

A CLINICAL AND EPIDEMIOLOGICAL STUDY OF LUMPY SKIN DISEASE IN CATTLE IN SULAYMANIYAH PROVINCE

ARI SARDAR ¹; RIZGAR R. SULAIMAN ²; KWESTAN N. ALI ²;
HARDI F. MARIF ²; BASIM A. ALI ²; DANA O. ISMAEEL ³ AND
MUHAMAD O. BABA SHEKH ⁴

¹ Directorate of Veterinary in Sulaimani, Salim Street, Sulaimaniyah, Kurdistan Region, Iraq

² Department of Clinic and Internal Medicine, College of Veterinary Medicine, University of Sulaimani; Sulaimani, Kurdistan Region, Iraq.

³ Department of Surgery and theriogenology, College of Veterinary Medicine, University of Sulaimani, Sulaimani, Kurdistan Region, Iraq.

⁴ University of Human Development, Sulaymaniyah, Kurdistan Region, Iraq.

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ABSTRACT

Lumpy Skin Disease (LSD) is a highly contagious and virulent viral infection that affects cattle and leads to enormous financial losses and economic impacts. The objective of this study was to examine the clinical and epidemiological features of LSD, with specific attention to the effects of season, breed, sex, and mortality rates. A comprehensive examination was conducted on a total of 387 cattle showing exhibited clinical symptoms of LSD, including excessive tearing, swelling of the legs, and the presence of nodules. A biopsy was obtained and subjected to polymerase chain reaction (PCR) analysis to verify the presence of infection. The findings revealed that rates of LSD infection exhibited significant seasonal variation, reaching their highest point in spring (50%) and declining to the lowest level in autumn (5%). Crossbreeds had a 32% infection rate, whereas local breeds had a higher rate of 68%. The study noted a significant disparity in infection rates between genders, impacting 12% of males and 88% of females and a mortality rate of 20%. These results underscore the significant influence of breed, gender, and seasonal fluctuations on vulnerability to LSD, underscoring the necessity of specific control methods to reduce the disease's transmission and consequences.

Keywords: Lumpy skin diseases, PCR, mortality rate, clinical and epidemiological features.

INTRODUCTION

Lumpy skin disease (LSD) is a highly contagious, notifiable, and emerging viral disease (Shah *et al.*, 2023), affecting cattle and Asian water buffaloes caused by the

nodular skin disease virus (LSDV) (Afzal *et al.*, 2024), of the genus *Capripoxvirus*, subfamily *Chordopoxvirinae*, *Poxviridae* family (Gupta *et al.*, 2020, Bodkhe *et al.*, 2023). It affects a variety of domestic cattle, buffaloes, sheep, and goats (El-Nahas *et al.*, 2011; Alkhamis and Vander Waal, 2016). LSD is most prevalent during the humid summer and autumn months, particularly in low-lying areas and along waterways (Banata and Geinoro, 2024). The disease was first recorded in Zambia in 1929 and later spread

Corresponding author: Hardi F. Marif

E-mail address: hardi.marif@univsul.edu.iq

Present address: Department of Clinic and Internal Medicine, College of Veterinary Medicine, University of Sulaimani; Sulaimani, Kurdistan Region, Iraq.

to countries in sub-Saharan Africa, southeastern Europe, and Asia (Namazi and Khodakarem Tafti, 2021).

Al-Salhi and Hussein, 2013, recorded the first outbreak of nodular skin disease in Iraq and confirmed LSDV by polymerase chain reaction method first in Nineveh Governorate, and then they discovered it in some Iraqi provinces in 2014 (Al-Salhi and Hassan, 2015). As for the Kurdistan Region, the first case was confirmed in 2013 in Sulaimaniyah Governorate in Zahrawa district and Sulaimaniyah Veterinary Laboratory by polymerase chain reaction as well as partial sequencing of LSDV virus registered in NCBI under accession number (KF996498).

According to the World Health Organization, the countries surrounding Iraq and the Kurdistan region (Turkey and Syria) recorded LSDV in 2013, and due to the uncontrolled animal trade and the long incubation period of LSDV, the disease can be transmitted to our region (Tuppurainen and Oura, 2012). Fever (41°C), swollen lymph nodes, streptococcal lesions on the skin (up to 5 cm in diameter), mucous membranes of the respiratory and gastrointestinal tracts, leg and chest edema, lymphadenitis, and sometimes death are the main symptoms of the disease (Das *et al.*, 2021; Afzal *et al.*, 2024; Banata and Geinoro, 2024). LSDV is transmitted by indirect contact and arthropod bites (Nesterov *et al.*, 2022). While direct and indirect contact are the most effective modes of virus transmission, LSDV is mainly spread mechanically by blood-feeding arthropods (Hamdi *et al.*, 2021).

Viruses can spread by touch, water, and food, but insects such as flies, mosquitoes, and ticks spread them in the first place (EFSA Panel, 2015). Advanced technologies such as virus isolation and culture, serological tests, polymerase chain reaction (PCR), point macular hybridization (DBH), agar gel immunoglobulin tests (AGID), enzyme-

linked indirect immunosorbent assays (ELISA), transmitting electron microscopy (TEM), immunoperoxidase staining (IMP), direct fluorescent antibody tests, western blotting, routine histology pathology, and pathological immunostaining can all be used *in vitro* to aid in confirmatory diagnosis (EFSA Panel, 2015; Kale *et al.*, 2023).

Antibiotics against secondary skin infections, pneumonia, and some anti-inflammatory drugs may be given to infected animals as supportive care, as certain antiviral drugs are not available (Vinothraj *et al.*, 2020). Skin lesions can also be treated with fly-repellent antiseptic ointment, which may be a wise choice in the tropics (Islam *et al.*, 2021). Pest control, quarantine, population evacuation, disinfection, and disinfection of infectious farms/herds are useful ways to reduce the disease burden, but vaccination is the most effective prevention and control strategy (Imran *et al.*, 2022). This study aimed to confirm the clinical cases of LSD by identifying the causative virus using PCR assay, to determine the incidence rate, morbidity and mortality rate, and finally to study some risk factors affecting the clinical cases of LSD.

MATERIALS AND METHODS

Study area:

This study was carried out in seven different districts (Sharazoor, Darbandikhan, Penjwen, Bakrajo, Chamchamal, Sangasar, and Piramagroon) in the Sulaymaniyah province of northern Iraq. The study area was located at 34-35°N and 45-46°E (Figure 1). This region has four distinct seasons with different rainfall in autumn, winter, and early spring that ranged between 750 and 400 mm. This area is characterized by having different habitats, such as a river, meadow pastures, and agricultural lands with high biological diversity.



Figure 1: Shows the geographical location of seven districts in Sulaymaniyah province on the map (the green patch). Savanna Style Simple Map of Sulaymaniyah

Animals

Three hundred eighty-seven cattle were investigated during this study. All those animals showed various symptoms of LSD, such as lachrymation of the eyes, swelling in one or more legs, and the development of small lumps on the body.

Clinical examination:

All the animals were subjected to a clinical examination according to Tuppurainen *et al.* (2017).

Samples:

Samples of the lumps were taken using standard sterile surgical techniques using tweezers, a scalpel, and a blade to remove the nodule, making sure that all layers of skin were included according to Bihonegn and Feyisa (2023). The collected samples were then prepared for further analysis.

Molecular examination:

DNA Extraction:

A sterile Eppendorf tube containing 20 mg of skin lesion samples was thoroughly mixed with 200 μ L of phosphate buffered saline. Subsequently utilized immediately for DNA extraction following the manufacturer's recommendations utilizing the genomic DNA extraction kit (tissue) (Geneaid, Korea). After purification, the DNA was kept at -20°C for the test by PCR that proceeded (Saleh *et al.*, 2024).

Amplification:

DNA amplification of the virus was carried out using a pair of primers according to Heine *et al.* (1999) Table (1). This primer targets a highly conserved region of the P32 gene, and its target band is 390 nucleotides. The PCR amplification reaction was performed in a 20 μ L reaction volume. The reaction was set up in a 0.2 mL PCR tube containing 10 μ L Master Mix (Bioneer, Korea), 5 μ L extracted DNA, 1 μ L forward primer (10 pmol), 1 μ L reverse primer (10 pmol), and 3 μ L ultrapure water. Amplification was performed using a thermal cycler (Biorad, USA) under the following conditions: initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes, then stored at 4°C .

Gel electrophoresis:

A 10 μ L aliquot of the PCR product was loaded onto a 1% agarose gel stained with ethidium bromide at a concentration of 0.4 $\mu\text{g}/\text{mL}$ and electrophoresed at 100 V for 1 hour using a 100 bp DNA ladder. The gel was then visualized using a gel documentation system (UVtec, UK) according to Saleh *et al.* (2024).

Table 1: The set of primer used in the study.

Primer name	Primer sequence	Amplicon size	Gene	Reference
B68	CTAAAATTAGAGAGCTATACTTCTT	390 bp	P32	Heine <i>et al.</i> , 1999; Peshnyar <i>et al.</i> , 2017)
B68	CGATTTCCATAAACTAAAGTG			

Statistical analysis:

All experiments were conducted with a significance level of 0.05. The binomial test was employed to ascertain the significance level following the mortality rate, sex, and breed, while the multinomial test was employed to evaluate the significance across various seasons using SPSS software, version 27.

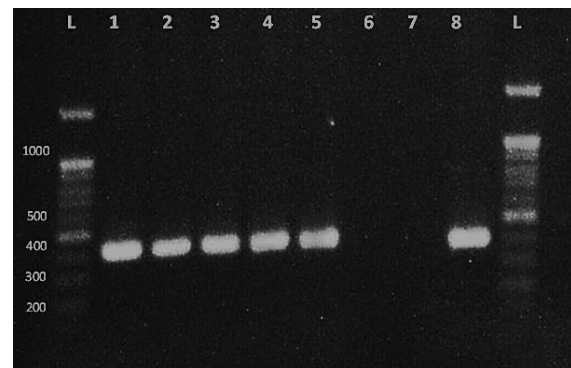
RESULTS**Clinical Examination**

Clinical examination revealed some common clinical signs such as fever, anorexia, respiratory distress, lacrimation, edema of one forelimb, and multiple intradermal nodules of different sizes on the skin of the animals (Figure 2).

**Figure 2:** Photo of cow showing sever form of the LSD lesions in the skin**Molecular Examination:**

The PCR assay confirmed the presence of the virus in all the samples tested, and the

positive results were confirmed by specific bands at 390 bp (Figure 3).

**Figure 3:** Gel electrophoresis showing DNA amplification of LSDV. Lane L: 100 bp DNA ladder. Lanes 1-5 are positive at 390 bp, Lane 6 is a Negative sample, Lane 7 is the Negative control and Lane 8 is the Positive control.**Epidemiological study**

The epidemiological results have shown that seasonal variation is one of the most important determinants. The lowest prevalence was observed in autumn, followed by winter, reaching its peak in spring and then declining throughout the summer. Crossbreeds had a higher infection rate than the native breed. The infection rate in female' animals was significantly higher than the prevalence of infection in male' animals. The mortality rate in this study was 21.19%, as shown in Figure (4).

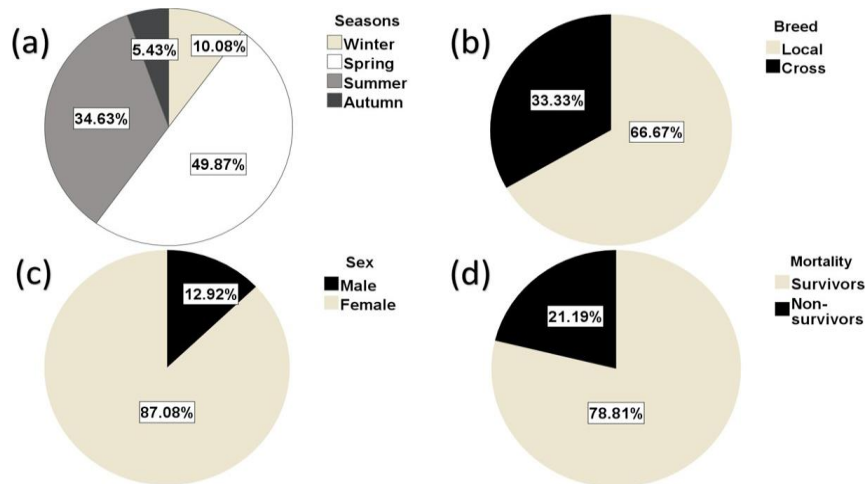


Figure 4: Lumpy Skin Disease infection rates based on: (a) Seasonal, (b) Breed, (c) Sex, and (d) Mortality rates

According to the locality, the infection rate was highest in the Darbandikhan region (2%) and the lowest in the Penjwen region. For the Sharazoor, Bakrajo, Piramagroon, Chamchamal, and Sangasar districts, the prevalence rates were 1.2%, 0.37%, 0.13%, 0.35%, and 0.2%, respectively.

DISCUSSION

The adverse impact of LSD lies in the fact that it has continued to spread and expand its scope to new regions (Tageldin *et al.*, 2014). In Iraq, several outbreaks have been reported over the last ten years. However, it is unclear how the disease is maintained during inter-epidemic periods (Gharban *et al.*, 2019). The diagnosis of LSD depends mainly on clinical signs. Clinical diagnosis of LSD is not difficult for those familiar with the disease, but those who are not experienced can easily confuse lesions with many other conditions (Amin *et al.*, 2021). However, it can be difficult to diagnose mild and unclear cases. Therefore, a quick laboratory assay like PCR was needed to confirm the diagnosis.

The finding revealed that the large variation in the prevalence of LSD between regions could be attributed to the following reasons: availability of warm, humid agricultural climate, rainy season, host's immune status, insect populations, defects in vaccination programs in these provinces, and introduction of new herd as risk factors for the spread of

LSDV (Abdelmohsen *et al.*, 2019; Selim *et al.*, 2021; Abebaw, 2024).

The prevalence of LSD in the current study is close to the average of 1-3%, which is accepted by some previous studies (Magori-Cohen *et al.*, 2012; Tuppurainen and Oura, 2012). The slight difference may be due to the immune status of the animal, and the veterinary authority makes some insect carriers and vaccination companions as they make a circular block to the site of the first outbreaks and later vaccinate the entire cattle herd in the area (Babiuk *et al.*, 2008). In contrast, the result obtained is lower than that previously recorded by the authors (Al-Salihi and Hassan, 2015; Jarullah, 2019; Kresic *et al.*, 2020; Gayal *et al.*, 2022), which were (9.11%, 77%, 85.91%, 55.85%), respectively. Furthermore, the score is higher than previously obtained data such as 12.3% (Sevik *et al.*, 2016), 6.5% (Zeynalova *et al.*, 2016), 7.1% (Sudhakar *et al.*, 2020), and 13.93% (Sethi *et al.*, 2021).

During the current study, the mortality rate was 21%, which is consistent with the rate recorded by Gari *et al.* (2012) and higher than the results recorded in some endemic areas. This could be attributed to the lack of protective immunity in this locality, lack of vaccination, and lack of proper wound treatment and management (Afridi *et al.*, 2023).

Moreover, the result of this study is not similar to those reported in Oman, Egypt, and Jordan, where morbidity and mortality rates were much higher. In Egypt, the morbidity rate was 100%, and the mortality rate was 1.8% (Salib and Osman, 2011). In the Sultanate of Oman, morbidity was 27.9% and mortality was 5.5% (Body *et al.*, 2012). In Jordan, the morbidity rate was 35.1%, and the mortality rate was 1.3% (Abutarbush *et al.*, 2013). This may be due to the change in climate and terrain that will affect the number of biting insects responsible for the transmission of LSDV, and the disease exists longer compared to our country.

The season plays a major role in the spread of the disease in Sulaymaniyah province. There is a sharp difference in temperature for the four seasons of the year, as we have cold winters and autumn, wet springs, and hot summers. This could be the reason for the high prevalence of LSD in the spring, as the climate helps in the vector and the disease spreading (Podshibyakin *et al.*, 2024). In contrast, a decrease in the prevalence of the disease was observed in the fall due to cold weather and drafts that restrict movement and reduce the number of insects (Al-Salhi and Hassan, 2015; Gari *et al.*, 2015; Sevik *et al.*, 2016; Abdelmohsen *et al.*, 2019).

According to gender, a higher rate of LSD was recorded in females than in males. This comes in agreement with Ayelet *et al.* (2014), Elhaig *et al.* (2017), and Gharban *et al.* (2019). This may be attributed to stress factors and physiological causes such as lactation and pregnancy (Mhemid and Hassan, 2016), lower numbers of males present in the field compared to females, and reduced exposure of bulls to biting insects (Gharban *et al.*, 2019). Owners breed cattle for dairy products more than meat products, so the number of cows is higher than for males (Gari *et al.*, 2011; Tageldin *et al.*, 2014).

In addition to the higher rate of LSD in local breeds compared to crossbreeds, this is due to the habit of the owner that was included in the

current study and due to the climate and topography of these areas, especially Darbandikhan and Sharzoor; the owners prefer the local breed to crossbreeds. The higher rate in local breeds than in the crossbreed is explained (Gari *et al.*, 2011; Tageldin *et al.*, 2014).

CONCLUSION

The study highlights the epidemiological characteristics of lumpy skin disease in livestock, with variations in infection rates between seasons, breeds, and sexes. Peak infection rates were observed in the spring season, highlighting the need for targeted prevention programs tailored to this period. The increasing susceptibility of local breeds and cows highlights the need for specific measures to protect these populations.

CONFLICT OF INTEREST: NONE

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مرض الجلد العقدي في الماشية: دراسة اكلينيكية ووبائية مع التركيز على الموسم والسلالة والجنس ومعدل الوفيات

اردي سردار ، زكار رحيم سليمان ، كويستان نجم على ، هاردي فتاح معاريف ،

باسم عبد الواحد على ، هانا سليمان ، محمد عمر بابا شيخ

E-mail: hardi.marif@univsul.edu.iq Assiut University website: www.aun.edu.eg

مرض الجلد العقدي هو عدوى فيروسية مؤثرة للغاية تؤثر على الماشية وتؤدي إلى خسائر مالية وأثار اقتصادية هائلة. كان الهدف من هذه الدراسة هو فحص السمات السريرية والوبائية لمرض الجلد العقدي. تم إجراء فحص شامل على إجمالي 387 رأساً من الماشية التي ظهرت عليها الأعراض السريرية لمرض الجلد العقدي بما في ذلك التمزق المفرط، وتورم الساقين، ووجود عقيدات. تم أخذ عينات وإخضاعها لتحليل تفاعل البلمرة المتسلسل للتحقق من وجود العدوى الفيروسية. أظهرت النتائج أن معدلات الإصابة بمرض الجلد العقدي أظهرت تبايناً موسمياً كبيراً، حيث وصلت إلى أعلى نقطة لها في الربيع (50٪) وتخفض إلى أدنى مستوى لها في الخريف (5٪). بلغت نسبة الإصابة بالسلالات الهجينة 32٪، في حين بلغت نسبة الإصابة بالسلالات المحلية 68٪. ولاحظت الدراسة وجود تفاوت كبير في معدلات الإصابة بين الجنسين، حيث بلغت 12٪ من الذكور و88٪ من الإناث، وبلغ معدل الوفيات 20٪. تؤكد هذه النتائج على التأثير الكبير للسلالة والجنس والتقلبات الموسمية على التعرض لمرض الجلد العقدي ، مما يؤكد ضرورة اتباع طرق مراقبة محددة للحد من انتقال المرض وعواقبه.