SHORT COMMUNICATION

INCIDENCE OF LUMPY SKIN DISEASE (LSD) IN JOHOR FOR THE YEAR 2021

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Abstract. Lumpy Skin Disease Virus (LSDV), which belongs to the genus Capripoxvirus of the family Poxviridae, is the virus that causes lumpy skin disease in cattle, a highly contagious viral disease. This study aims to report the incidence of Lumpy Skin Disease (LSD) disease in Johor according to districts and to determine the most suitable specimen for disease detection using RT-PCR. A total of 424 clinical specimens were sent to the Southern Zone Veterinary Laboratory (MVZS) for diagnosis in the year 2021, consisting of whole blood in EDTA, serum, nasal swab, scab, saliva swab, meat, and lymph nodes which were obtained from cattle with clinical signs and suspected of LSD. However, only 347 specimens were fit for analysis, and a total of 187 out of the 347 specimens that were tested were found positive (53.9 %) for LSDV by TaqMan real-time PCR. Based on the specimens sent to MVZS, it was found that Pontian district has the highest positive cases (87 %) of LSD compared to other districts.

Keywords: Lumpy Skin Disease, cattle, Johor

INTRODUCTION

Lumpy skin disease (LSD) is one of the most essential transboundary and emerging diseases in cattle and has been included in the OIE list of notifiable terrestrial animal disease (Dejan *et al*., 2016). The outbreak of lumpy skin disease causes severe economic losses as the cattle suffer from skin destruction, weight gain, decreased milk production, and infertility which greatly impacted the livestock industry (Lin *et al.*, 2022). LSD virus (LSDV) is transmissible via vectors such as midgets, mosquitoes, and stable flies (Sanz-Bernardo *et al.*, 2021). LSD signs can possibly be subclinical or clinical that include fever, as well as appearance of skin nodules which cover the entire animal's body, oedema of the brisket, and limbs with lameness and enlargement of the superficial lymph node (Lubinga *et al*., 2013). LSD was first reported in Asia and the Pacific in 2019 in north-west China, India, and Bangladesh. In May 2021, Veterinary Research Institute (VRI) Ipoh received reports and specimens of unknown skin disease in dairy cattle for laboratory test to confirm provisional LSD clinical diagnosis. The results showed that all skin nodule specimens received were positive for *Capripoxvirus*, confirming the first molecular detection of LSDV in Malaysia. The laboratory diagnosis was conducted by PCR technique in which the specimen's nucleotide sequence homology analysis showed high nucleotide homology to LSDV (Khoo *et al*., 2021). Thus, the Department of Veterinary Services (DVS) has widened the surveillance of cattle with LSD symptoms throughout Malaysia. In September 2021, Southern Zone Veterinary Laboratory (MVZS) detected the first case of LSD in Batu Pahat, Johor.

For the confirmation of the disease, the diagnosis of LSD requires rapid and reliable laboratory diagnostic methods. Several PCR techniques have been developed for LSDV molecular based diagnostics. Nowadays, real-

time PCR (RT-PCR) is the molecular approach that is more efficient, quick, closed system, less contaminations, specific, and very sensitive (Gamil Sayed *et al*., 2019). This study aims to report the incidence status of LSD in the state of Johor via RT-PCR detection and to determine the most suitable specimen for disease detection using RT-PCR.

MATERIALS AND METHODS

Specimens Collection

A total of 424 clinical specimens, including whole blood in EDTA, serum, nasal swab, scab, saliva swab, meat, and lymph node were obtained from cattle with clinical signs and/or suspected of LSD. These specimens were sent to MVZS for diagnosis. All specimens were collected accordingly in sterile manner before being transported in ice to MVZS.

Specimens Preparation and DNA Extraction

Nasal swab specimens in Medium Essential Media (MEM) were filtered up to 2 ml before DNA was extracted. Scab specimens, meat, and lymph node were cut into small pieces and homogenized using sterile sand and 5 ml MEM before being grinded with a mortar and pestle. The suspension was then centrifuged at 5000 rpm for 3 mins and the resulting supernatant was removed for further testing. Whole blood specimens were directly used in the next step of nucleic acid extraction (Lin Li *et al*.,2022). A total of two hundred microlites (200 µl) homogenates supernatant was extracted using QiAmp cador Pathogen Mini kit (Qiagen, Manchester, UK) and 50 µl AVE buffer according to the manufacturer's instructions. DNA extracts were stored at -20°C before further analysis.

Primers and Probes

The Malaysian strain of LSDV from Veterinary Research Institute (VRI) Ipoh was used as a control in this study. The quantitative real-time PCR (qPCR) within LSDV ORF074 was used to determine the viral DNA in clinical specimens. Forward Primers CaPV-074F1 5'-AAA ACG GTA TAT GGA ATA GAG TTG GAA-3' and Reverse Primers CaPV – 0741R1 5'-AAA TGA AAC CAA TGG ATG GGA TA-3' were used with the Taqman probe CaPV 074P1 5'-6FAM-TGG-CTC ATA GAT TTC CT-IABkFQ-3'. (Bowden *et al*., 2008). DNA amplication was performed in a final volume of 20 µl containing the following reagents from Quantinova Probe PCR (Qiagen, Germany): 2x QuantiNova Probe PCR Master Mix 10 µl, 1.6 µl 0.10 mM of each primer, 0.5 µl 0.10 mM of probe (IDT, Singapore), and 1.3 µl of RNase Free water with 5 µl DNA specimens.

qPCR analyses

The assays were performed on Eppendorf realplex4 machine using the following amplification program: 95°C for 2 minutes; 45 cycles of 95°C for 5 seconds and 60°C for 30 seconds. Results were generated by determination of the threshold cycle (CT), the fractional cycle number at which the change in the fluorescence of each reporter dye passed a fixed threshold value set in the log (exponential) amplification phase. Clinical specimen templates with CT values less than 37 were considered positive. The positive and negative controls were included in all PCR reactions performed.

Table 1. Incidences of LSD in the state of Johor according to district in 2021

District	Number of specimens	Number of positive specimens
Batu Pahat	69	3
Johor Bahru	11	6
Kluang	40	12
Kota Tinggi	103	55
Kulai	13	0
Mersing	33	22
Muar	34	18
Pontian	77	67
Segamat	20	2
Tangkak	24	2
Total	**347**	**187**

Based on the Table 1, it was found that all districts in the state of Johor were affected by LSD virus except Kulai district. However, the district that showed the highest number of LSD specimens was Pontian with 67 positive specimens out of 77 received specimens (87 %), followed by Kota Tinggi. A total of 103 specimens were received from Kota Tinggi district for LSD diagnosis where it was found that 55 of the specimens were diagnosed positive (53.4 %). Meanwhile, 33 specimens from Mersing district were analyzed and 22 of the specimens were positive for LSD (66.7%).

The LSDV CT values achieved by qPCR assays following automated extraction are shown in Table 2.

Table 2. CT values generated using qPCR assays.

Type of specimens	Number of specimens (n)	Taqman real time PCR		
		CT value Mean	Positive	%
Blood	196	30.38	98/196	50.00
Nasal Swab	64	32.24	25/64	39.06
Scab	57	27.39	57/57	100.00
Saliva Swab	24	35.62	7/24	29.17
Meat	3	38.02	0/3	0.00
Lymph Node	3	37.56	0/3	0.00
Total	**347**	**33.53 (average)**	**187/347**	**53.90 (average)**

77 out of 424 specimens (18 %) were not analyzed due to the hemolyzed specimens (9), media transportation for the specimens is not suitable (4), the diagnosis method for serum specimens has not yet been developed (64). In total, 187 out of 347 specimens (53.90 %) were found positive for LSD by Taqman real time PCR. As shown in Table 2, specimens that showed 100 % positive result of LSDV were scabs. Scab also showed the lowest CT value means at 27.39, which indicates that the scab specimens are more sensitive. These results are similar to the study of Lin Li *et al*. (2022). We also discovered that only 50 % of blood specimens were LSDV positive, indicating that LSDV DNA was less likely to be detected in cattle's blood compared to scab specimens. This result is consistent with studies that showed LSD viremia to be a very short-lived condition; blood specimens were positive for PCR for 4-11 days after infection, whereas the virus was found in skin lesions for up to 92 days (Lin Li *et al*., 2022). Twenty-five (25) out of 64 (39.06 %) nasal swab specimens and 7 out of 24 (29.17 %) saliva swab specimens were positive for LSDV. The remaining specimens (meat and lymph nodes) showed negative results for LSDV which indicates that they are less susceptible to virus amplification than other specimens.

1(a)

1(b)

1(c)

1(d)

Figure 1. (a, b, c): There are multifocal skin nodules of variable sizes covering the face, neck, body and testicles. (d): Erupted skin nodules were samples from affected cattle.

Clinical LSD signs found in cattle from all districts were skin nodules all over the body, including the head, neck, and genital area (Fig. 1). Further investigation revealed that contributing factors towards the spread of LSD in Johor are illegal trade and movement of animals, coupled with extensive farming method with minimized practise of vector control. It is challenging to control flying biting insects, ticks, and other potential vectors for the LSD virus when livestock are raised in an integrated farming or free grazing (Samuel, 2020).

The Department of Veterinary Services Malaysia (DVS) has issued *Protokol Veterinar* Malaysia: Lumpy Skin Disease (LSD) (PVM 2(11): 1/2021) as a guide for the veterinary officials and the public to manage and control LSD. In order to prevent LSDV from further spreading, great efforts for disease prevention and control must be taken which include raising biosafety awareness, increasing public knowledge of LSDV, reducing the density of LSDV vectors, tightening regulations on illegal animal movements, and implementing massive vaccination campaigns in affected areas. DVS officials have been actively conducting LSD vaccination program in local farms to prevent LSD infection. However, no comprehensive study on the effectiveness of this vaccine has been conducted in Malaysia. Therefore, further study on evaluation of the safety, immunogenicity, and efficacy of LSD vaccine brought into Malaysia needs to be done so that the government money that has been spent on the purchase of this vaccine is worthwhile in the vaccination program carried out in the country. All farm owners also are advised to immediately quarantine animals showing clinical signs of LSDV and report to the closest veterinary authority. All animals showing clinical signs of LSDV are required to be slaughtered at government or other licensed slaughterhouses nearest to the farm. Apart from that, thorough inspection must be done before deciding which parts of the cattle are fit for human consumption.

CONCLUSION

In summary, based on the specimens sent to MVZS, it was found that Pontian district has the highest positive cases (87 %) of LSD compared to other districts. This study also concludes that scabs are the most suitable specimens for clinical diagnosis of LSDV. Scab specimens are easy for clinical examination and considered as the most sensitive clinical specimens as they show the highest detection rate. We recommend that when collecting LSD samples, emphasis should be focused on scab samples. As for farmers, they can also observe any signs of scabs on the cattle's skin for early detection of LSDV infection and take prompt corrective action before the disease spreads to other livestock.

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