Molecular Identification of LSD Virus whitin RPO30 Gene Isolate from Cattle in Malang Area

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Abstract

Lumpy Skin Disease (LSD) is a disease that affects cattle and buffalo, caused by the *Lumpy Skin Disease Virus* (LSDV). LSD causes economic losses to farmers. Several cattle in the Malang area, namely in Batu City and Malang Regency, suffered from symptoms of decreased appetite accompanied by nodules and lesions on the skin. Based on these clinical symptoms, the cattle are suspected of having LSD. The study aimed to identify the molecular LSD gene RPO30 from the Malang area. The samples were swabs of skin lesions on dairy and beef cattle that showed clinical symptoms of LSD. The samples were identified by conventional PCR using specific primers encoding the RPO30 gene with a target of 554 bp. The results of molecular identification of LSD virus RPO30 gene showed that seven cows were positive for LSD.

Keyword*:* Lumpy Skin Disease Virus, Gene RPO30, PCR

1. Introduction

Lumpy Skin Disease is caused by the *Lumpy Skin Disease Virus* (LSDV) of the Poxviridae family, Capripoxviridae sub-family, Capripox genus [1]. Although LSD is not a zoonotic disease, LSD infection can reduce production and reproductive performance in cattle and buffalo, causing significant economic losses in various aspects, including decreased milk production, weight loss, abortion, infertility, and death [2].

The incubation period in animals infected with LSDV naturally reaches 28 days. Clinical symptoms in cattle are nodules on the skin; other signs are lacrimation, nasal discharge, high fever (>40.5°C), loss of appetite, enlarged subscapular and prefemoral lymph nodes, and a drastic decrease in milk production [3].

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LSD was first discovered in Zambia in 1929 and became endemic in most countries on the African continent. In 2012, the LSD virus spread widely to the Middle East, Southeast Europe, the Balkans, Caucasus, and Kazakhstan. In 2013, the LSD virus was found to be endemic in Turkey and further endemic in Bulgaria in 2016 [2]. In July 2019, LSD disease broke out in Bangladesh, China, and India. In 2020, LSD disease spread to other parts of China, India, as well as Nepal and Bhutan [3]. On October 5, 2020, the country of Vietnam reported the first case of LSD, and based on epidemiological analysis LSD spread throughout Southeast Asia [4]. In 2021, LSD has been reported in Thailand, Cambodia and Ma,laysia [5].

The first case of LSD in Indonesia was found in Riau Province based on the Decree of the Minister of Agriculture Number 242/KPTS/PK.320/M/3/2022 concerning the establishment of a lumpy skin disease outbreak area in Riau Province on March 2, 2022 (Kepmentan, 2022). LSD cases in Indonesia based on data from the National Animal Health Information System (ISIKHNAS) on November 18, 2022, 11,474 LSD cases were found in six provinces in Indonesia. On February 9, 2023, there were 32 districts/cities with confirmed positive cases of LSD in Central Java. There were seven districts/cities in East Java and four districts/cities in Yogyakarta [6].

In September 2023, information was obtained that several cattle in Malang, namely Batu City and Malang Regency, were sick with symptoms of decreased appetite accompanied by nodules and lesions on the skin. Based on these clinical symptoms, cattle possible that they suffered from LSD. Clinical symptoms of mild and subclinical forms require rapid laboratory testing to confirm the diagnosis [7]. Disease diagnosis is immediately established as the basis for treatment and disease control. The disease's presence will threaten the 358,811 cattle population in Malang District and Batu City based on BPS data in 2022.

A sample of skin lesions was taken from that problem, and molecular identification of the cause of the disease was carried out using the polymerase chain reaction (PCR) test. A commonly used method to diagnose LSD is to detect viral DNA by PCR [7]. The RPO30 gene is one of the antigenic proteins of the LSD virus [8]. Viral polymerase plays a central role in the replication and transcription of the viral genome [10].

2. Material and Methods

2.1. Material

The sampling materials were VTM (viral transport media) ice pack gel. Materials for DNA extraction are QIAamp Viral DNA Mini Kit (Qiagen, Cat 51306). Materials for DNA Amplification are Specific primers for the RPO30 gene of the LSDV: forward primer 5'-CAGCTGTTTGTTTACATTTGATTTTT-3', reverse primer 5'-TCGTATAGAAACAAGCCTTTAATAGA-3' [9]. The master mix used Ki Viantis Cat.No.PL.1202. Materials for electrophoresis visualization of PCR products were 1% agarose gel, TBE buffer, sybersave, and DNA markers.

2.2. Methode

.Sampling

Samples were taken from skin lesions of livestock with clinical symptoms of LSD. Samples were collected by swabbing the skin lesions, and then, the samples were put in VTM bottles and carried to the laboratory using a cool box containing ice-pack gel [11].

DNA Extraction

Swab samples were extracted to obtain DNA. DNA extraction was performed using the QIAamp Viral DNA Mini Kit (Qiagen, Cat 51306), and the 100µl ATL swab solution sample was vortexed. The sample was added 20µl proteinase K, then vortexed for 15 seconds, incubated at 56^{0}C for 1 hour, and then centrifuged. The solution was added 200 µl AL, then vortexed for 15 seconds and incubated at 70^{0}C for 10 minutes. The solution was then centrifuged/spindown. The solution was added to 200 µl ethanol absolute, vortexed for 15 seconds, and then spun down. The solution was transferred to a mini spin column and then centrifuged at 8000 rpm for 1 minute. The collection tube was replaced with a new one, then the solution was added with 500 µl Buffer AW1, then centrifuged at 8000 rpm for 1 minute. The collection tube was replaced with a new one and 500 µl Buffer AW2 was added to the solution and centrifuge at 14,000 rpm for 3 minutes. The collection tube was replaced with a new one, and then the centrifuge was blank at 14,000 rpm for 1 minute. Collum was placed in a new 1.5 microtube, and then 50 µl Buffer AE was added. The solution was incubated at room temperature for 5 minutes. Next, the solution was centrifuged at 8000 rpm for 1 minute. The spin column was discarded, and the DNA obtained was stored at -20^{0}C or immediately used for other tests.

DNA Amplification

PCR master mix using Ki Viantis Cat.No.PL.1202. The composition of the master mix with a total volume of 25 µl (1 reaction) is as follows:

Table 1. PCR master mix composition

No	PCR Components	Concentration	Vol Rx (µl)
1	RNAse free water		13,75
2	10 x PCR Buffer	10	2,5
3	dNTPs	2 mM	2,5
4	Primer Forward (10 µM)	10 mM	0,5
5	Primer Revers (10 µl)	10 mM	0,5
6	Taq Pol (vivantis)	5 U/ µl	0,25
	Quantity		20 µl
	DNA templat		5 µl

The above components are inserted one by one (sequentially) into a 0.2 ml PCR tube. Negative control, sample, and positive control DNA molds were inserted last. The PCR tube is closed tightly, and then spin down is carried out. PCR tubes were inserted into a thermal cycler machine with reaction conditions according to the primers used with the PCR program: initial denaturation 950C (2 minutes), 34 cycles including denaturation 950C (45 seconds); annealing 520C (50 seconds); extension 720C (1 minute); additional extension stage 720C (2 minutes),

40C (). PCR results (in PCR tubes) were removed from the PCR machine and immediately electrophoresed or stored at 40C until ready for electrophoresis.

Electrophoresis and Visualization of PCR Products

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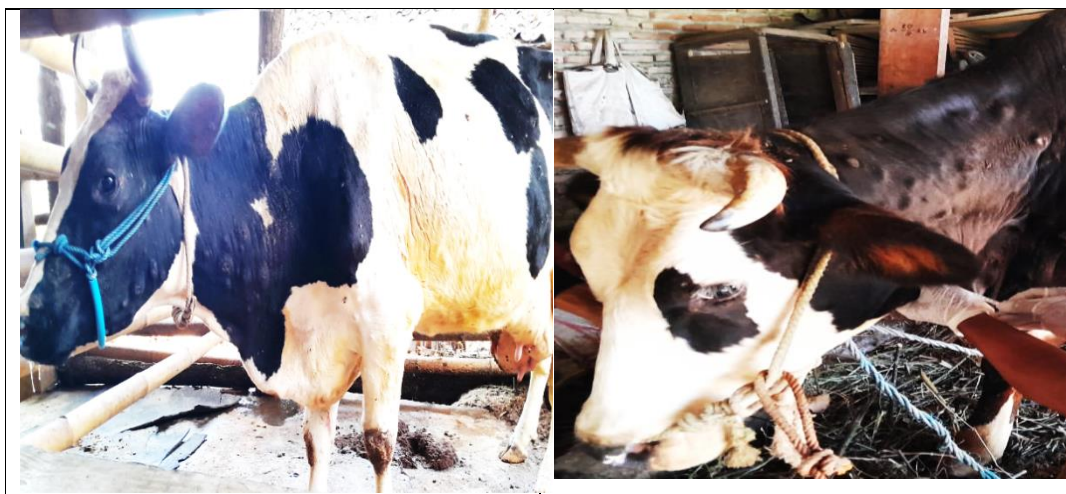
Agarose weighed 1 gram of agarose, and agarose was included in 50 ml of 1 X TBE buffer (Tris-Borate-EDTA). Agarose is put in the microwave until it becomes liquid. The liquid agarose was cooled at room temperature until the agarose temperature became about 600C. Add sybersave to the liquid agarose with a final concentration of 0.06%. For example, for 50 ml of agarose, approximately 3 µl of sybersave is added and mixed thoroughly. Agarose is poured into an agarose gel mold (first mounted comb) and left until it forms a gel. The gel is inserted into the electrophoresis vessel, and 1 X TBE buffer is added until it reaches the electrophoresis line. In a 0.2 PCR tube, a mixture consists of 1 part of DNA loading buffer plus nine parts of PCR product (for example, 1 ml loading buffer + 9 ml PCR product). Insert the mixture into the agarose gel wells carefully. A 100 bp DNA marker is included to calculate the DNA molecule's length. For validation of PCR results, negative control, and positive control were included in the gel wells. Electrophoresis of PCR results (DNA) for 50 minutes, with a constant voltage (100-120 volts). After the electrophoresis process, the gel was taken and placed on the surface of the UV transilluminator. The gel was photographed with a digital camera or gel doc. Identify the molecular length (in base pairs or bp) of sample DNA and control DNA concerning the DNA marker used.

3. Results

Sample collection was conducted at community-owned farms in Batu City and Malang Regency. Sample collection was carried out based on the "purposive sampling" method based on clinical symptoms of suspected LSD disease, namely nodules on the skin and accompanied by lesions on the skin, as shown in Figure 1. The sample size in this study was seven cows from Batu City, three heads from Malang Regency, and four from Malang.



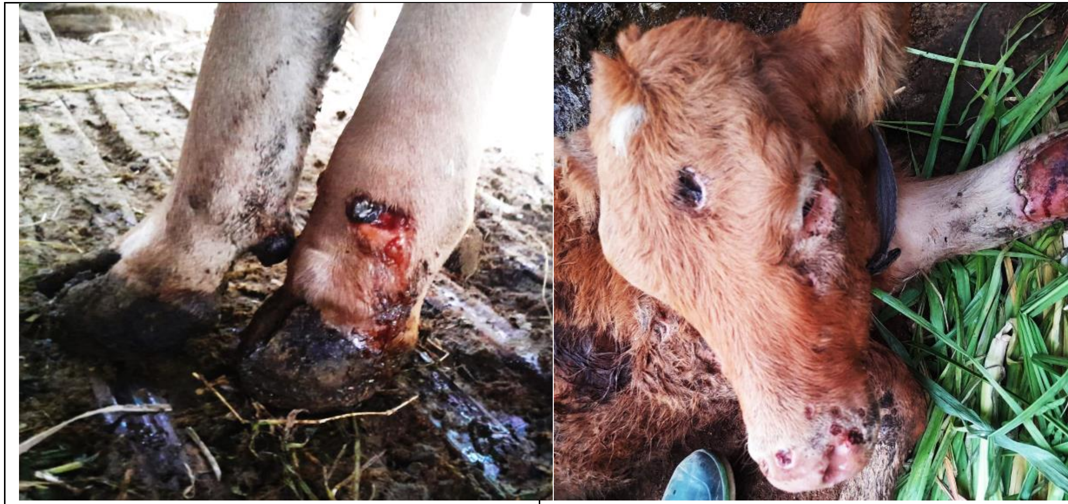


Figure 1. Cattle with clinical symptoms of suspected LSD in Batu City and Malang Regency

Samples were tested by conventional PCR, and the PCR products were visualized using gel electrophoresis to detect the presence of LSDV based on the RPO30 gene. Each sample was placed in two electrophoresis gel wells. A band indicates electrophoresis visualization of positive samples at 554 bp; the complete results can be seen in Figure 2.

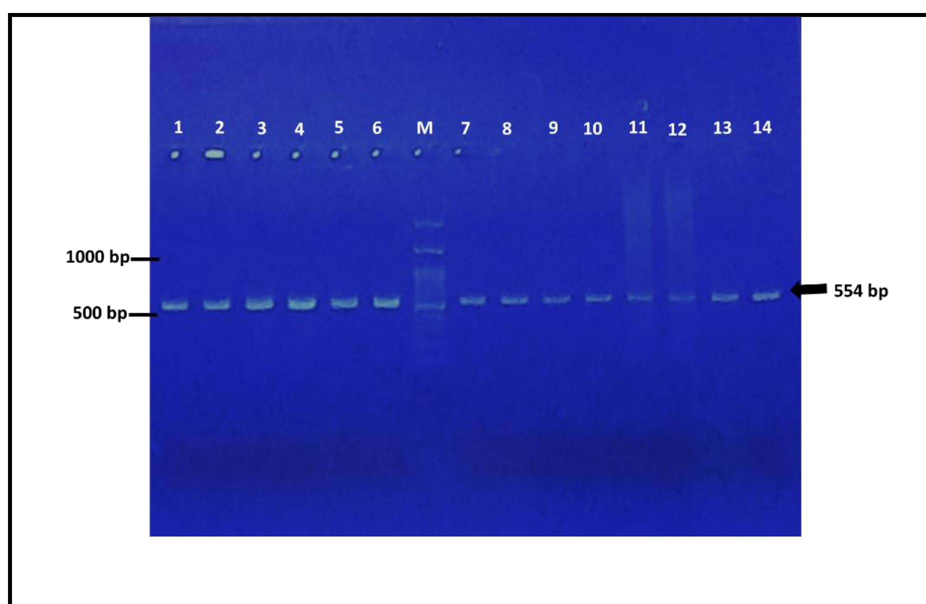


Figure 2. Electrophoresis results of the RPO 30 gene of LSDV. M indicates 500 bp marker. Numbers 1-6 indicate Batu City samples. Numbers 7-14 indicate Malang Regency samples. Numbers 1,2: sample 01/IGB; numbers 3,4: sample 02/IGB; numbers 5,6: sample 03/IGB; numbers 7,8: sample 04/IGM; numbers 9,10: sample 05/IGM; numbers 11,12: sample 06/IGM; numbers 13,14: sample 07/IGM.

The results of electrophoresis on the RPO30 gene of LSDV in seven samples from both regions can be concluded that 7 samples are positive for LSD with details in Table 2.

Table 2. Electrophoresis results of conventional PCR products.

No	Sample Origin	Sample Code	Virus LSD gen RPO30
1	Batu	01/IGB	Positive (+)
2	Batu	02/IGB	Positive (+)
3	Batu	03/IGM	Positive (+)
4	Malang district	04/IGM	Positive (+)
5	Malang district	05/IGM	Positive (+)
6	Malang district	06/IGM	Positive (+)
7	Malang district	07/IGM	Positive (+)

4. Discussions

The results of molecular identification of cattle samples from Batu City and Malang Regency were all positive for LSD, as evidenced by the presence of sample DNA fragments on electrophoresis with a length of 554 bp, which can be seen in Figure 2. The size of the amplicons for PCR with the RPO30 gene target is 554 bp, which is used to confirm the presence of LSD virus [9].

Samples taken were skin swabs from cattle that showed clinical symptoms of nodules and lesions on the skin of the head, neck, legs and body of cattle confirmed to have LSD. Nodules on the skin will necrose and cause sitfast, which leaves a deep hole. In cattle, it can cause permanent or temporary infertility, while in females, it causes abortion and temporary infertility. Generally, affected cattle have difficulty recovering completely [11].

The results showed that cattle suffering from LSD occurred in dairy and beef cattle. LSD causes clinical symptoms on the skin that are more severe in Friesian Holstein dairy cows than in local cattle breeds (Bos Indicus) because of genetic factors and farm management that make dairy cows easily stressed, causing more severe disease levels [2].

5. Conclusion

Based on the results of the study, the sick cattle in the Malang area were positive for LSD with molecular identification of the LSD virus RPO30 gene.

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Conflict of Interest

Regarding this inquiry, there are no conflicts of interest for the author.

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