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Molecular Detection *of Lumpy Skin Disease* in Cattle Samples *(Bos taurus) at* Lampung Disease Investigation Center

Elfita Nova Yunior[1], Elly Lestari Rustiati[2], Eko Agus Srihanto[3], Firwantoni[4], Ahyul Heni[5], Fransiska Panasea Anggy[6], Dwi Ayu Febriyani[7]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia. Email elfitanovayunior21@gmail.com
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia. Email ely.jazdzyk@gmail.com
[3] Lampung Veterinary Center, Indonesia. Email aguseko.dvmmsc@gmail.com
[4] Lampung Veterinary Center, Indonesia. Email irwantonitoni@gmail.com
[5] Lampung Veterinary Center, Indonesia. Email f heniahyul3@gmail.com
[6] Lampung Veterinary Center, Indonesia. Email fransiskapanasea@gmail.com
[7] Lampung Veterinary Center, Indonesia. Email dwiayufebriyani02@gmail.com
Corresponding Author Email: ely.jazdzyk@gmail.com

Abstract. *Lumpy Skin Disaese*, LSD, was first discovered in Indonesia in Riau Province in February 2022. Although LSD is not a zoonotic disease, its infection can affect the production and reproductive performance of cattle, resulting in various impacts including reduced milk production, weight loss, miscarriages, and infertility as well as significant economic losses. It is a vector-borne smallpox disease of cattle and buffalo. Characterized by the appearance of pustules on the skin. Identification of LSDV can be carried out by three methods, namely *Polymerase Chain Reaction* (PCR), virus isolation, and electron microscopy. PCR is the cheapest and fastest method to detect LSDV. LSD disease testing at the Biotechnology Laboratory, Lampung Disease Investigation Center using the RT- PCR method. No LSD was found over 13 blood samples and 2 nasal swab samples from January 2024 sampling showed that all of them were negative to LSD.
Keywords: lumpy skin disease; polymerase chain reaction; cattle; farm; molecular analysis

INTRODUCTION

Animal husbandry is a business activity of a person or group of people who maintain and breed livestock with the aim of obtaining profit from the results of these activities (Rasyaf, 1994). One of the controls that a farmer needs to consider and manage is disease control. Disease control is an effort to prevent the spread of disease and treat disease (Yustendi et al., 2022), including in oxen or often called cattle.

Lumpy Skin Disease (LSD) is a disease that often affects cattle, first discovered in Indonesia in Riau Province in February 2022. Not recognized as a zoonotic disease, LSD infection can affect production and reproductive performance of cows and causes various impacts including reduced milk production, weight loss, miscarriage, and infertility as well as significant economic losses (Abutarbush et al., 2015).

A vector-borne pox disease of Asian cattle and buffalo, LSD is characterized by the appearance of pustules on the skin (Tuppurainen, 2017). The disease is caused by Lumpy Skin Disease Virus (LSDV), a virus from the Family Poxviridae, Genus Capripoxvirus along with two other viruses, goat pox and sheep pox (OIE, 2017). Identification of LSDV can be done by three methods, namely Polymerase Chain Reaction (PCR), virus isolation, and by electron microscopy. The PCR method is the cheapest and fastest method to detect LSDV. Skin nodules, crusts, saliva, nasal secretions and blood are suitable samples for detecting LSDV using PCR. Virus isolation followed by PCR to confirm virus identity is time-consuming and expensive, but has the advantage of detecting the presence of live virus in the sample. Although identifiable using electron microscopy, classic poxvirus virions cannot be distinguished at the genus or species level (OIE, 2017). Rapid, accurate diagnosis of bovine disease is important for proper farm management. Cattle diseases can have a serious impact on cattle production and welfare (Hilmi and Latifah, 2023). Sample testing in cattle (Bos taurus) with the aim of identifying LSD disease in blood samples and nasal swabs of cattle has been conducted at the Biotechnology Laboratory, Lampung Veterinary Center. The purpose of sample testing is to identify cattle samples from farmers infected with LSD, both symptomatic and asymptomatic.

METHOD

Tools and Materials

Tools and materials used were ABI 7500 Real-Time PCR Thermo Cycler, PCR Work Station, biosafety cabinet (BSC) class II, laminar air flow, waterbath, centrifuge, vortex, micropipette with filter tip, 0.2 ml optical tube, optical plate, DNA isolation kit from QIAGEN (QIAamp® DNA Mini Kit (250) cat. no. 51306) consisting of buffer AL, proteinase K, buffer AW1, buffer AW2, buffer AE, and 2 ml collection tube, amplification kit from bioline SensiFAST Probe Lo-ROX Kit (Lot No. SFPL 222110A), forward primer, reverse primer, probe, and Nuclease Free Water (NFW).

How it Works

The initial stage of sample testing was carried out by the extraction process using a DNA extraction reagent kit from QIAGEN (QIAamp® DNA Mini Kit) with stages, namely lysis, binding, washing or purification, and elution.carried out in a biosafety cabinet so that DNA derived from chromosomal DNA is obtained. The next step is to add a template with the aim of inserting the extracted DNA into a microtube that has been filled with master mix reagents. Amplification is done to multiply the target DNA through cycles that have been programmed on the ABI 7500 Real-Time PCR Thermo Cycler. RT-PCR results were analyzed qualitatively (positive/negative) with a descriptive analysis method, including the proportion of positive and negative results in the total sample.

Using the ABI 7500 Real-Time Polymerase Chain Reaction Thermo Cycler, LSD test results can be viewed on the amplification plot screen. As the instrument collects luminescence data during a run, the screen will display the sample amplification process. On the plate display layout tab, the amplification plot screen displays data to the well-selected when the continuous data collection method is set. Each cycle is marked with a normalized color luminescence graph of the amplification plot.

Results and Discussion

Samples obtained from survey and passive sampling activities in January 2024 and tested for LSD at the Biotechnology Laboratory, Lampung Veterinary Center totaled 15 samples (Table 1, Figure 1). Specimen types for LSD detection were 13 blood specimens and 2 swabs. The proportion of samples tested was 100% negative.

Table 1. LSD Sample Test Results in Cattle at the Biotechnology Laboratory of Lampung Veterinary Center in January 2024

No. Agenda	Total Sample	Test results	
		Positive	Negative
PR 0002, 0004, 0005, 0006	4	-	4
PR 0008, 0010	2	-	2
PR 0009	1	-	1
PR 0031, 0032, 0033, 0034	4	-	4
PR 0056, 0057	2	-	2
PR 0059	2	-	2
Sum	**15**	**0**	**15**

RT-PCR is able to detect and present PCR reaction results in real time on the screen of a connected computer. This makes it possible to quantitatively count the number of DNA initial reactions and has time efficiency in virus detection speed.

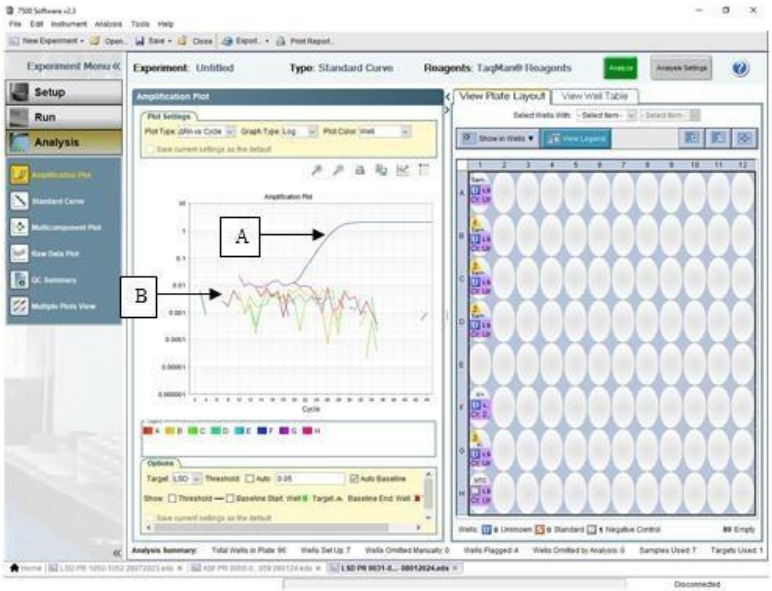

Figure 1. Visualization of LSD Negative Test Computer Results
(Source: Biotechnology Laboratory, Lampung Disease Investigation Center)

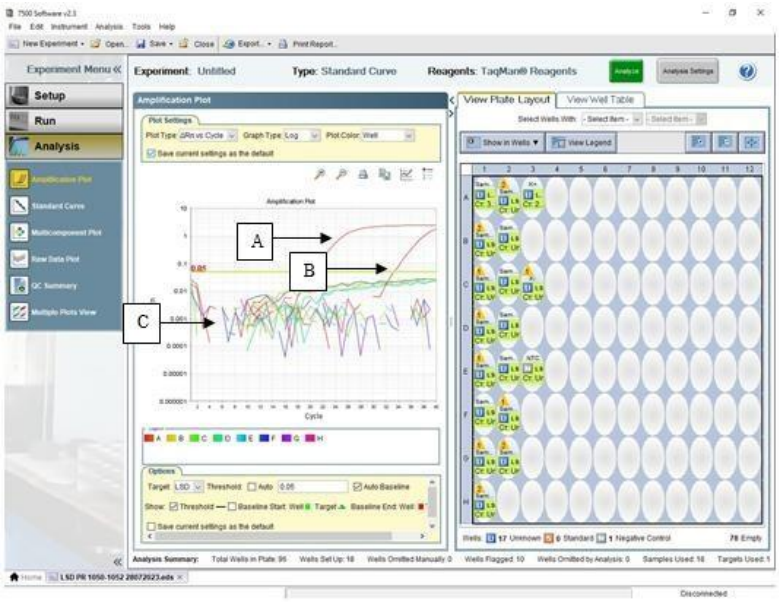

Figure 2. Visualization of LSD Positive Test Computer Results
(Source: Biotechnology Laboratory, Lampung Disease Investigation Center)

Based on the predetermined range, the interpretation of CT values is divided into high (positive and negative) and low (negative) values. A low CT count indicates a high concentration of viral genetic material in the sample, while a high CT count indicates a low concentration of viral genetic material. Selection of wells on the plate layout tab to view the high and low CT values on the amplification plot screen. The amplification plot is set with a range of 0.05 and a luminescence graph with various colors.

The blue color as a positive control indicates a graph that crosses the range, while the color The other tested samples show a graph that rises and falls and is below the range line, which means that the sample test results are negative (Figure 1). In the positive RT-PCR test results, the graph for the negative control in red and the graph for test sample number 8 in magenta show a graph that crosses the range line so that it can be said that the sample is positive for LSD (Figure 2). The graph line below the light green range line is the negative control, while graph below the other range line is the sample, so it can be said that the sample is LSD negative.

Clinical symptoms LSDV infection include fever up to 41.5°C, anorexia, decreased milk production, runny nose, conjunctivitis, hypersalivation, depression, and swollen lymph nodes. Generally, nodules/bumps are found on the head, neck, back, abdomen, tail, and genitals. These nodules become necrotic, causing a mass that leaves a deep hole (Sendow et al., 2021). LSD disease can also cause permanent or temporary sterility in bulls. Affected cattle generally have difficulty making a full recovery. Secondary infections, especially pneumonia and fly pustules, are common and cause deep wounds. Some animals do not show clinical symptoms even if antibodies are detected (Issamoy et al., 2020).

Transmission of the virus is through various vectors, namely flies (Stomoxys calcitrans) and mosquitoes (Aedes aegypti). The virus can be transmitted to susceptible animals through direct contact with infected secretions or through indirect contact, such as contaminants from animal owners or objects (Ratyoha et al., 2022). In addition, LSD infection can also occur in utero. The disease is transmitted from infected mothers to calves through milk or injured skin. Apart from vectors, LSD transmission can occur through the consumption of contaminated food or water, direct contact, natural mating, and artificial insemination. Mass vaccination is the most effective way to control the spread of this disease (Tuppurainen, 2017).

CONCLUSION

The proportion of LSDV test results in 15 cattle samples in January 2024 at the Biotechnology Laboratory of Lampung Veterinary Center showed 100% negative results.

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