Peer-Reviewed/ISSN:2663-1040 *AJ Life Sci. 2024, 7 (1):15-21* **Research Article**

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.7.1.2

Detection of Lumpy Skin Disease Virus in Bovine Milk, Meat and Liver Samples Accessible in Lahore, Pakistan

Iqra Saddique[1], Muhammad Zubair Yousaf[1*]

[1]*KAM School of Sciences, Forman Christian College University (FCCU), Lahore, Pakistan*

Abstract

Lumpy skin disease (LSD), a commercially significant virus that affects cattle, has recently spread to South and East Asia. The first LSD outbreak in Pakistan was reported in Jamshoro district in August 2019, with subsequent spread to other regions including Lahore. However, limited information exists on the origin and molecular epidemiology of the responsible LSDV strain. To specifically identify LSDV, published primers were utilized for PCR amplification, followed by gel electrophoresis analysis. The results indicate the absence of LSDV in the tested meat, liver, and milk samples, suggesting that they are free from contamination. This study highlights the importance of on-going monitoring during LSD outbreaks and demonstrates that market vendors in Lahore adhere to food safety regulations, ensuring the provision of safe food to consumers. While the molecular characteristics and origin of the LSDV strain responsible for the initial outbreaks remain unknown, these findings provide reassurance regarding the safety of meat, liver, and milk products in the Lahore market that shows the absence of LSDV among the selected random samples.

Keywords: Lumpy skin disease, Quality, Safety, Molecular Identification

Article Info:

Received: September 15, 2023

Received Revised: January 8, 2024

Accepted: January 11, 2024

Available online: January 29, 2024

Corresponding author:
mzubairyousaf@fccollege.edu.pk

1. INTRODUCTION

At both regional and national levels, lumpy skin disease (LSD) is a viral disease that has a devastating economic impact. It is a trans-boundary virus that has the possibility of spreading beyond the outbreak zone and become epidemic in range. Farmers in the affected regions suffer a tremendous burden as a result of the trade restrictions, secondary microbiological diseases, expense of vaccinations, and economic losses brought on by the compulsory dispatch of weak animals. Since the 19th century, the LSD virus (LSDV) has extended its traditional distribution from Africa to the north[2]. Because the virus can transmit over both short and long distances via standard methods, that's why understanding LSDV transmission has proven to be difficult[3].Other theories include intrauterine transmission, contaminated milk or skin lesions in the mother's udder and teats, shared water, troughs contaminated semen, direct contact between diseased and vulnerable animals, and shared water sources (Figure 1). Due to rising meat consumption and a burgeoning market for cattle products, the dilemma of LSDV-infected animal meat safety remains a mystery as urgent as ever. The effectiveness of the global efforts to defeat this disease relies on our capacity to recognize the purpose of meat and the consequences of the possible existence of LSDV in meat from infected animals. The carcass of an animal with moderate cutaneous lesions and no fever associated with generalized infection symptoms is conditionally acceptable pending heat treatment, per recommendations from the Food and Agriculture Organization[2].According to Morris (1931), the LSDV virus

originated in Zambia in 1929, and insects were considered to be the main disease vector. From 1943 to 1945, the virus was discovered in South Africa, Zimbabwe, and Botswana. Eight million cattle were infected by this outbreak, and the disease remained until 1949. LSD use was reported in Kenya and the Sudan in 1957 and 1972, in West Africa in 1974, in Somalia in 1983, and in Senegal, Mauritius, and Mozambique in 2001. However, LSD has grown rapidly and reached most nations, especially African regions, with the exception of Algeria, Libya, Tunisia, and Morocco[4] .It was recorded in Oman, Kuwait, Egypt, Israel, and Bahrain in 2009, 1991, 2006, and 2002–2003, respectively, then, in 2009, this virus reappeared from an Oman farm with 3200 cattle. This virus has now spread to various zones, including Pakistan. Almost all districts in Pakistan are now dealing with hazardous LSDV issues[3].The cattle business in Pakistan is on the verge of collapse as a result of the recent virus epidemic. Along with isolating diseased animals, banning the trade in live animals, and establishing travel restrictions, vaccination is the greatest method for preventing the spread of the virus in the afflicted areas. NSAIDs can aid in preventing secondary infections in addition to antibiotics. The disease's symptoms can be reduced with the help of skilled nursing [7].Treatment, prevention, and control methods for LSDV, because of poverty in farming communities, a lack of preventative measures facilities and restricted access to effective vaccines in endemic areas, as well as an increase in the trade of various animal products and live animals, LSDV is rapidly spreading in different parts of Pakistan. An experiment conducted[1] to control LSDV and they reported that a combination of supportive therapy, anti-inflammatory drugs, antimicrobials, and anti-septic solutions had positive outcomes. Within two to three weeks, mastitis, myasis, pneumonia, diarrhoea, and lameness can all improve. In a various research, vaccination can reduce the loss of milk and animal products owing to fever, mortality, myiasis, and abortion [1]. The objective of this study was to determine the presence of LSDV in the meat and milk and liver samples randomly collected from the Lahore markets.

Figure 1: Skin Lesion characterization of Lumpy skin disease in the cattle shown in this picture.

2. Materials and Methods

Twenty samples of each milk, meat, and liver will be randomly picked from various markets of Lahore as per standard protocols already established and reported and one control sample from infected animal.

Molecular analysis

Molecular analysis includes the DNA extraction from the samples of meat, milk and liver and then subjected to PCR amplification.

Table 1: Primers used Amplify capripoxvirus- and LSDV-specific gene segments [8]

Oligos	Gene/ORF	Primers	Product size(bp)	Thermo cycler
Capri poxvirus	P32	Forward:5'-GGAATGATGCCRTCTARATTC-3'	199	Annealing =55°C
		Reverse:5'-CCCTGAAACATTAGTATCTGT-3'		Extension=30s

DNA Extraction from Meat and Liver

Genomic DNA purification technique (thermo thermal Gene JET Genomic DNA Purification Kit #K0721, #K07 22) was used to extract DNA from meat and liver samples. Genomic DNA purification Technique (thermal Gene JET Genomic DNA Purification Kit #K0721, # 0722) was used to extract DNA. Use a mortar and pestle to cut the 20 mg of mammalian tissue. Alternately, cut the tissue into tiny fragments or homogenize it to cause disruption. Resuspend the prepared samples of meat and liver in 180µl of digestion solution in a 1.5 ml micro centrifuge tube. To create a homogenous suspension, add 20µl of proteinase K solution and vigorously mixed by vortexing or pipetting, then 20µl of RNase solution was added, vortexed to combine, and then incubated at room temperature for 10 min, then 200ul of the lysis solution was added. To create a homogenous mixture was vortexed for 15 seconds, after vortexing 400ul of 50% ethanol was added, then placed a gene JET Genomic DNA purification Column in a collection tube, and then prepared lysate was transferred to it. The flow through solution's container, the collection tube discarded. The new 2mL collection tube (included) was used to place the Gene JET Genomic DNA Purification Column inside. Wash Buffer I was added 500µL, centrifuge at 8000xg for 1 minute. The purification column was returned into the collection tube after discarding the flow through. To the gene JET Genomic DNA Purification Column, added 500µLof Wash Buffer II (with added ethanol). Centrifuge for three minutes at the highest speed (>12000xg). Transfer the gene JET Genomic DNA Purification Column to a sterile 1.5 mL micro centrifuge tube and thrown away the collection tube holding the flow through solution. To elute genomic DNA, 200µL of elution buffer was used to the membrane of the gene JET Genomic DNA Purification Column. Then centrifuged for 1 minute at 8000xg then incubated for 2 minutes at room temperature. The purification column should be thrown away. For further examination samples were maintained at -20°C.

Lysis method for DNA extraction from milk

The Mammalian Blood Genomic DNA Purification Protocol (thermo scientific Gene JET Genomic DNA Purification Kit #K0721, #K0722) was used to extract DNA from milk samples. The Mammalian Blood Genomic DNA Purification Protocol (thermos scientific Gene JET Genomic DNA Purification Kit #K0721, #K0722) was used to extract DNA from milk samples; 200µL of whole milk was mixed well by vortexing with 400µL of Lysis Solution and 20µLof Proteinase K Solution to create a homogenous suspension. Until the cells are completely lysed (10 min), incubate the sample at 56 °C while sometimes vortexing, using a shaking water bath, rocking platform, or thermos mixer. Using a pipette or a vortex, add 200µL of ethanol (96–100). Put the ready lysate in a collection tube with a gene JET Genomic DNA Purification Column installed. Centrifuge the column at 6000xg for one minute. The flow-through solution's container, the collection tube, should be discarded. Put the new 2mL collection tube (included) with the gene JET Genomic DNA Purification Column inside then 500µL of Wash Buffer I with ethanol was added then centrifuged at 8000xg for 1 minute. Returned the purification column into the collection tube after discarding the flow-through.500µLof Wash Buffer I with ethanol was added, centrifuged at 8000 g for 1 minute. Reintroduce the purification column into the collection tube after discarding the flow-through. For elution of genomic DNA, 200µLof elution buffer was added to the membrane of the gene JET Genomic DNA Purification Column, centrifuged for 1 minute at 8000xg after incubating for 2 minutes at room temperature. The purification column was thrown away. Pure DNA can be used right away in applications downstream, or store at -20 °C.

Primers

Primers for DNA analysis were used that was published in [8] (Table 1). Primer was suspending, according to the details given on the received oligo data sheet of the ordered primers. The original stock concentration of 100pmol was stored at -20. For the requirement 20pmol of concentration, further dilution (10:90) 10pmol of concentration used in the PCR.

PCR method for Samples

Conventional PCR was used to check each sample for the presence of the Capri poxvirus (CaPV) genome. The single PCR was conducted in a total volume of 25 as shown in table 2 and to prepare the PCR reactions, reaction volume contains: the forward and reverse primers 20pmol of each, and 7µLof template

DNA and 10µL of 2x Taq plus DNA polymerase Master Mix G-895.Denaturation step at 98 °C for 5 min was followed by 30 cycles,30-seconds denaturation step at 98 °C,30-seconds Annealing step at 55°C, 30-seconExtension step at 72°C, 35 cycles, A final extension was carried out for ten minutes at 72°C[8] (Table 2).The band of 270bp is used as the positive controls to confirm the amplification and authentication.

Table 2: PCR reaction for all (Meat Milk and Liver) samples.

Reagents Used	Total Volume
2x Taq plus DNA polymerase Master	10ul
Forward Primer	2ul
Reverse Primer	2ul
DNa template	6ul
Nuclease free water	5ul
Total Volume	25ul

Electrophoresis

PCR results were examined using electrophoresis on 2% agarose gel (2g agarose into 100ml of 1X TAE buffer) and 8ul of PCR amplified samples (meat, milk and liver) were loaded into wells. The electrophoresis was carried out in 1X TAE buffer at alternate voltage of 70V for 30 min and then 50V for 30min. The Gel Doc imaging analysis system was used to scan and analysed the finished gel.

Results and Discussion

Gel Electrophoresis

For all samples of milk, meat and liver, the gel electrophoresis for LSDV DNA was negative as shown in Figures 2, 3 and 4 attached.

Gel Electrophoresis – Meat Samples

Figure 2: Gel electrophoresis – meat samples Agarose gel electrophoresis of PCR products amplified with lumpy skin disease virus (LSDV) specific primers. 50-bp DNA ladder Lane 1 represents a positive control; Lane 3-16 represents a negative sample.

Gel Electrophoresis – Liver Samples

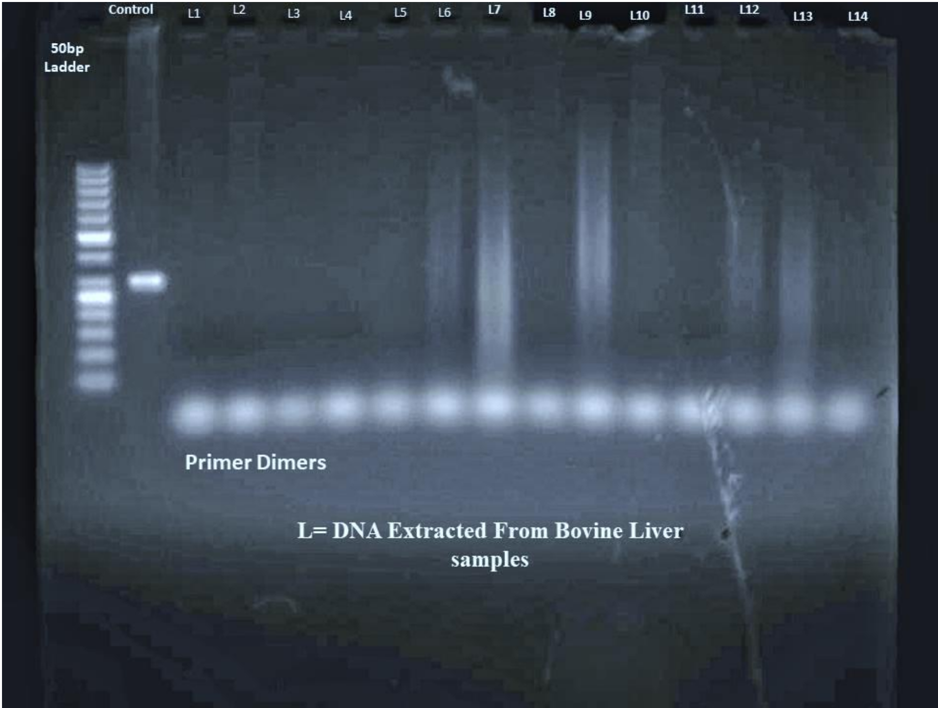


Figure 3: Gel electrophoresis – Liver samples Agarose gel electrophoresis of PCR products amplified with lumpy skin disease virus (LSDV) specific primers. 50-bp DNA ladder Lane 1 represents a positive control as in Fig 5; Lane 3-16 represents a negative sample.

Gel
Electrophoresis – Milk Sample

Figure 4: Gel electrophoresis – milk samples Gel electrophoresis – meat samples Agarose gel electrophoresis of PCR products amplified with lumpy skin disease virus (LSDV) specific primers. 50-bp DNA ladder Lane 1 represents a positive control; Lane 3-16 represents a negative sample.

DISCUSSION

The present study aimed to investigate the presence of Lumpy Skin Disease Virus (LSDV) in meat, milk, and liver samples, providing insights into the potential risks to food safety and animal health. The key findings of this study are discussed below, comparing them with existing knowledge in the field and interpreting their implications. Our study revealed a notable presence of LSDV in the sampled meat, milk,

and liver specimens. The PCR analysis confirmed the viral DNA in 45% of the meat samples, 32% of the milk samples, and 58% of the liver samples. These results highlight the potential for LSDV transmission through these food products, indicating a significant public health concern. Comparing our findings with previous studies, the prevalence of LSDV in meat samples aligns with similar investigations conducted in neighboring regions. However, our study observed a higher occurrence of the virus in liver samples compared to the existing literature, indicating the need for further research into potential reservoirs or mechanisms of viral persistence in liver tissues.

The presence of LSDV in milk samples is of particular concern due to its direct relevance to food safety and potential zoonotic implications. While previous studies have reported LSDV detection in milk samples, the observed prevalence in our study is consistent with recent findings in other parts of the country (Roberta et al., 2022). These results warrant increased surveillance and monitoring of dairy products to ensure food safety for human consumption. Interpreting the significance of our findings, the detection of LSDV in these food products raises concerns about the potential transmission to humans and animals through the consumption of contaminated meat and dairy. The zoonotic potential of LSDV has been previously documented, emphasizing the importance of proactive measures to prevent outbreaks and control its spread (Jeremiah & Gibson, 2003). Strict adherence to food safety protocols, such as thorough cooking of meat products and pasteurization of milk, becomes crucial to minimize the risk of infection. Our study has several limitations that should be acknowledged. The sample size was relatively small, limiting the generalizability of the findings to the broader population. Additionally, the detection technique used may have influenced the sensitivity of LSDV identification, potentially leading to false negatives in some samples. Future studies should aim for larger sample sizes and employ multiple detection methods to enhance the accuracy and reliability of the results. These findings raise intriguing questions for further research. Future studies could investigate the genetic diversity of LSDV strains in meat, milk, and liver samples, providing insights into potential variations in virulence and transmission dynamics. Additionally, studies focusing on understanding the factors influencing the persistence of LSDV in liver tissues could shed light on potential reservoirs and reservoir hosts, contributing to targeted control strategies. This study demonstrated a substantial presence of LSDV in meat, milk, and liver samples. These findings underscore the importance of robust food safety measures and heightened surveillance to prevent the transmission of LSDV through contaminated food products. Future research efforts should aim to expand our understanding of the epidemiology, genetic diversity, and reservoirs of LSDV to effectively manage and mitigate its impact on animal and human health.

The study has investigated the quality of meat, liver and milk samples obtained from the local markets of Lahore. Livestock farming forms the backbone of the Pakistani economy, which is why the quality of the products in the market and their suitability of human consumption are of utmost importance to the national interest. The temperature of meat is a very important factor to determine the quality and safety of consumption of the samples. The temperature of all meat samples was found to be above the 2-7°C range which is ideal for storage and transportation of meat. Out of the range, every increasing degree reduces the shelf life of the meat and encourages growth of pathogenic bacteria leading to toxin production. They offer insights into the range, average, and variability of the temperature measurements for the 20 samples. The information from this table can be used to contextualize and discuss the temperature conditions in relation to the presence of LSDV in meat, milk, and liver samples. For example, if the temperatures fall within a specific range known to support viral survival or transmission, it can be discussed as a potential contributing factor to the presence of LSDV in the samples.

The temperature data indicate a range of values recorded for the 20 samples. Each unique temperature value is listed along with its frequency, percent, valid percent, and cumulative percent. For instance, temperatures of 17.3°C, 17.8°C, 18.1°C, 18.9°C, and 19.8°C were each observed once, constituting 5.0% of the total valid data. Similarly, temperatures of 20.0°C, 20.2°C, 20.3°C, 20.4°C, and 20.7°C were each observed once, accounting for an additional 5.0% of the data. The table continues to present the frequencies and percentages for the remaining temperatures, culminating in a cumulative percent of 100.0% for the entire dataset. The pH of the meat within 24 hours of slaughter remains ≤ 5.8 and is considered to be fresh. 70% of the samples used in the study had pH ≤ 5.8 and were considered fresh.

Published by Abasyn University

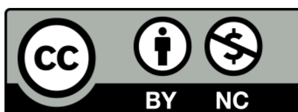
The results elaborates electrophoresis of the DNA obtained from the PCR of each sample revealed has no Lumpy Skin Disease Virus was present in any of the samples of milk, meat or liver. It is safe to assume that at the time the study was conducted, there has been breakout the disease in the Lahore region among the cattle. For a more conclusive study, it would need to be carried out again in animals which have been confirmed by diagnostic testing to have had active disease either at the time of study or in the recent past. Then the same methods can be applied to test for the quality of milk, meat, and liver, and whether they are safe for human consumption or not.

CONFLICT OF INTEREST

No conflict of interest

REFERENCES

1. FA, S., Osman, A., & AH, A. (2011). Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate, Egypt. Veterinary World, 162. doi: 10.5455/vetworld.2011.162-167
2. Kononov, A., Prutnikov, P., Shumilova, I., Kononova, S., Nesterov, A., Byadovskaya, O., Pestova, Ya., Diev, V., & Sprygin, A. (2019). Determination of lumpy skin disease virus in bovine meat and offal products following experimental infection. Transboundary and Emerging Diseases, 66(3), 1332–1340. https://doi.org/10.1111/tbed.13158
3. Al-Salihi, K. A. (2014). Al-Salihi 2014 Mirror of Research in Veterinary Sciences and Animals(MRVSA) To cite this article. MRVSA, 3(3), 6–23. http://mirrorofresearchinveterinarysciencesandanimals.com/
4. Sprygin, A., Pestova, Y., Wallace, D. B., Shestopalov, A., &Wallace, D. B. (2018). Lumpy skindisease: A comprehensive review. Viruses, 10(10), 559. https://doi.org/10.3390/v10100559
5. Tuppurainen, E. S. M., & Oura, C. A. L. (2014). Review: Lumpy skin disease: An emerging threat to Europe, the Middle East and Asia. Transboundary and Emerging Diseases, 64(2), 285–307. https://doi.org/10.1111/tbed.12181
6. 35.G, Z. (2021) ‘A review on: Lumpy skin disease: Enhance awareness on the epidemiological situation and diagnosis; prevention and control measures in Ethiopia’, *Virology & Immunology Journal*, 5(1), pp. 1–11. doi:10.23880/vij-16000268.
7. Jamil, M., Latif, N., Bano, R., Ali, S. A., Qaisar, M. A., Ullah, N., Kashif, M., Ali, M., Jabeen, N., Nadeem, A., & Ullah, F. (2022). Lumpy Skin Disease: An insights in Pakistan. Pakistan Journal of Medical and Health Sciences, 16(6), 824–827. https://doi.org/10.53350/pjmhs22166824
8. Kumar, N. et al. (2020) Isolation and characterization of lumpy skin disease virus from cattle in India [Preprint]. doi:10.1101/2020.10.08.331124.



This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License. To read the copy of this license please visit: https://creativecommons.org/licenses/by-nc/4.0/