- 2015. Transboundary and Emerging Diseases 64, 1268–1279. 10.1111/tbed.12501.

Sharawi, S.S.A., Abd El-Rahim, I.H.A., 2011. The utility of polymerase chain reaction for diagnosis of lumpy skin disease in cattle and water buffaloes in Egypt. Revue Scientifique et Technique (International Office of Epizootics) 30, 821–830. 10.20506/rst.30.3.2075.

Suvarna, S.K., Layton, C., Bancroft, J.D. (Eds.), 2019. Bancroft's theory and practice of histological techniques. Elsevier. 10.1016/C2015-0-00143-5.

Tassew, A., Assefa, A., Gelaye, E., Bayisa, B., Ftiwi, M., 2018. Identification and molecular characterization of lumpy skin disease virus in East Hararghe and East Shoa Zone, Oromia Regional State. Journal of Animal and Veterinary Sciences 4, 1–16.

Tulman, E.R., Afonso, C.L., Lu, Z., Zsak, L., Kutish, G.F., Rock, D.L., 2001. Genome of lumpy skin disease virus. Journal of Virology 75, 7122–7130. 10.1128/JVI.75.15.7122-7130.2001.

Tuppurainen, E., Galon, N., 2016. Lumpy skin disease: current situation in Europe and neighboring regions and necessary control measures to halt the spread in southeast Europe. Technical Report. World Organisation for Animal Health. 10.20506/TT.2554.

Tuppurainen, E.S.M., Oura, C.A.L., 2012. Review: lumpy skin disease: An emerging threat to Europe, the Middle East and Asia. Transboundary and Emerging Diseases 59, 40–48.

Tuppurainen, E.S.M., Pearson, C.R., Bachanek-Bankowska, K., Knowles, N.J., Amareen, S., Frost, L., Henstock, M.R., Lamien, C.E., Diallo, A., Mertens, P.P.C., 2014. Characterization of sheep pox virus vaccine for cattle against lumpy skin disease virus. Antiviral Research 109, 1–6. 10.1016/j. antiviral.2014.06.009.

Tuppurainen, E.S.M., Venter, E.H., Coetzer, J.A.W., 2005. The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. The Onderstepoort Journal of Veterinary Research 72, 153–164. 10.4102/ojvr.v72i2.213.

Tuppurainen, E.S.M., Venter, E.H., Shisler, J.L., Gari, G., Mekonnen, G.A., Juleff, N., Lyons, N.A., De Clercq, K., Upton, C., Bowden, T.R., Babiuk, S., Babiuk, L.A., 2017. Review: capripoxvirus diseases: current status and opportunities for control. Transboundary and Emerging Diseases 64, 729–745. 10.1111/tbed.12444.

Zeedan, G.S.G., Mahmoud, A.H., Abdalhamed, A.M., El-Razik, K.A.E.H.A., Khafagi, M.H., Zeina, H.A.A.A., 2019. Detection of lumpy skin disease virus in cattle using real-time polymerase chain reaction and serological diagnostic assays in different governorates in Egypt in 2017. Veterinary World 12, 1093–1100. 10.14202/vetworld.2019.1093-1100.

Zewdie, G., Mammo, B., Gelaye, E., Getachew, B., Bayssa, B., 2019. Isolation and molecular characterization of lumpy skin disease virus from dairy farms of Central Ethiopia. World Applied Sciences Journal 37, 764–775. URL: https://www.idosi. org/wasj/wasj37(9)19/7.pdf.

Zeynalova, S., Asadov, K., Guliyev, F., Vatani, M., Aliyev, V., 2016. Epizootiology and molecular diagnosis of lumpy skin disease among livestock in Azerbaijan. Frontiers in Microbiology 7, 1022. 10.3389/fmicb.2016.01022.