**Alqalam Journal of Medical and Applied Sciences**. 2026;9(6):1493-1496
https://doi.org/10.54361/ajmas.269606

*Original article*

# Detection of Circulating Strains of Lumpy Skin Disease Virus in The City of Benghazi Using Polymerase Chain Reaction

**Z. Akriem[1], Mohammd Abd Alati[1], Randa Mahmoud[2], Abdulsalam Saleh[2]*, Fathi Muhammad[3], Mahmoud Al-Jahmi[3], Ahmed Al-Awami[3]**

[1]*Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Al-Bayda, Libya*
[2]*Department of Health Food Hygiene, Omar AL Mukhtar University, AL Beida, Libya*
[3]*National Center for Animal Health, Benghazi, Libya*
***Email.*** *abdulsalam.abdullah@omu.edu.ly.*

**Abstract**
Lumpy Skin Disease Virus (LSDV) is a disease that causes significant economic losses in livestock, particularly in Benghazi, Libya. It is listed as an endangered species (OIE) and is caused by the lumpy skin disease virus (LSDV). When the disease occurs, attenuated strains of the virus are used to control it successfully, provided a vaccination policy is implemented. However, rapid adverse reactions in animals after vaccination are possible. Therefore, it is essential to use diagnostic methods that differentiate between the pathogenic virus strain and the vaccine itself. This study, conducted in 2025 at the Central Animal Health Laboratory in Benghazi, investigated the reaction using quantitative polymerase chain reaction (PCR) on 181 samples from newly infected and vaccinated animals. The results were positive, as real-time PCR is more sensitive than interfering PCR. This allows for the rapid detection of animals exposed to a highly virulent virus. In conclusion, the KV-2/FLI device used in this study is characterized by its rapid sensitivity for routine laboratory use and can be used instead of the traditional polymerase chain reaction to detect bovine lumpy skin disease virus strains and pox viruses in all clinical samples collected.
**Keywords.** LSDV, PCR, Benghazi, Vaccination, Animals.

## Introduction

Lumpy skin disease is a viral disease affecting cattle, characterized by skin nodules, intermittent fever, and swollen lymph nodes. These symptoms can lead to death[1]. Lumpy skin disease is a transboundary animal disease (TDD) registered with the World Organization for Animal Health (OIE)[2]. The danger of this disease lies in the economic losses it causes, as it leads to decreased milk production and weight loss in animals, especially fattening calves, and can result in permanent infertility[3]. The infection rate ranges from 5% to 80%, and the mortality rate is relatively low compared to other viral diseases, ranging from 1% to 5%[4].

Lumpy skin disease is widespread during peak insect activity, particularly among arthropods and mosquitoes[5]. The severity of infection and mortality depends on the animal's immunity, age, sex, and breed; the duration of infection can range from days to weeks[6]. None of the pets contracted this disease during the outbreak on infected farms[7]. This virus belongs to the poxvirus family. A weakened strain of the lumpy skin virus is used as a vaccine[8]. The sheep pox virus genome is known to be 151 serotypes, characterized by a high degree of conservation. There is a high degree of similarity between sheep pox and sheep pox, and therefore, the polymerase chain reaction (PCR) was readily used to detect the virus[9]. The study aimed to work on a manual design for a reliable real-time polymerase chain reaction for use in laboratories so that it would have the effective ability to accurately detect strains of the streptococcal skin disease virus within a short period. Therefore, it is necessary to work on developing this protocol in order to detect samples within a few hours.

## Methods

Table 1 shows the types of samples used in the study. The test was performed using a field strain of bovine lumpy skin disease virus from Egypt and two strains from Türkiye, and the SIS vaccine was used.

***Table 1. The samples that were used in the clinical study***

| Sample type | Number of samples |
|---|---|
| Skin nodules | 30 |
| Blood | 30 |
| Nasal swab | 13 |
| Milk | 10 |
| Flies | 13 |
| Swabs from the surrounding environment | 20 |
| DNA from the internal organs of cattle | 15 |
| Blood from animals vaccinated against the | 50 |


***Received****: 01-04-2026 -* ***Accepted****: 28-05-2026 -* ***Published****: 04-06-2026*

| | |
|---|---|
| disease | |
| Total | 181 |

***Step 1***

Viral DNA extraction using a 100 µL Thermo Fisher reagent. This extraction was performed by cutting the samples into small pieces using a sterile scalpel. The pieces were then rapidly transferred to sterile mL tubes containing glass beads to enhance extraction and sterile saline solution. The samples were homogenized for 4–5 minutes and then centrifuged for 1 minute at 1000 rpm. Approximately 200 µL of the resulting filtrate was used for DNA extraction. Blood and milk samples were then extracted according to the manufacturer's instructions**.** Probes and primers were designed manually using all the information published in GenBank.

To determine the LOD, several sequential dilutions of the virus were performed using the known decimal sequence.

***Polymerase Chain Reaction (PCR)***

The PCR MASTER MIX was used to prepare a 26 µL reaction volume containing 2.5 µL of extract, 0.2 µL of each primer, 0.50 µL of probe, 1.5 µL of master mix, and 10 µL of distilled water. The reaction was quantified in real time using two different instruments according to the following parameters: 94% for 4 minutes, then 45 cycles at 95 degrees Celsius for 15 seconds, and finally 65 degrees Celsius for 30 minutes. In this study, ARIA MX software was used to calculate the reaction efficiency. Electrophoresis was then performed on gels using 2% 1 gram cassettes. In this study, a virus was isolated by culturing nodules on MDBK. The sample was cut into 1-gram portions, homogenized, and then diluted in a 10:1 saline solution with added antibiotics such as penicillin and gentamicin to inhibit bacterial growth. The sample was then centrifuged at 2000 rpm for 15 minutes. One milliliter of the filtrate was used to inoculate a 24-hour culture. The culture was then incubated and monitored under a microscope for one week. At the end of the incubation period, the viral titer was calculated using the standard predominance method.

## Results

(Table 2) shows the probe sequences and their comparison with primers and polymerase chain reaction (PCR) products, as well as their positions with the genome of strain AF 22 66 27.1.

***Table 2. Comparison of primer and probe sequences, PCR product lengths, and their positions with the complete genome of strain AF325528.1***

| **PRIMER/ P. N** | **PRIMER/ P. S (5.3)** |
|---|---|
| KV.2A | |
| LSD.KV.2.FF | TGGATGAACCGGCATTGCTA |
| LSD.KV.2.RR | AGCCGGTACAGGATTGACA |
| LSD.KV.2.PRO.FIELDD | TTAACCGATCAGGTAAGTACCAG |
| FLII, A | |
| LSD.FILL.FF | AGGTACTTGATAAGCT |
| LSD.FILL.R | CTGTCCGATCTGATAA |

***Table 3. The interaction of Q, P, C, R, DR, and RSQ, and determines the slope for both tests***

| **ASSAY** | **TARGET** | **EFFICIENCY** | **RSQ** | **Slope** |
|---|---|---|---|---|
| KV.2.A | VIC | 102,3 | 0.889 | -4356 |
| F.A | FAM | 103,5 | 0.769 | -2.421 |

***Table 4. The sensitivity and specificity through manually designed quantitative polymerase chain reaction (PCR) protocols and cross-linked polymerase chain reaction (CLCR) protocols from virus-infected and vaccinated animal samples***

| **ASSAY** | **positive sample** | **negative sample** |
|---|---|---|
| B. QPCR | 97 | 10 |
| K.F.STRAIN | 66 | 39 |
| F.F.STRAIN | 66 | 39 |
| N.PCR | 86 | 24 |
| N.PCR.FIELD.STRAIN.AFTER.RESTRICTION | 60 | 22 |
| N.PCR.VACCIN.STRAIN. AFTER.RESTRICTION | 25 | 60 |


***Received****: 01-04-2026 -* ***Accepted****: 28-05-2026 -* ***Published****: 04-06-2026*

Ninety-seven out of 107 samples tested positive using the qPCR test for smallpox virus. K.F.STRAIN tests showed 66 positive samples for lumpy skin disease virus, while n.PCR showed 86 positive samples. Four samples had Ct values above 35. A low viral load of the vaccine strain was not detected by the n.PCR test, so it was classified as inconclusive. Both qPCR tests identified a field strain from Egypt and two field strains from Turkey as field strains. The mean threshold value [11] was 24.6 for biopsy samples, 26.3 for nasal swabs, and 28.5 for blood samples. Thirty-nine samples known to be negative for lumpy skin disease virus were tested, and all yielded negative results.

## Discussion

Quantitative PCR is more sensitive, reliable, and faster than traditional and nested methods[5]. Biopsy samples were the most suitable for testing because they have the lowest CT values. Recently, smallpox viruses have been detected using real-time polymerase chain reaction (PCR) [12]. As of 2026, no studies or research had been published describing real-time PCR using the known Taq MAN technique for the qualitative detection of the virulent field strain of this virus. This study describes the validity of real-time polymerase chain reaction (PCR) using the Taq-Man technique, which is considered sensitive and specific for the virus circulating in Libya. It is known that KV2 (qPCR) and FLI are tests designed to detect the field virus. Therefore, they must be used in conjunction with, or after, (qPCR), which allows for the simultaneous detection of both the field and vaccine strains with high accuracy[13].

In this study, it was found that the two field tests (qPCR) on six samples, compared to the traditional method, showed positive CT values in the real-time test and negative values in the overlapped test above 35. Therefore, it was not sensitive compared to real-time. The overlapped PCR cannot definitively detect mixed infections, especially if the vaccine strain is more prevalent. In this study, real-time reaction did not show any reaction with any poxviruses, including those from unvaccinated or uninfected vaccinated animals. In conclusion, the technique described in this study is highly sensitive, allowing for rapid and accurate detection of lumpy skin virus strains. This technique is useful in countries where the disease is prevalent, and vaccination strategies are employed [14]. However, further research is needed to validate these tests using isolated strains from other countries to confirm their effectiveness.

## Conclusion

This study aimed to provide a reliable diagnosis of lumpy skin disease virus in Benghazi and its surrounding areas by extracting DNA from blood, milk, and superficial skin lesions of animals, as well as using polymerase chain reaction (PCR) technology. PCR is a reliable and rapid diagnostic tool that reveals the genetic relationships between viruses, enabling early detection. It is also essential to curb smuggling across borders. International reports have confirmed that lumpy skin disease can only be eradicated through the implementation of a clear and effective strategy involving all stakeholders in animal health.

***Conflict of interest***. Nil

## References

1. Saleh A, Kawafi WI, Zafir ZA, Mahmoud R. Isolation of Salmonella, Yersinia, and Vibrio bacteria from some seafood sold in retail stores in the Libyan cities of Sousse and Hamama. Derna Acad J Appl Sci. 2025;5(1):1-9.
2. Gomaa A, Abdulhadi S, Mohamad N, Saleh A. Comparison of reported antibiotic treatment in chicken farming and antibiotic-resistant (E. coli) in commercial poultry meat. AlQalam J Med Appl Sci. 2025;96-100.
3. Mahmoud R, Gaballa M, Alsadi I, Saleh A, Abd Alati M, Abid AA. Microbiological evaluation of retail veal meat in the city of Al Bayda, Libya. AlQalam J Med Appl Sci. 2024;335-40.
4. Alsadi I, Zafir A, Alati M, Hamad R, Saleh A, Gaballa M, et al. Risk analysis of brucellosis in country level Libya. J Vet Sci Res. 2023;01-5.
5. Abdalnaser B, Bellhamad N, Saleh A, Mahmoud R, Abd Alati M, Hamd F. Prevalence of chronic Toxoplasma gondii infection among women who had undergone abortion in Tobruk and surrounding areas, Libya. AlQalam J Med Appl Sci. 2025;2064-7.
6. Saleh A, Farhat RR, Zafir ZA, Mahmoud R, Abd Alati M, Othman H. Evaluation of veterinary antibiotic residues in commercial and local farms in Al-Bayda city, Libya (2024-2025), with a molecular docking study to analyze their effect on target microbial proteins. Afr J Adv Pure Appl Sci. 2025;219-26.
7. Zafir AG, Almardi A, Alorfi S, Saleh A, Hamad RM. Occurrence of vancomycin resistant Enterococcus faecalis in chicken flocks. Alex J Vet Sci. 2023;76(1).
8. Saleh A, Mahmoud R, Am Zafir Z, Abd Alati M, Hasan A. Nutritional evaluation of porcupine meat compared to sheep and cow meat. Libyan Med J. 2025;526-8.
9. Amraga F, Akriem Z, Mohammed N, Akriem A, Saleh A. Evaluation of haematological and biochemical parameters in feedlot Libyan local lambs. Derna Acad J Appl Sci. 2026;6(1):96-102.
10. Abrayk SES, Akraeim AM, Saleh A, Almabruk MI, Mahfoth N, Othman H, et al. Urinary retention syndrome associated with Cistus salviifolius ingestion in cattle: a case report from eastern Libya. Derna Acad J Appl Sci. 2025;5(2):96-104.


***Received***: *01-04-2026* - ***Accepted***: *28-05-2026* - ***Published***: *04-06-2026*

11. Mohamed AH, El Hawy AS, Sawalhah MN, Squires VR. Middle East and North Africa livestock systems. In: Livestock: production, management strategies and challenges. Hauppauge (NY): Nova Science Publishers; 2019.
12. Haib SH, Kaur H, Mohamed FEB, Benkhyal FAK, Bouzid G, Belaidi M, et al. Evaluation of extraction techniques, chemical composition, antioxidant properties, computational modelling, and biological insights of the novel truffle species (Tirmania nivea) for enhanced human health benefits. Trends Sci. 2026;23(7):12762-.
13. Kumar R, Anjum N, Tripathi Y. Phytochemistry and pharmacology of Santalum album L.: a review. World J Pharm Res. 2015;4(10):1842-76.
14. Mahmoud R, Mohamad NA, Saleh A, Bufarwa SM, EL-Seifat R. Exploring the effect of heat treatments on eliminating the remains of antibiotic residues (colistin). Afr J Adv Pure Appl Sci. 2024;132-7.


***Received****: 01-04-2026 -* ***Accepted****: 28-05-2026 -* ***Published****: 04-06-2026*