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Lumpy skin disease virus isolation, experimental infection, and evaluation of disease development in a calf

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Lumpy skin disease (LSD) is one of the most economically significant viral diseases of cattle caused by the Lumpy Skin Disease Virus (LSDV), classified as a member of the genus *Capripoxvirus* and belongs to the family *Poxviridae*. Nodular skin samples were collected from clinically sick cattle in the districts of Amuru and Wara Jarso Ethiopia to isolate LSD virus. The virus was isolated using primary lamb testis and kidney cells. The isolated LSDV was infected into a healthy calf while maintaining the necessary biosecurity measures to generate skin lesions and to assess disease progression using postmortem examinations. On the fourth day after virus inoculation, the calf developed typical LSD skin nodules with increased rectal temperature, which lasted until the 12th day, when they began to decrease. Viral shedding was detected in nasal, oral, and conjunctival swabs from 6 to 14 days after infection using real-time PCR. Post-mortem tissue specimens tested positive for LSD virus using real-time PCR and virus isolation. This study showed that LSDV were responsible for the LSD outbreaks, and the appearance of typical skin nodules accompanied by fever (> 39.5 °C) defined the virus's virulent status. The experimental infection with the isolated infectious LSDV could serve as a platform for future vaccine evaluation study using an LSDV challenge model.

Keywords Calf, Disease development, Experimental infection, Lumpy skin disease, Virus isolation

Lumpy skin disease (LSD) is a serious skin disease of cattle caused by Lumpy Skin Disease Virus (LSDV), which is a member of the family *Poxviridae* and the genus *Capripoxvirus;* and is antigenically related to Sheeppox virus and Goatpox virus[1]. LSD is characterized by nodular cutaneous eruption, lymphadenitis, and edema of one or more limbs, and it causes significant economic losses in infected cattle due to chronic debility, reduced milk production, poor growth, infertility, abortion, and in some cases, death. Furthermore, severe and permanent damage to hides can occur, lowering their commercial value. Because of its rapid spread and the potential for severe economic loss, the World Organisation for Animal Health (WOAH/OIE) considered LSD as notifiable disease[2]. LSD infection has been reported in fine-skinned breeds such as Holstein Friesian (HF) and Jersey breeds[3,4].

Morbidity and mortality vary greatly depending on the breed of cattle, the population's immunological status, insect vectors involved in mechanical transmission, and virulence nature of the virus isolates. LSD morbidity varies greatly, ranging from three to 85% in various epizootic situations. Morbidity is estimated to be 10% in endemic areas[3,5]. LSD related mortality ranges between 1 and 3%, but up to 40% had been reported in severe outbreak situations[6]. A recent cross-sectional study in Ethiopia across different agro-ecological zones showed an overall observed LSD animal-level prevalence of 8.1% and a mortality rate of 2.12%[7].

Vaccination, quarantines, livestock movement controls, vector control, slaughter of infected and exposed animals, and cleaning and disinfection of the premises are all used to control and prevent LSD[1]. However,

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adequate financial, infrastructural, and human resources, as well as information systems, are required. Because it is not possible to implement all of these strategies in Ethiopia, vaccination has been adopted as the most important practical approach to LSD control for many years. According to the World Organisation for Animal Health (WOAH) recommendations, the Kenyan Sheep and Goat pox vaccine strain KS-1 has been used because it confers cross-protection against LSD[8].

Within the genus, Capripoxviruses are cross-reactive. As a result, cattle can be protected against LSD by using Capripoxvirus derived strains from sheep or goats[9]. However, it is recommended that controlled trials be conducted using the most susceptible breeds before introducing a vaccine strain not commonly used in cattle. The vaccine seed strain must be of sufficient quality to be used in all breeds of cattle for which it is intended, including young and pregnant animals. It must also be non-transmissible, remain attenuated after additional tissue culture passages, and provide complete protection against virulent field strain challenge for at least one year. When administered through the recommended route, it must produce a minimum clinical reaction in all cattle breeds[1]. As a result, the current study focused on isolating and identifying lumpy skin disease virus from outbreaks, and measuring disease development and virus shading through the mucous membrane on experimental calf infection, which may pave the way for future vaccine studies using the LSDV challenge model.

Results

Active outbreak investigation

Two active outbreaks were investigated in this study between August 2019 and December 2019. The first outbreak was reported from Amuru District Sidan Peasant association, and the second was reported from Wara Jarso District four PAs. During these outbreaks, a total of 138 cattle were affected and eight died. The disease affected all age groups of cattle with both local and cross breeds. Initial fever, skin nodules on various body parts, enlarged peripheral lymph nodes, depression, lameness, and lacrimation were the most commonly observed clinical signs of LSD (Fig. 1).

Virus isolation

From the 12 samples collected, six (two from Amuru and four from Wara Jarso) were grown on primary lamb testis and lamb kidney cells. Starting from the third day of culture, cytopathic effects (CPE) were observed in all plates. Single cell rounding, aggregation of dead cells, and monolayer destruction were all observed as characteristic CPEs; whereas CPE was not observed in the negative control cultures (Fig. 2).

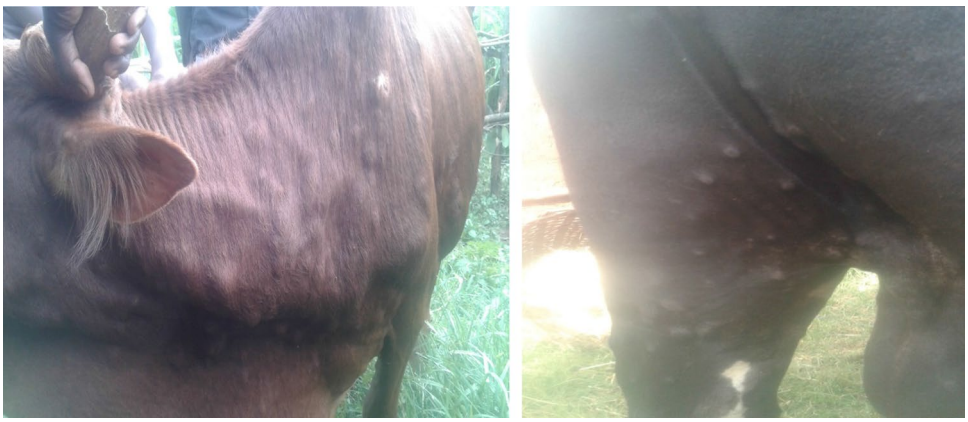

Figure 1. Cattle showing typical clinical signs of lumpy skin disease with generalized nodular skin lesions covering the entire body and perineum area.

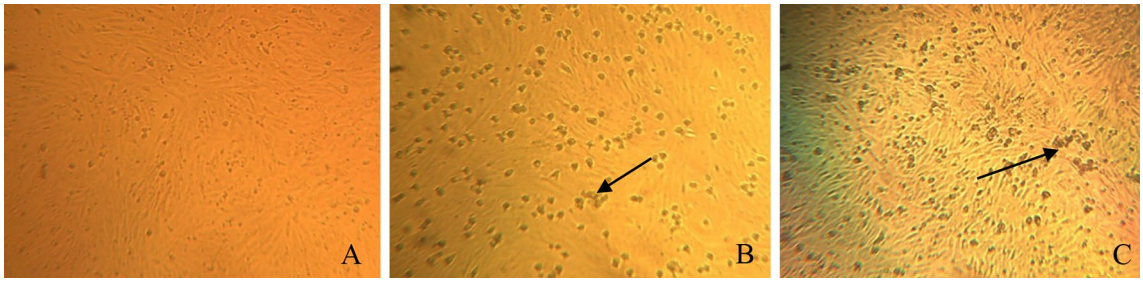

Figure 2. Monolayer of normal lamb testis cells (**A**); Lamb testis monolayer cells infected with LSDV where cytopathic effects developed at day 6 (**B**) and day 10 (**C**) (the arrows indicate infected cells aggregation and destruction).

Molecular examination

Polymerase chain reaction

All (12) of the extracted DNA samples amplified by conventional and real time PCR were positive. On species specific conventional PCR, the tissue suspected of LSD revealed that all isolates collected from outbreaks were LSD viruses (Fig. 3). Supplementary Fig. 1 shows original images of the conventional PCR results on LSD outbreak samples.

Species-specific real-time PCR

To confirm the Capripoxvirus identity and genotype of the field isolates, a species-specific real-time PCR method using unlabeled snapback primer and dsDNA intercalating dye assay targeting the CaPVRPO30 gene was used. The PCR assay detects differences in SPPV, GTPV, and LSDV melting temperatures determined by fluorescence melting curve analysis. Melting curves were analyzed to determine the CaPV strain using Low Profile Hard-Shell® 8-well PCR strips (BioRad). In all PCR runs, appropriate positive controls and RNase free water were used as a negative control. All screened isolates had the same melting profile as that of the LSDV reference strain, the amplicons 73.0 °C, and the snapback 50.0 °C, as shown in the plots (Fig. 4). The Ct values for LSD positive skin nodule samples ranged from 19 to 30, and when compared to positive LSD controls, all of the values were lower, indicating high virus concentrations. Supplementary Fig. 2 depicts real-time PCR data displaying melting curve profiles of tested materials, normalized melt curve profiles, and melting curve analysis of LSDV field isolates and controls.

Animal experiment

Animal inoculation, monitoring, sample collection, and postmortem examination

This experiment used a calf that was 4½ months old. The calf was inoculated intradermally and intravenously in the neck area on the right side with $10^{3.9}$ 50% tissue culture infectious doses (TCID50). Rectal temperatures were recorded every day, and the calf was examined for clinical signs such as the development of lesions at the inoculation site and elsewhere on the body. The calf developed typical LSD skin nodules with increased rectal temperature on day 4 post inoculation, which lasted until the 15th day and was considered a positive case. A severe case was defined as cattle with the appearance of typical skin nodules and fever (> 39.5 °C) (Figs. 5 and

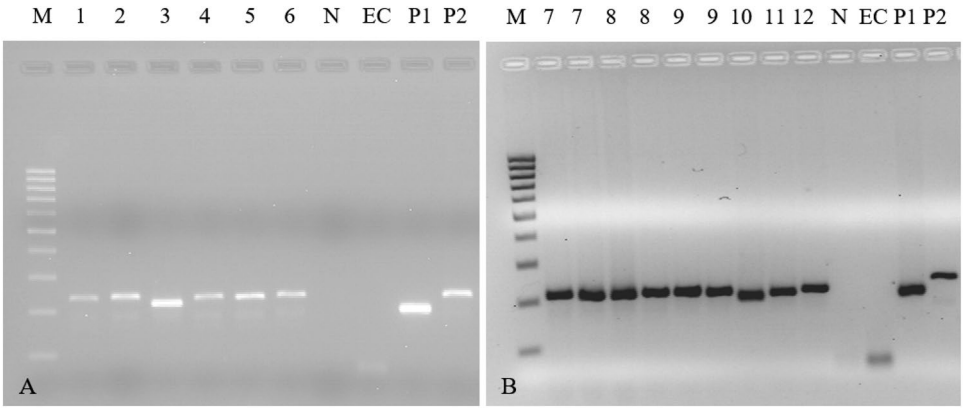

Figure 3. Conventional PCR results on LSD outbreak samples.

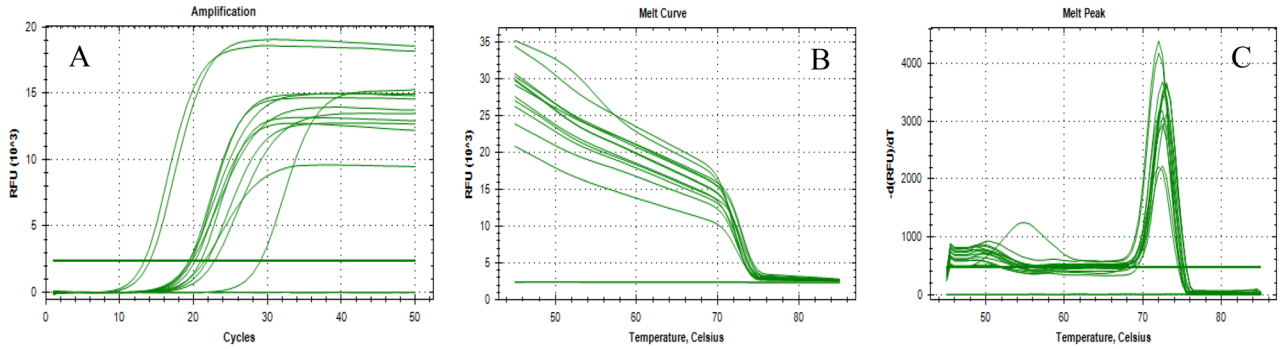

Figure 4. Real time PCR results. (**A**) Melting curve profiles of the tested samples and CaPV controls after PCR amplification of the RPO30 gene, (**B**) Representative normalized melt curve profiles, (**C**) Melting curve analysis of LSDV field isolates and controls.

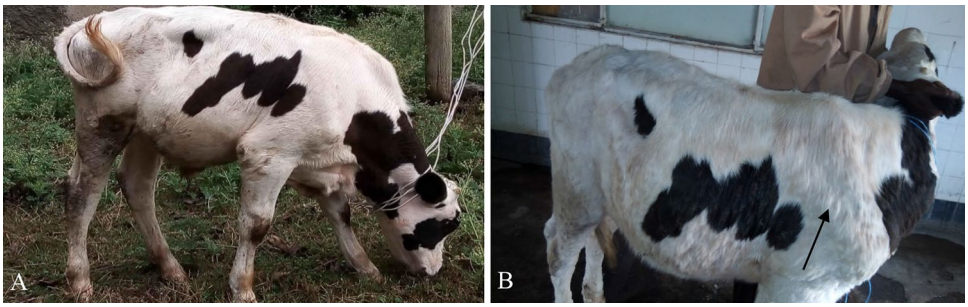

Figure 5. The experiment's healthy calf (**A**); and the calf after clinical disease development with typical skin nodules distributed all over the body surface (**B**).

6). Supplementary Table 1 depicts the daily rectal temperature record of an experimentally infected calf with the infectious LSD virus.

During necropsy in addition to skin nodule, the calf showed ulcerative lesions on the mucous membranes of the oral and nasal cavities, and organs of the respiratory tract, such as lung tissue and trachea, mandibular, cervical, mesenterial lymph nodes, and nodule on the rumen clearly indicating a generalized infection (Fig. 7).

To verify that these nodules were caused by LSD infection, samples were taken from skin nodule, trachea as well as mesenterial lymph node were tested using PCR and showed positive result for LSD virus genome.

Observation of infectious virus shed through mucus membrane by rt-PCR

Swab samples from the nasal, conjunctival, and oral cavity were analyzed by real time PCR for the detection of LSDV in mucosal secretions collected on days 2, 4, 6, 8, 10, 12, and 14 post infection. The virus was detected on the sixth day after inoculation from conjunctiva, the eight-day from oral cavity, and the tenth day from nasal

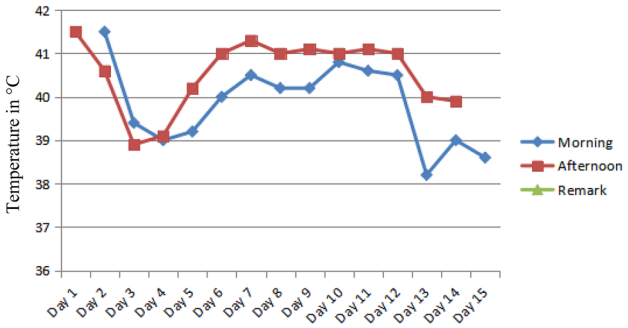

Figure 6. The daily rectal temperature of an experimentally infected calf with the LSD virus.

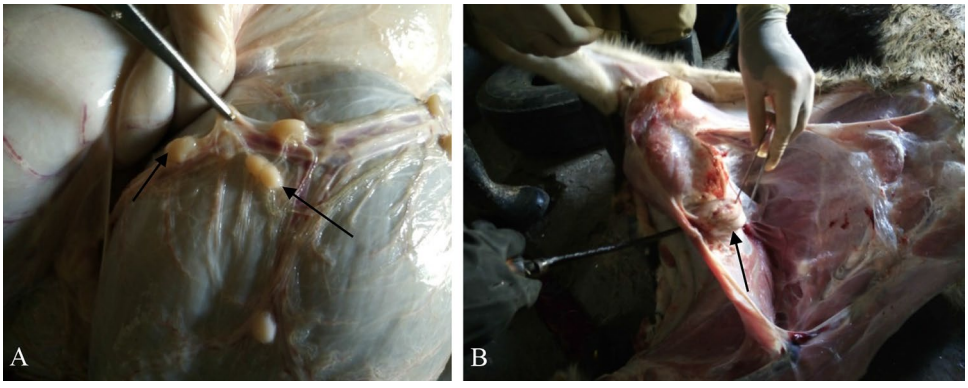

Figure 7. Gross lesions in cattle following experimental LSD virus infection. (**A**) Development of typical nodule on the rumen. (**B**) Swelling of the superficial lymph node.

cavity as shown in Table 1. The results of the rt-PCR of LSD virus shedded across mucosal membranes are described in Supplementary Fig. 3.

Discussion

Clinical signs, virus isolation, and PCR analysis all confirmed that LSD virus caused the outbreaks. The presence of LSD in the current study area indicates that this insidious disease continues to affect different parts of the country. Several authors have reported the occurrence of LSD outbreaks in different parts of Ethiopia at different times to support the current study[7,10,11]. Furthermore, LSD has been an epidemic in Ethiopia since 2008[11].

Depression, lacrimation, nasal discharge, loss of body condition, and circumscribed skin nodules over the skin were observed in the sick animals studied. These clinical manifestations were also observed in LSD affected animals[6,7,10–14]. Swelling limbs, lameness, and reluctant involvement of epithelial cells of the digestive organs and abortion have been reported on cross breeds[15].

The virus isolation method was used to determine the viability of the virus in the samples. LSDV can be propagated in a variety of primary cells or cell lines of bovine, ovine, or caprine origin. In the current study, primary lamb kidney and primary lamb testis cells were used for LSDV isolation as reviewed by[16]. Lumpy skin disease virus was isolated from skin nodule samples suspected of causing LSD in Amuru and Werejarso districts. Samples were passaged three times in primary lamb kidney and testis cells. LSDV CPEs on cell lines were characterized by rounding of single cells, aggregation of dead cells, and destruction of cell monolayer.

Previously described a species-specific real-time polymerase chain reaction (PCR) was used to examine the outbreak samples[17]. A gel-based PCR method was also used for differentiation of Sheep poxvirus (SPPV) and Goat poxvirus (GTPV)/LSDV isolate[18]. This is a published CaPV species-specific molecular assay[19–21]. To confirm the presence of the LSD virus genome, a Capripoxvirus detection method recommended by the World Organisation for Animal Health[1] and an LSDV specific gel-based PCR was used[22,23]. Following testing with conventional and real-time PCR methods, the isolates were identified as LSDV.

In this study, isolated LSDV was inoculated into calf to cause generalized skin lesions, and the virus shaded through mucous membrane at the onset of clinical signs and the level of disease development was measured at postmortem examination. Shedding of the virus was detected in nasal, oral, and conjunctival swabs from 6 to 14 days post infection using real-time PCR. LSDV shedding is low in body secretions, with the highest concentration of viral particles found in skin lesions, which is consistent with previous experimental findings[5,16]. The postmortem findings were consistent with what has previously been reported in the literature[24–26]. The experimental infection of a calf with an isolated infectious LSDV could serve as a foundation for future vaccine assessment research employing an LSDV challenge model. It is recommended that the experimental infection utilizing the infectious LSDV be repeated with a small number of calves with strict biosecurity measures, as using only one calf may be considered a study limitation. Considering clinical course and molecular data, the isolated virus signifies a useful candidate for LSDV challenge model in future vaccine studies. In endemic countries such as Ethiopia, control and prevention of LSD was undertaken mainly through vaccination. Due to the nature of the disease and its rapid spread into more countries and the lack of protection observed in countries such as Ethiopia with the locally produced vaccines, there has been increased interest and concern about the effectiveness of LSD vaccines in recent years[27]. As a result, studies should be conducted to evaluate the effectiveness of the LSD vaccines for disease control.

Materials and methods

Study area

From August to December 2019, the outbreak investigations study was conducted in Horo Gudru Wollega zone of Amuru district from one peasant association (Siden) and in Central Ethiopia North Shewa Zone of Wara Jarso district (Gohatsion) from four peasant associations (01, Jemo Berbeda, Wali-Chilelo and Lencho Bursu) of Oromia Regional State (Fig. 8). Amuru woreda/district is located 392 km from Addis Ababa. The district has altitudes ranging from lowland of 760 to midland of 2002 m above sea level (masl). The Woreda has moist and hot climate, with average annual temperature of 11.1 to 23.6 °C and rainfall ranging from 1167 to 1737.9 mm, respectively (Shambu metrological office, 2017). The district Wara Jarso is located in the North Shewa Zone. Kuyu borders it on the south, on the west by the Muger River, which separates it from the Horo Guduru Welega Zone, on the north by the Abay River, which separates it from the Amhara Region, on the northeast by the Jamma River, which separates it from Dera, and on the east by Hidabu Abote. Filiklik, Gohatsion, and Tullu Milki are among the towns in Wara Jarso. Geographically, the district is located between 10° 10" North latitudes and 38° 34" East longitudes. The district's average elevation is 1282masl. To obtain representative samples, study areas were chosen based on the presence of active LSD suspected outbreaks.

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Conjunctival swab				+ ve	+ ve	+ ve	+ ve	+ ve
Nasal swab						+ ve	+ ve	+ ve
Oral swab					+ ve	+ ve	+ ve	+ ve
Skin nodule (visual observation)			+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

Table 1. LSD virus shedding through mucus membranes as detected by rt-PCR. + ve Virus detected using rt-PCR.

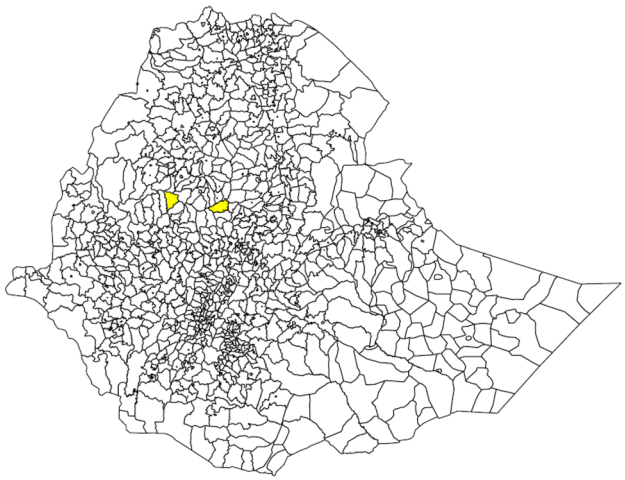

Figure 8. Map of Ethiopia with sample collection sites identified in yellow in the Amuru district of Horo Guduru Wellega zone and Wara Jarso district of North Shewa zone (generated using QGIS version 3.10).

Study animals

In the study areas, 39,432 cattle were at risk of LSD infection and 138 cattle were infected, with eight dead. The disease affected all age groups of both local and crossbred cattle managed by smallholder farmers with two to 20 cattle using extensive and semi-intensive management systems. This study focused on cattle with clinical signs of pox-like skin lesions.

Outbreak investigation and specimens' collection

Two natural outbreaks suspected of LSD in different geographical locations were chosen and investigated based on reports from the Oromia Regional State Agricultural Office Animal Health Directorate. During the visit, a visual inspection was performed to detect the presence of typical clinical signs of lumpy skin disease, and sick cattle were thoroughly examined. Twelve skin nodule samples were collected from twelve sick cattle in order to identify the causative agents (four from Amuru and eight from Wara Jarso). Following the procedures outlined in the WOAH, samples for virus isolation and antigen detection were collected from clinically sick animals (2017). Cleaning the area with 70% Ethanol and removing the hairs with a sterile scalpel blade, 12 skin nodules samples were collected aseptically from representative cattle that developed severe clinical signs of the disease. Antibiotics was administered topically on skin nodules to prevent secondary bacterial infections. Tissue samples were placed in a sterilized universal bottle containing phosphate buffered saline (PBS) with antibiotics and antifungals, and transported to the NVI virology laboratory using a cold chain system and stored at − 20 °C. Furthermore, data on clinical observations, the species and age of affected animals, and vaccination history were gathered. Supplementary Table 2 contains information on clinical observations, affected animal breed and age, and vaccination history.

Laboratory techniques

Sample processing

Skin biopsy samples were thawed at room temperature and washed three times with sterile phosphate buffer saline (PBS) containing antibiotics and antifungals in a Bio-safety cabinet class II. Tissue homogenates (10% w/v) were prepared in sterile PBS and centrifuged for 10 min at 4000xg in a refrigerated centrifuge. The supernatant was collected and stored at − 20 °C (WOAH, 2023). One ml of tissue homogenates was submitted for molecular analysis.

Virus isolation on primary lamb testis and lamb kidney

The supernatant from tissue homogenates was taken and inoculated onto a confluent monolayer of primary lamb testis and lamb kidney cells in a 25 cm^2 tissue culture flask containing 10 ml of Glasgow Minimum Essential Medium (Sigma Aldrich, Germany) supplemented with 2% fetal calf serum (Himedia). Cell cultures were incubated at 37 °C/5% CO_2 and observed daily for the development of LSDV specific cytopathic effects (CPE). When no CPE was observed after three blind passages, a sample was considered negative. Cell cultures that showed CPE were frozen at − 20 °C and thawed three times at room temperature to release the virus particle. Finally, the virus suspension was stored at − 80 °C until processed for DNA detection and animal experiment.

DNA extraction

DNA was extracted from tissue homogenate and infected cell culture suspension using DNeasy® Blood and Tissue kit (Qiagen, Germany) following the manufacturer's instructions. In a 1.5 ml eppendorf tube, 200 µl of tissue homogenate or cell culture suspension was transferred, and 20 µl proteinase K (QIAGEN protease) was added and thoroughly mixed. 200 µl AL buffer was added to the virus suspension and gently mixed by pulse-vortexing

and incubated in a water bath at 56 °C for 10 min. After adding 200 µl of 95% ethanol and thoroughly mixed, the mixture was transferred in to DNeasy mini column in a 2 ml collection tube and centrifuged at 8000xg for 1 min. The spin column was placed in to a new 2 ml collection tube and 500 µl of AW1 buffer was added and centrifuged at 6000xg for 1 min. The collection tube was discarded, and the mini spin column placed in a new 2 ml collection tube and 500 µl of AW2 buffer was added and centrifuged for 3 min at 6000xg. The mini spin column was carefully transferred into a new 1.5 ml of microcentrifuge tube, and 50 µl elution buffer added and incubated for 1 min at room temperature and centrifuged at 8000xg for 2 min. The spin column was discarded, and the microcentrifuge containing the eluted DNA was labeled and stored at − 20 °C freezer until tested by PCR.

Conventional PCR

The Capripoxvirus genome was detected using PCR with Capripox virus specific primers of SpGpRNAPol Forward: 5'-TAGGTGATTTTGGTCTAGCTACGGA-3′ and SPGpRNAPol-Reverse: 5′-AGTGATTAGGTGGTG TATTATTTTCC-3′ previously designed by[18]. PCR was performed in a 20 µl reaction volume containing 10 µl supper mix, 3 µl temple DNA, 2 µl forward primer, 2 µl reverse primer, and 3 µl RNase-free water. The PCR tube was placed in a thermal cycler, and amplification began. The program was as follows: initial denaturation at 95 °C for 4 min, followed by 40 cycles at 95 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for 5 min.

Agarose gel electrophoresis

To confirm the presence of DNA, amplified products were analyzed by agarose gel electrophoresis as described previously by[18,28]. A 3% agarose gel prepared in Tris Acetate EDTA (TAE). Aliquots of PCR products were analyzed using 3% agarose gel stained with GelRed (Biotium, inc.) for 1 h at 100 V. The DNA bands were visualized under UV transilluminator and photographed in gel documentation system (UVI TEC, UK). The PCR results were considered positive for LSD DNA when 172 bp was observed.

Real-time PCR

To confirm the Capripoxvirus identity and genotype of the field isolates, a recently developed species-specific real-time PCR method using unlabeled snapback primer and dsDNA intercalating dye assay targeting the CaPVRPO30 gene was used[21]. Real-time PCR was performed at the Molecular Biology Laboratory of the NVI using the amplification primers and PCR protocol described by[21]. Briefly, the PCR was set up in a reaction volume of 20 µL; where 4.84 µl of RNase-free water, 2µL of forward primer (CP-HRM-sb 5′-GGTGTAGTACGTATAAGA TTATCGTATAGAAACAAGCCTTTA-3′, 0.16µL reverse primer CP-HRM1 5′-AATTTCTTTCTCTGTTCC ATTTG-3′, 10µL of Safest EvaGreen IQ Super mix (BioRad) and 3 µL sample template. PCR was carried out with an initial denaturing step at 95 °C for 3 min, followed by 40 cycles at 95 °C for 15 s and 58 °C for 80 min using Low Profile Hard-Shell® 8-well PCR strips (BioRad). After denaturation at 95 °C for 1 min (held for 1 min), the product was cooled to 40 °C (held for 1 min), and heated continuously at 0.5 °C/10 s with fluorescence acquisition from 45 to 85 °C. Finally, for genotyping of the tested isolate, a pair of melting temperatures each for snapback tail and full amplicon were recorded as LSDV (50 °C/73 °C), GTPV (56 °C/72.5 °C), and SPPV (51 °C/72.5 °C).

Animal experiment

Animal inoculation, monitoring, sample collection, and postmortem examination

The calf was obtained from NVI dairy farm and has no history of LSD infection and vaccination. Due to a calf shortage, only one calf was used for the experimental infection. The calf was inoculated intradermally and intravenously in the neck area on the right side with $10^{3.9}$ 50% tissue culture infectious dose (TCID50). Rectal temperatures were taken every day, and the calf was examined for clinical signs such as the development of lesions at the inoculation site and elsewhere on the body. On 2, 4, 6, 8, 10, 12, and 14 days post inoculation (dpi) nasal, conjunctival, and oral swabs were collected and placed in 1.25 ml of viral transport medium (phosphate buffered saline containing 1% (w/v) bovine serum albumin, 200 U/ml penicillin, 200 µg/ml streptomycin, 50 µg/ml gentamicin and 5 µg/ml amphotericin B).

Serum samples were taken at defined time points during the animal trial to examine sero-conversion. Sampling was performed on 0, 7, 14, 21, 28, and 35 dpi. During necropsy, the following organ samples were taken: lung tissue and trachea, mandibular, cervical, and mesenteric lymph nodes. In addition, samples from skin areas displaying characteristic nodular lesions were also taken. On the 15th dpi, the calf was euthanized by cutting the neck and examined for any gross pathological lesions. The necropsy samples were collected aseptically and meticulously. To access all tissues sampled, separate sterile scissors and forceps were used, and tissues were harvested onto individual sterile disposable universal bottles for further processing with separate sterile forceps and scalpels.

Data analysis

Data collected during observation from all field and laboratory investigations, such as clinical signs while investigating the outbreak, sample collection, virus isolation using cell culture, and CaPV targeted gene amplification using classical and real-time PCR, was coded, stored, and analyzed in Excel spreadsheets. The data was interpreted and presented in biological terms, such as an increased body temperature, depression, decreased in feed intake, nasal and ocular discharges, and nodular skin lesions.

Ethics approval and consent to participate

The study is reported in accordance with ARRIVE guidelines. Animal experiment was carried out in accordance with the international guidelines for care and handling of experimental animals. The Animal Research Ethic Committee of the National Veterinary Institute reviewed and approved the protocol. The calf for the experiment was obtained from NVI's dairy farm.

Data availability

All data generated or analyzed during this study are available upon request from the corresponding authors.

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References

1. World Organisation for Animal Health (WOAH). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. WOAH/OIE, Paris. 2023, Chapter 3.4.12, pp: 1–16. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.04.12_LSD.pdf.
2. Tuppurainen, E., Venter, E. & Coetzer, J. A. W. The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. *Onderstepoort J. Vet. Res.* **72**, 153–164 (2005).
3. Davies, F. G. Lumpy skin disease, an African Capripoxvirus disease of cattle. *Vet. J.* **147**, 489–502 (1991).
4. Barnard, B. J., Munz, E., Dumbbell, K. & Prozesky, L. Lumpy Skin Disease. In *Infectious Disease of Livestock with Special Reference to Southern Africa* (eds Coetzer, J. A. W. *et al.*) 604–612 (Oxford University Press, Cape Town, 1994).
5. Babiuk, S., Bowden, T., Boyle, D., Parkyn, G. & Copps, J. Quantification of lumpy skin disease virus following experimental infection in cattle. *Transbound. Emerg. Dis.* **55**, 299–307 (2008).
6. Coetzer, J. A. W. Infectious Diseases of Livestock. In *Lumpy Skin Disease* (eds Coetzer, J. A. W. & Tustin, R. C.) 1268–1276 (Oxford University Press, Cape Town, 2004).
7. Gari, G., Waret-szkuta, A., Grosbois, V., Jacquiet, P. & Roger, F. Risk factors associated with observed clinical lumpy skin disease in Ethiopia. *Epidemiol. Inf.* **138**, 1657–1666 (2010).
8. Brenner, J. *et al.* Appearance of skin lesions in cattle populations vaccinated against lumpy skin disease: Statutory challenge. *Vaccine* **27**(10), 1500–1503 (2009).
9. Coakley, W. & Capstick, P. B. Protection of cattle against lumpy skin disease. Factors affecting small scale production of tissue culture propagated virus vaccine. *Res. Vet. Sci.* **2**, 369–371 (1961).
10. Gelaye, E. *et al.* Capripox disease in Ethiopia: Genetic differences between field isolates and vaccine strain, and implications for vaccination failure. *Antiviral Res.* **119**, 28–35 (2015).
11. Ayelet, G. *et al.* Lumpy skin disease: Preliminary vaccine efficacy assessment and overview on outbreak impact in dairy cattle at Debre Zeit, central Ethiopia. *Antiviral Res.* **98**, 261–265 (2013).
12. Alaa, A., Hatem, M. & Khaled, A. Polymerase chain reaction for rapid diagnosis of a recent lumpy skin disease virus incursion to Egypt. *Arab J. Biotechnol.* **11**, 293–302 (2008).
13. Salib, F. A. & Osman, A. H. Incidence of lumpy skin disease among Egyptian cattle in Giza governorate. *Egypt. Vet. World* **4**(4), 162–167 (2011).
14. Ayelet, G. *et al.* Lumpy skin disease in cattle in central Ethiopia: Outbreak investigation and isolation and molecular detection of the virus. *Rev. Sci. Tech. Off. Int. Epiz.* **33**(3), 877–887 (2014).
15. Babiuk, S., Bowden, T., Boyle, D., Wallace, D. & Kitching, R. Capripoxviruses: An emerging worldwide threat to sheep goats and cattle. *Transbound. Emerg. Dis.* **55**, 263–272 (2008).
16. Bowden, T. R., Babiuk, S. L., Parkyn, G. R., Copps, J. S. & Boyle, D. B. Capripox virus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats. *Virology* **371**, 380–393 (2008).
17. Lamien, C. E. *et al.* Use of the Capripox virus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: Development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus. *Vet. Microbiol.* **149**, 30–39 (2011).
18. Grooms, D. Raising awareness about lumpy skin disease department of large animal clinical sciences. *Michigan Dairy Rev.* **48**, 824–1225 (2005).
19. Lamien, C. E. *et al.* Real time PCR method for simultaneous detection, quantitation and differentiation of Capripoxviruses. *J. Virol. Methods* **171**, 134–140 (2011).
20. Le Goff, C. *et al.* Capripoxvirus G-protein-coupled chemokine receptor, a host-range gene suitable for virus-animal origin discrimination. *J. Gen. Virol.* **90**, 67–77 (2009).
21. Gelaye, E. *et al.* Development of a cost-effective method for Capripox virus genotyping using snapback primer and dsDNA intercalating dye. *PLoS One* **8**, e75971 (2013).
22. Ireland, D. C. & Binepal, Y. S. Improved detection of capripoxvirus in biopsy samples by PCR. *J. Virol. Methods* **74**, 1–7 (1998).
23. Stram, Y. *et al.* The use of lumpy skin disease virus genome termini for detection and phylogenetic analysis. *J. Virol. Methods* **151**, 225–229 (2008).
24. Prozesky, L. & Barnard, B. J. H. A study of the pathology of lumpy skin disease in cattle. *Onderstepoort J. Vet. Res.* **49**, 167–175 (1982).
25. Nagi, A. A. Lumpy skin disease: Cutaneous and testicular lesions. *Assiut. Vet. Med. J.* **23**, 90–99 (1990).
26. Annandale, C. H., Irons, P. C., Bagla, V. P., Osuagwuh, U. I. & Venter, E. H. Sites of persistence of lumpy skin disease virus in the genital tract of experimentally infected bulls. *Reprod. Domest. Anim.* **45**, 250–255 (2010).
27. Gari, G. *et al.* Evaluation of the safety, immunogenicity and efficacy of three Capripoxvirus vaccine strains against lumpy skin disease virus. *Vaccine* **33**, 3256–3261 (2015).
28. Mangana-Vougiouka, O. P., Markoulatos, G., Nomikou, K., Bakandritsos, N. & Papadopoulos, P. Sheep poxvirus identification from clinical specimens by PCR, cell culture, Immunofluorescence and agar gel immunoprecipitation assay. *Mol. Cell Probes* **14**, 310–305 (1999).

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Author contributions

KA, EG conceived and designed the experiments; KA, TA, BG, HM, GD, MZ, WC, EG performed the experiments and analyzed the data; TA, EG contributed reagents/materials and supervised the study; KA wrote the paper; EG edited the final manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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