SHORT COMMUNICATION

MOLECULAR DETECTION OF LUMPY SKIN DISEASE VIRUS IN PAHANG DURING THE FIRST OUTBREAK IN 2021

SITI FATIMAH, M.A.A.[1*], NOOR FAEZAH, M.S.[1], ZULRIYANA, M.[1], NORAIHAN, M.Y.[1], KHAIRUL ANUAR, M.[2] AND KAMALIAH, G.[2]

1 Eastern Zone Veterinary Laboratory (Pahang), Department of Veterinary Services, Jalan Sri Kemunting 2, 25100 Kuantan, Pahang

2 Department of Veterinary Services of Pahang, Jalan Sri Kemunting 2, 25100 Kuantan, Pahang

**Corresponding author:* fatimahaziz@dvs.gov.my

ABSTRACT. In the first half of 2021, an alarming disease outbreak of Lumpy Skin Disease (LSD) has virulently expanded in Malaysia and could potentially cause a severe economic loss to the cattle industry, notably the state of Pahang. Following the surveillance actions and suspected cases in Pahang, several clinical samples including scabs, saliva and nasal swabs, whole blood, bone marrow, and organs were presented to Eastern Zone Veterinary Laboratory (Pahang) for laboratory diagnosis of LSD. This study aims to molecular detect and confirm the presence of LSD virus (LSDV) nucleic acids via quantitative polymerase chain reaction (qPCR) method which was performed on all the processed and extracted samples. The results showed 37 % of the samples tested were positive for LSDV. Subsequent control measures applied has proved to be effective as the positive cases were gradually reduced towards the end of the outbreak. Even though LSD is currently endemic in Malaysia, there is an urge for the continuous efforts of LSD awareness, strict regulations on animal movement and import protocol, as well as proper vaccination programs for cattle in Malaysia.

Keywords: lumpy skin disease, lumpy skin disease virus, quantitative polymerase chain reaction (qPCR)

INTRODUCTION

Lumpy skin disease (LSD) is an emerging transboundary viral disease listed by World Organisation for Animal Health (WOAH) as notifiable disease due to its significant economic losses and the potential of rapid spread (Namazi & Tafti, 2021). Lumpy skin disease virus (LSDV); an enveloped double-stranded DNA virus of *Capripoxvirus* genus within *Poxviridae* family is capable of infecting cattle and water buffalo by causing high fever, swollen lymph nodes, cutaneous nodules of 2 to 5 cm in diameter on the head, neck, limbs, udder, genitalia, and perineum; lachrymation, reduction in milk production, and sometimes death (OIE, 2021; Tuppurainen *et al*., 2005).

Originated from Africa continent, LSD has never been reported in Malaysia before and the recent outbreak in May 2021 is categorized as an emerging disease. The incident is likely due to the illegal movement of smuggled animals from infected neighboring countries (Khoo *et al*., 2021; WAHIS, 2022). Following this outbreak, surveillance activity was intensified by Department of Veterinary Services (DVS) Malaysia to cover the ruminant farms all over Malaysia including Sabah and Sarawak leading to the submission of clinical samples to the several veterinary laboratories of DVS.

This paper intends to molecular detect and present the findings of laboratory confirmation of LSDV by quantitative polymerase chain reaction (qPCR) using Taqman real time PCR assay from the clinical samples received in Eastern Zone Veterinary Laboratory during the outbreak in 2021.

MATERIALS AND METHODS

Samples

The Department of Veterinary Services of Pahang has observed that there were six farms in Pahang that were having LSD-like lesions affecting some of the cattle population. Thus, a total of 38 clinical samples were collected from those farms based on the recommended type of specimens stated in the LSD Technical Disease Cards. The clinical samples that consist of scabs (n=2), saliva and nasal swabs (n=13), whole blood (n=19), bone marrow (n=1) and pooled organs such as lung, heart, spleen, kidneys, and liver (n=3) from cattle with and without clinical signs and highly suspected for LSD were submitted to the Eastern Zone Veterinary Laboratory (Pahang) for diagnostic analysis. DNA was extracted from 200 µL of whole blood in ethylenediaminetetraacetic acid (EDTA), tryptose phosphate broth containing scabs, saliva and nasal swabs which acted as a transport media for the samples and also supernatant samples that had been processed and homogenized. Extraction process was performed by using Indispin Pathogen Kit (Indical, Germany) according to manufacturer's instruction.

Quantitative Real Time PCR

A quantitative real time PCR (qPCR) Taqman assay that amplified and detected an 89 bp region, which encodes the intracellular mature virion envelope protein P32 within LSDV ORF074 was used to determine the viral DNA in clinical samples. Forward primer CaPV-074F1 5'- AAA ACG GTA TAT GGA ATA GAG TTG GAA-3' and reverse primer CaPV- 074R1 5'- AAA TGA AAC CAA TGG ATG GGA TA-3' were used with the probe CaPV-074P1 5'-6FAM-TGG CTC ATA GAT TTC CT-IABkFQ-3' (Bowden *et al.,* 2008). DNA amplification was performed with Quantinova Probe PCR (Qiagen, Germany) and operated using Bio-Rad CFX96 real time PCR machine. Results were generated by determination of the threshold cycle (CT). Clinical sample templates with CT values less than 37 were considered as positive result. The positive and negative controls were also included in all PCR reactions.

RESULTS AND DISCUSSION

The Taqman real time PCR assay has successfully detected 37 % of the clinical samples as positive for LSD. Scab samples showed the highest percentage of positive result which is 100 % (Table 1); with low CT values of 20.31 and 22.36 respectively. This suggests that this sample is most suitable for LSD diagnosis than other types of samples such as saliva swab (42 %), whole blood (37 %), and nasal swab, bone marrow or animal organs which recorded 0 % positive result as tabulated in Table 1. This is probably due to the samples from nodular cutaneous lesions were frequently positive by PCR and preserve higher concentration of virus (WOAH, 2021) than blood and pooled internal organ samples. Moreover, LSD virus replicates in epithelial cells, thus projecting high accumulation of virus in skin scabs (Irshad *et al*., 2022). In addition, viruses persist in skin lesions/scabs for long duration. Therefore, LSD virus can be detected in skin scabs several months after infection (Calistri *et al*., 2018). This is coherently reported by Tuppurainen *et al.* (2005) that LSDV could be detected on skin biopsies up to 92 days after infection while LSDV in blood was only positive for 4 to 11 days after infection. Additionally, LSD viruses can be detected in blood and swab samples in early stages of infection when the infected animals have fever (Irshad *et al*.,

2022), which could be the reason why LSD virus was not detected from the blood and swabs. In short, the negative results in blood samples and swabs were probably due to the condition that the virus is only present for a short duration which is fourth to eleventh day, hence the virus may have been undetected.

Table 1. Detection of LSDV in clinical samples of cattle via Taqman real time PCR assay

Type of samples	Number of samples (n)	Positive LSDV	Percentage of positive LSDV (%)
Scabs	2	2	100
Saliva swab	12	5	42
Whole blood EDTA	19	7	37
Nasal swab	1	0	0
Bone marrow	1	0	0
Pooled organs	3	0	0
Total	38	14	37

These findings also clearly indicated that the disease outbreak is still occurring while the eradication control implemented by DVS Pahang has gradually reduced the positive cases. This is because the partial stamping-out was immediately applied once the case was positively confirmed for LSD. Other control measures that subsequently practiced were suspension of importation of live animals and vaccination. In addition, effective control of LSD demands accurate and rapid laboratory techniques to confirm a tentative clinical diagnosis unexceptionally during disease outbreak. As virus isolation technique may be too time-consuming, PCR is a fast and sensitive diagnostic method to demonstrate viral DNA in clinical samples, thus allowing for rapid detection of the disease and outbreak confirmation. Up to March 2022, our laboratory has no longer received suspected LSD case from Pahang as the outbreak is over and currently endemic in Malaysia.

CONCLUSION

In conclusion, we detected and confirmed the presence of LSDV in suspected clinical samples from Pahang by Taqman Real Time PCR assay which suggests that scab is the most preferable sample for LSD diagnosis by molecular method.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding on the publication of this paper.

REFERENCES

1. Bowden T.R., Babiuk S.L., Parkyn G.R., Copps J.S., and Boyle D.B. (2008). Capripox virus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats. *Virology* 371: 380-393.
2. Calistri P., DeClercq K., De Vleeschauwer A.D., Gubbins S., Klement E., Stegeman A., Abrahantes J.C., Antoniou S., Broglia A., and Gogin A. (2018). Lumpy skin disease: scientific and technical assistance on control and surveillance activities. *EFSA Journal* 16(10): 5452.

3. Irshad H., Ahsan A., Hussain M., Zaheer M.U., Farooq U., Latif A., Asadullah A., Ullah R.W., Kalhoro N.H., Andrabi S.M.H., and Akram M. (2022). Epidemiological findings of Lumpy skin disease outbreak investigations carried out in Cattle Colony Karachi, Pakistan. *Agricultural Sciences Journal* 4(2): 28-34.
4. Khoo C.K., Dahlan R., Mat Desa Z., Syarina P.N.A., Mohd. Salim S.S.H., Barker Z., Abu Hassan M.H., Hassan R., and Mohd Saied F.H. (2022). Molecular detection of lumpy skin disease virus in Malaysia 2021. *International Journal of Infectious Diseases* 116: S1-S130.
5. Namazi F., and Tafti A.K. (2021). Lumpy skin disease, an emerging transboundary viral disease: A review. *Veterinary Medicine and Science* 7: 888-896.
6. OIE (2021). Lumpy skin disease. In OIE Terrestrial Manual 2021, Chapter 3.4.12: 1-13. World Organisation for Animal Health: France.
7. Tuppurainen E.S.M., Venter E.H., and Coetzer J.A.W. (2005). The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic technique. *The Onderstepoort Journal of Veterinary Research* 72: 153-164.
8. WAHIS (WOAH) (2022). Follow up report Lumpy Skin Disease virus, Malaysia: 1-124.
9. WOAH (2021). Lumpy Skin Disease Technical Disease Card. Retrieved from https://www.woah.org/en/document/lumpy-skin-disease-technical-disease-card/

ACKNOWLEDGEMENT.

The authors would like to thank all the staff from Eastern Zone Veterinary Laboratory (Pahang) and Department of Veterinary Services of Pahang who were involved in assisting the sample collection and distribution process.