A Systemic Review of Lumpy Skin Disease Virus (LSDV) And its Emergence in Pakistan

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ABSTRACT

Lumpy skin disease (LSD) is an infectious disease caused by the virus lumpy skin disease virus (LSDV). The family of LSDV is Poxviridae and the genus Capripoxvirus (CaPV). The GTPV (goat pox virus) and SPPV (sheep pox virus) also belong to the same genus. LSDV causes disease in livestock animals except for dogs. LSDV causes vast economic losses in the country in the livestock industry and dairy industries. LSDV also affects the industries belonging to these industries like the leather industry. The sequence of 21 strains of LSDV were taken from NCBI database and their fasta files were retrieved. After that, phylogenetic analysis was performed using these sequences. This study provides an overall overview of the lumpy skin disease (LSD) its genome, causative agent, transmission, epidemic, molecular characterization, phylogenetic analysis, control, and treatment of the LSDV. We also give a short review of the emergence of LSD in Pakistan.

Keywords: Lumpy skin disease; Lumpy skin disease virus (LSDV); Livestock animals.

1. Background

Lumpy skin disease (LSD) is an animal ailment resulting from the lumpy skin disease virus (LSDV). LSDV belongs to the Capripoxvirus (CaPV) genus within the Poxviridae family. This virus is characterized by its stable and large double-stranded DNA structure. Although sharing its genus with other viruses such as goat pox virus (GTPV) and sheep pox virus (SPPV), LSDV is phylogenetically distinct from them [1]. The initial recorded occurrence of LSD dates back to 1929 in Zambia, subsequently spreading across South-Eastern European nations, Sub-Saharan Africa, and various Asian countries. In Asia, the first documented emergence of LSD was reported in Bangladesh, subsequently extending its presence to Nepal, Bhutan, Hong Kong, India, Vietnam, Thailand, and Myanmar. The Sindh Livestock Department declared an epidemic of LSD after around 36,000 cattle were infected by the conclusion of April 2022, resulting in a mortality rate of 0.8%. The emergence of LSD had a severe impact on five million dairy meat vendors and dairy farmers, leading to substantial economic consequences. Additionally, there is a concern that the virus can potentially transmit to humans through the consumption of meat and milk from infected animals [2]. Lumpy skin disease (LSD) is an infectious disease caused by the lumpy skin disease virus (LSDV), which belongs to the Poxviridae family and the Capripoxvirus (CaPV) genus. This virus affects various animals except for dogs. The Poxviridae family comprises two subfamilies: Entomopoxviridae, which infects invertebrate hosts, and Chordopoxviridae, which causes diseases in vertebrate hosts. Within the Chordopoxviridae subfamily, there are ten genera, including the Capripoxvirus genus. This genus consists of three species: lumpy skin disease virus (LSDV), sheeppox virus (SPPV), and goat poxvirus (GTPV). LSDV primarily infects sheep, goats, and cattle [3].

Lumpy skin disease (LSD) is an emerging viral disease with the potential to become epidemic, spreading beyond localized outbreaks. The primary clinical manifestation is the presence of nodular lesions on the skin and mucous membranes of infected animals [4]. These skin nodules typically develop on various parts of the cattle's body, including the head, neck, back, perineum, and breast. Additionally, affected cattle may experience varying degrees...
of leg edema and lameness [5]. In live animals, LSD often manifests with symptoms including mucosal ulcerations, high fever, and enlarged lymph nodes. Mucopurulent nasal discharge is not a characteristic clinical symptom of LSD [6]. While a significant number of affected cattle can recover from the disease after a prolonged illness, they may experience long-term complications such as mastitis, pneumonia, and the development of deep skin lesions. Given its highly contagious nature, the World Organization for Animal Health (OIE) mandates that LSD is a notifiable communicable disease. LSDV can spread through various means, including indirect contact between animals, transmission via vectors, lactation, blood-feeding insects, semen, and iatrogenic transmission [7].

2. Genome of LSDV

Lumpy skin disease virus (LSDV) is a member of the capripoxvirus genus within the Poxviridae family and causes a significant disease in cattle in Africa. The total genome of LSDV is around 151-kbp, which consists of a central coding region flanked by identical 2.4 kbp-inverted terminal repeats and contains 156 putative genes. Comparative analysis with other chordopoxviruses reveals conserved genes involved in various viral processes, including transcription, replication, protein processing, virion assembly, virulence, and host range.

LSDV shares similarities with mammalian poxviruses in the central genomic region, while exhibiting distinct differences in the terminal regions, particularly in genes associated with virulence and host range. Notably, LSDV also contains genes found in other poxvirus genera, such as interleukin-10 (IL-10), IL-1 binding proteins, G protein-coupled CC chemokine receptor, and epidermal growth factor-like protein. These findings highlight LSDV's unique gene repertoire responsible for its specific host range and virulence characteristics. Even though, LSDV shows similarities with leporipoxvirus. This study shows the relationship between LSDV and members of the Chordopoxivirinae [8].

3. Symptoms of LSDV

LSDV infections exhibit a wide range of clinical signs, varying from subclinical cases to severe outcomes leading to death. Common clinical manifestations include the onset of fever, the emergence of nodules on the skin, the presence of lesions in the mouth and pharynx, enlargement of superficial lymph nodes, skin edema, and, in some cases, fatality. Following an initial incubation period of 6 to 9 days in acute cases, affected animals experience a significant rise in body temperature, with fever exceeding 41°C. The diversity in clinical signs highlights the complexity and varying outcomes of LSDV infections, emphasizing the importance of early detection and appropriate management to minimize the impact on livestock populations [9]. Infection with the lumpy skin disease virus (LSDV) leads to virus replication, fever, and the formation and progression of nodules. Experimental studies have revealed the following events following virus inoculation into the host. Swelling of nodules (1-3cm) occurs at 4-7 days post-infection (dpi). Shedding of the virus through oral and nasal discharge takes place between 6-18 dpi. Development of nodules and lymphadenopathy is observed at 7-19 dpi. Furthermore, after 42 days of infection, the presence of the virus in semen has been detected [10].

4. Stability of Virus

Lumpy skin disease virus (LSDV) is the causative agent of lumpy skin disease (LSD), and it belongs to the family of double-stranded DNA viruses with enveloped linear DNA. The serogroup known as "Neethling" was initially
identified in South Africa and shares close properties with the sheep and goatpox virus [11] Lumpy skin disease virus (LSDV) shows higher susceptibility to acidic pH or high alkalinity, although it remains stable within a pH range of 6.6 to 8.6. The virus can be effectively inactivated using different detergents such as 20% chloroform, 1% formalin, and liquid solvents containing detergents. Additionally, ultraviolet light can degrade LSDV, which is why the vaccine is prepared and stored in dark glass bottles to protect it from light exposure [12].

5. Transmission of LSDV

Lumpy skin disease virus (LSDV) primarily affects cattle and water buffaloes as host-specific viruses. While the infection rate in buffaloes is lower compared to cattle, LSDV can also affect goats and sheep to some extent. Clinical signs of the disease have been observed in giraffes (Giraffa camelopardalis) and impalas (Aepyceros melampus), and cases of LSD have been reported in Arabian oryx (Oryx leucoryx) and springboks (Antidorcas marsupialis). It's important to note that LSDV does not pose a risk of infection to humans [1]. To investigate the transmission of LSDV among animals without the involvement of a vector, a 60-day experiment was conducted. The study involved inoculating bulls with a vaccine-derived virulent recombinant strain of LSDV (Saratov/2017) and placing them in a vector-free environment. Two groups of animals, namely C1 and C2, were utilized in the experiment. In the first trial, C1 was in direct contact with the inoculated bull, followed by the introduction of C2 for a duration of 33 days. Through the use of molecular tools, the infection was confirmed in both groups of animals through serological, clinical, and virological assessments [13].

Figure 1. The transmission mechanism of LSDV through vector and without vector [11]

LSDV primarily spreads through arthropod vectors. Outbreaks of LSDV often coincide with wet and warm seasons, and mosquitoes, particularly Aedes aegypti, serve as efficient mechanical vectors for transmitting and maintaining the virus. It is important to note that direct and indirect contact between infected and susceptible
animals is not considered a significant pathway for transmission. While LSDV can infect sheep and goats, it does not typically cause clinical disease in these animals. However, the contact between cattle herds and sheep or goats in grazing or watering areas has been identified as a potential risk factor for the mechanical transmission of LSDV [14].

6. Disease Cycle

Lumpy skin disease (LSD) has been observed exclusively in cattle. The incubation period for the disease typically ranges from 4 to 12 days. Clinical symptoms begin with a fever of 40-41.5°C, which lasts for 1-3 days. Concurrently, there is an increase in nasal and pharyngeal secretions, excessive tearing, lymph node enlargement, reduced appetite, decreased milk production (dysgalactia), overall depression, and a reluctance to move [15]. Within 1-2 days, skin nodules begin to emerge, gradually becoming hardened and necrotic, causing significant discomfort, pain, and lameness. Over a span of 2-3 weeks, these nodules either regress or lead to necrosis of the skin, resulting in raised areas (known as sit-fasts) that are clearly distinguishable from the surrounding skin. Some sit-fasts may slough away, leaving behind deep holes in the skin, making it susceptible to bacterial infections or infestation by maggots. Severe emaciation can occur in some affected animals, and in such cases, euthanasia may be considered. It is important to note that bulls may experience temporary or permanent infertility and can shed the virus for an extended period. The morbidity rate in LSD varies from 50% to 100%, while the mortality rate is typically low, ranging from 1% to 5%, although occasional reports have indicated higher mortality rates [16].

7. Worldwide Outbreak

A skin disease known as 'pseudo urticaria' was initially reported in cattle in 1929 in Northern Rhodesia (now Zambia) [17]. By the 1940s, the disease had spread to other southern African countries, gradually extending its reach northwards. Currently, lumpy skin disease (LSD) is prevalent throughout Africa, including Madagascar, with only a few countries in the region remaining free from the disease. LSD has demonstrated increased pathogenicity over time, leading to extensive epidemics and pandemics across the continent, with sporadic cases occurring during non-epidemic years. The first LSD outbreak in Egypt was recorded in May 1988, possibly linked to high insect populations [18] In August 1989, the disease made its first appearance outside of Africa, reaching Israel, potentially transmitted by stable flies [19]. After a 17-year absence, LSD reemerged in Egypt in 2006, introduced by infected cattle from the African Horn countries. The disease rapidly spread throughout Egypt despite vaccination efforts. LSD outbreaks have also been reported in various Middle Eastern countries since 1990, possibly facilitated by the importation of live animals and animal products. Factors such as dense cattle populations, favorable arthropod environments, uncontrolled animal movements, and limited animal health resources contribute to the spread of the disease within the region. The politically unstable nature of the Middle East and inadequate communication between countries further increase the risk of LSD spreading to neighboring regions [20].

In 2019, multiple outbreaks of LSD occurred across the Asia and Pacific region, affecting countries such as India, China, Nepal, Sri Lanka, Bhutan, Thailand, and Pakistan, with a significant outbreak in Bangladesh [21]. A notable recent outbreak occurred in mid-2022 in the states of Gujarat and Rajasthan, India, spreading to seven states and resulting in the unfortunate death of over 80,000 cattle within a brief three-month period. The mortality rate of this current outbreak in India has been reported as high as 15%, with Rajasthan being particularly affected. It is worth
noting that during a previous LSD outbreak in India in 2019, the morbidity rates were reported to be as low as 7.1%, with no recorded mortality [22]. These recent outbreaks highlight the significant impact and varying severity of LSD on cattle populations in the region [23].

Figure 2. Lumpy skin disease prevalence worldwide and over time from 1929 to 2022

The impacted nations are shown in yellow circle between 1929 and 1970, yellow square between 1971 and 1988, blue rhombus between 1989 and 2011, and red mark between 2012 and 2022. This map was created using Goodnotes app.

(a) Outbreak in Pakistan

In November 2021, the first recorded case of LSD in Pakistan emerged in the district of Jamshoro, Sindh. The outbreak affected a substantial number of animals, with approximately 36,000 infections reported. By April 2022, the severity of the situation prompted the Sindh Livestock Department to declare it an epidemic, as the disease posed a significant threat to the livestock industry. Unfortunately, the absence of a vaccine against LSD and the challenges associated with controlling the movement of animals contributed to the spread of the disease across other provinces in Pakistan. The consequences of the outbreak had a detrimental impact on the livestock industry, causing economic instability. Despite these challenges, the mortality rate remained relatively low, allowing for a significant proportion of infected animals to recover. To mitigate the impact of the outbreak, various treatment and control measures were implemented. These included conducting breeding tests on bulls to identify potential carriers, administering antibiotics, vitamin injections, and glucose to alleviate symptoms and reduce body temperature in affected animals. Implementing proper care practices became crucial in managing the disease's effects and minimizing its spread [24].

(b) Economic losses in Pakistan

LSD's emergence in a country like Pakistan, heavily reliant on agriculture and home to the world's second-largest cow population, would be catastrophic. With 49.6 million cattle and 41.2 million buffaloes, the livestock sector is a
vital component of Pakistan's economy, contributing significantly to its GDP. The livestock trade engages around 8 million households, comprising 35%–40% of their income. The economic impact of such a devastating disease on a nation already grappling with a fragile economy could be severe and long-lasting. Anticipated livestock trade restrictions and a decline in the rural economy would further exacerbate the challenges faced by eight million families [25]. The widespread impact of LSDV on more than 190,000 animals in Pakistan has far-reaching implications for the nation's economy. As the third-largest milk-producing country globally, Pakistan's annual milk production exceeds 47 million tons. However, with LSD-infected cows unable to produce milk for extended periods, there will be a significant reduction in milk production. Even after recovery, it will take considerable time for these cows to regain their previous production levels, further affecting the country's economy [4]. To estimate the financial losses faced by Pakistan, we can draw insights from the experience of Ethiopia, which reported a median financial loss of USD 375 per deceased animal and a financial loss of USD 141 in milk production per affected cow. Such losses can have a profound impact on the overall economic situation of Pakistan, underscoring the need for effective measures to control and mitigate the effects of LSDV outbreaks [26].

(c) Call of Action (Why it is disappeared?)

To combat the spread of LSD in cattle and buffaloes, Pakistan's Ministry of National Food Security and Research has established a task force to develop preventive measures. Importing 500,000 vaccinations is a key component of their strategy, but in the interim, the effective "Goat Pox" vaccine is being used as a temporary substitute [35]. A ban on livestock markets has been imposed, and cattle owners are advised to separate sick animals from healthy ones and regularly use anti-mosquito spray to protect their livestock. Special teams are being dispatched to dairy farms for vaccination campaigns. Awareness campaigns are being conducted nationwide, and in Sindh, 30,000 vaccines were administered in Karachi. Restricting cattle movement, ensuring veterinary certificates accompany authorized movements, and treating animals with insect repellents are essential preventive measures. Vector control by eliminating breeding grounds like stagnant water and improving farm drainage is recommended. Education campaigns target veterinarians, students, farmers, truck drivers, and inseminators. Vigilant surveillance includes clinical monitoring, laboratory testing, and thorough cleaning and disinfection of affected areas [1] vaccination, with a commercially available live attenuated vaccine estimated to be 75% effective, is crucial for outbreak prevention. Uniform vaccination coverage across all areas is vital to avoid pockets of unvaccinated farms [26].

8. Special About Epidemic Strains

In the past decade, there has been a growing focus on sequencing historical and emerging LSDV strains worldwide, particularly following the incursion of LSDV into Europe. Characterizing and classifying these strains often relies on the sequencing of one or a limited number of genomic regions, such as GPCR and RPO30. However, this approach provides low-resolution or poorly resolved phylogenetic trees, limiting the detailed analysis of phylogenetic relationships. The recent advancements in third-generation sequencing methods have played a significant role in providing more whole genome sequences (WGS) of LSDV strains. This availability of WGS data has opened up opportunities for more comprehensive and detailed analyses of LSDV strain patterns and their phylogenetic relationships [27]. Various methods, including PCR, real-time PCR, and HRM, have been developed.
for the detection of the LSDV genome. Molecular epidemiological studies of LSDV often involve analyzing different genomic regions, such as the GPCR, RPO30, P32, and EEV glycoprotein genes. These regions serve as targets for specific molecular assays, allowing for the identification and characterization of LSDV strains. By analyzing these genomic regions, researchers gain valuable insights into the genetic diversity and evolution of the virus, contributing to our understanding of its epidemiology [9].

9. Molecular Characterization of LSDV

Laboratory tests can be conducted to confirm the presence of lumpy skin disease (LSD) by detecting the viral DNA or proteins. Polymerase chain reaction (PCR) techniques, including real-time PCR and conventional PCR, can be employed for this purpose. These tests enable the detection and identification of the lumpy skin disease virus (LSDV) with high specificity and accuracy [28] Samples, including scab, nasal swab, skin nodules, and serum, were processed at the Central Veterinary Laboratory (CVL). The skin nodules were minced and homogenized in phosphate-buffered saline (PBS). Scabs and nasal swabs were collected in PBS, centrifuged, and the resulting supernatant was transferred to sterile vials. DNA extraction from 200 μL of the supernatant was performed. The eluted DNA was stored at -80 °C until further analysis. A Snapback assay, designed to detect and genotype capripoxviruses, was utilized for the collected samples [29].

(a) PCR

Specific primers were used in the assay to distinguish the genotypes of capripoxviruses (SPPV, GTPV, or LSDV) based on differences in the melting temperature (Tm) of the snapback stem and PCR amplicons. The PCR reaction was carried out in a 20μL volume, comprising iQsupermix (Bio-Rad), Snapback forward and reverse primers, and template DNA. The amplification protocol consisted of denaturation, cycling, and melting steps, followed by fluorescence readings. Additionally, four genes (RPO30, GPCR, EEV glycoprotein, and CaPV homolog of the variola virus B22R genes) were amplified by PCR to confirm the identity and further characterize the LSDV positive samples. Gel electrophoresis was performed to visualize the PCR products on a 2% gel, which were then analyzed using a Gel Documentation System [30].

(b) ELIZA Test

The Capripoxvirus P32 protein-coding gene was cloned and expressed in Escherichia coli as a fusion protein with glutathione-S-transferase. The expressed protein was purified using glutathione Sepharose. To screen for antibodies against capripoxviruses, an indirect enzyme-linked immunosorbent assay (ELISA) was employed. Samples obtained from experimentally infected animals were tested using ELISA and virus neutralization test (VNT). The results showed that ELISA is a more sensitive test for detecting antibodies at earlier stages of infection compared to VNT [31].

10. Phylogenetic Analysis

A comprehensive phylogenetic analysis of 21 LSDV strains from different countries was conducted using sequences obtained from the NCBI database. The first step involved retrieving the fasta files for these LSDV strains. Multiple sequence alignment was performed using the ClustalW algorithm, which allowed for the alignment of the sequences based on their similarities and differences. Following the alignment, a phylogenetic tree
was constructed using the neighborhood joining method, employing FastTree as the tree-building algorithm. This method enabled the estimation of evolutionary relationships among the LSDV strains based on the sequence data. To visualize the phylogenetic tree, iTol (Interactive Tree of Life) was employed, providing an interactive and visually appealing representation of the evolutionary relationships between the LSDV strains. Through this phylogenetic analysis, valuable insights can be gained into the genetic relatedness and diversity of LSDV strains from different geographic regions, aiding in understanding the evolutionary dynamics and potential spread of the virus.

![Phylogenetic tree](image)

**Figure 3.** Multiple sequence alignment was performed using clustalw and phylogenetic tree constructed using neighborhood joining method using FastTree. Tree visualization performed using iTol

### 11. Treatment and Control

Currently, there is no specific treatment available for lumpy skin disease (LSD). The primary approach involves providing supportive care and implementing proper wound care measures to prevent bacterial infections. Antibiotics may be used to address secondary infections. Additionally, efforts should be made to help control the animal's body temperature, as LSD can cause high fever. Cooling methods and techniques can be employed to help regulate the animal's temperature and provide comfort [32].

Currently, there is no specific antiviral drug available for the treatment of LSDV. Supportive treatment measures involve the administration of antibiotics, vitamin injections, and anti-inflammatory drugs. It is important to isolate sick animals from healthy ones to prevent the spread of the disease. The mortality rate among affected animals is estimated to be around 3%, with the majority of animals recovering from the disease [33]. Arthropods such as biting flies, ticks, and mosquitoes serve as vectors for the transmission of LSDV. To control the spread of the virus, it is crucial to create a vector-free environment for infected animals. Immunization against LSDV involves two approaches. In South Africa, the Neethling strain of
LSDV undergoes a process of attenuation through 20 passages in the chorio-allantoic layers of a hen's egg. However, this method is still under development. In Kenya, a vaccine derived from goat pox and sheep poxvirus is utilized to provide solid immunity against LSD in cattle. It is important to note that this vaccine can only be used in countries where sheep pox and goat poxvirus are endemic [32].

12. Future Aspects

Attenuated vaccine strains of LSDV have gained popularity as recombinant vaccine vectors for targeting both LSDV and other pathogens, including human infectious agents. Traditionally, these vaccine strains and recombinants were generated in primary lamb testis cells, MDBK cells, or eggs. However, these methods have drawbacks such as laborious processes, potential pathogen introduction, and the presence of bovine viral diarrhea virus in MDBK cells. This study demonstrates the growth of an attenuated LSDV strain in BHK-21 cells, a type of baby hamster kidney cells. A recombinant LSDV vaccine was successfully generated in BHK-21 cells. Limited growth was also observed in RK13 cells, rabbit kidney cells, when the vaccinia virus host range gene K1L was expressed. Although growth was limited, the expression of K1L served as a positive selection marker for generating recombinant LSDV vaccines in RK13 cells. This simplified approach for generating (recombinant) LSDV vaccines holds promise for future livestock vaccine development, and with BHK-21 cells approved for good manufacturing practices, it can be expanded to human vaccines as well [34].

Declarations

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Authors have declared no competing interests.

Consent for Publication

The authors declare that they consented to the publication of this research work.

Author’s Contribution

All the authors took part in data collection and manuscript writing equally.

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