Ann PUBLISHER
Journal of Agricultural Sciences (Agrosci)
e ISSN 3074-2423
p ISSN 3032-7547

Molecular Detection *of Jembrana Disease* in Balinese Cattle (*Bos sondaicus*) Samples at the Lampung Veterinary Center

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Abstract. *Jembrana Disease* is an infectious disease in Balinese cattle, which is characterized by fever and inflammation of the oral mucous membranes (stomatitis, enlarged lymph nodes of the preskapularis, prefemolaric, and parotid, sometimes accompanied by blood *sweating*. This disease reaches a mortality rate of no more than 20% caused by *the Jembrana Disease Virus* (JDV). According to data from the Directorate of Animal Health, Jembraa Disease cases continue to increase from year to year, with as many as 398 cases in 2016 and increased to 4,797 cases in 2018. The presence of the Jembrana virus can be detected through tests. One of them is by identifying and isolating the pathogen. Technological breakthroughs in DNA-based testing systems are becoming an available alternative. Viruses can be detected through commonly used molecular analysis such as *Polymerase Chain reaction* (PCR). The *Jembrana Disease* test was carried out at the Biotechnology Laboratory of the Lampung Veterinary Center using PCR and then visualized by agarose gel electrophoresis. The test results of 53 samples consisting of 50 types of *buffycoat* samples and 3 types of organ samples showed that 1 type of organ sample was detected with JDV

Keywords: jembrana disease; balinese cow; polymerase chain reaction; buffycoat; organ.

INTRODUCTION

The development of the livestock sub-sector is part of the development of the agricultural sector and national development and makes a very important contribution to supporting the provision of nutritious food and animal protein and increasing national income (Fatimah and Rahim, 2019). The development of cattle farming or better known as cattle in Indonesia, has great potential to improve genetic quality in preserve it in the future. Sukarno and Setyawan (2015) stated that one of the cows in Indonesia that has great potential to be developed is the

Balinese cow. Balinese cattle are developed as a natural livestock resource that has distinctive characteristics and has good reproductive abilities in various environments in Indonesia

The problem that occurs in Balinese cattle breeders is diseases that attack their livestock, such as *Jembrana Disease*. *Jembrana Disease* is an infectious disease in Balinese cattle, which is characterized by fever, inflammation of the oral mucous membranes (stomatitis), enlarged lymph nodes of preskapularis, prefemoral and parotid, can be accompanied by blood *sweating* (Mardiatmi, 2015). According to Su *et al*. (2018), the mortality rate of this disease reaches 20% caused by *the Jembrana Disease Virus* (JDV). The Directorate of Animal Health stated that *Jembrana Disease* cases continue to increase from year to year, as many as 398 cases in 2016 and increased to 4,797 cases in 2018 (Firison et al., 2022).

Jembrana virus is an RNA virus that infects target cells by attaching to their surface and inserting RNA genetic material into the target cell. The viral RNA then undergoes reverse transcription into cDNA. Viral DNA moves from the cytoplasm to the nucleus, where the integrase enzyme integrates viral DNA into the DNA of the host cell, thus forming a provirus (Krisnayanti *et al*., 2020). Proviruses (DNA) are found in the blood of infected target cells, for example Balinese cattle. The presence of the Jembrana virus can be detected through the test process. One of them is the diagnosis of the disease, which can be identified by isolating the pathogen. Technological breakthroughs in DNA-based testing systems are becoming an available alternative. Indriawati *et al*., 2013, stated that this virus can be detected at the DNA level through molecular-based techniques. A commonly used molecular-based technique is the *Polymerase Chain Reaction* (PCR) method.

Research to identify the existence of the jembrana virus molecularly by the *Polymerase Chain Reaction* (PCR) method under the Lampung Veterinary Center program.

METHOD

Tools and Materials

The tools and materials used are Thermo Cycler, PCR Work Station, biosafety cabinet (BSC) class II, laminar air flow, waterbath, centrifuse, vortex, micropipette and filter tip, 0.2 ml optical tube, optical plate, chamber, agarose gel electrophoresis, TAE buffer, RNA isolation kit from QIAGEN, RNA carrier, AVL buffer, AW1 buffer, AW2 buffer, and AE buffer.

How it works

The samples used were *buffycoat samples* and organ samples. Extraction was carried out using an RNA extraction kit inside a biosafety cabinet. RNA extraction was performed according to the protocol of the QIAamp® RNA Mini Kit (250). The extraction process aims to take RNA from the sample through four stages, namely lysis, *binding*, *washing* or purification, and *elution* using a DNA extraction reagent kit derived from QIAGEN (QIAamp® DNA Mini Kit (250) cat. no. 51306) carried out in a *biosafety cabinet* by following the predetermined protocol. The next stage is amplification which has the goal of multiplying the target DNA through several cycles that have been programmed on the ABI 7500 Real-Time PCR Thermo Cycler. The amplified product is further electrophoreseized in 2% agarose gel in TAE buffer (Tris-acetate-EDTA), and added with ethidium bromide for visualization of results. The electrophoresis process lasts for 30 minutes at a voltage of 100V and the agarose gel is then visualized with UV light inside the GelDoc machine.

DISCUSSION

The samples tested were *buffy coat, blood with anticoagulant (EDTA-Ethylene diamine tetra-acetic acid)*, and organs (Figure 1). The anticoagulant blood is processed by the centrifuge and washing method to obtain a buffy coat. Blood plasma is also collected if the cattle are known to be in the phase of showing clinical symptoms such as high fever characterized by diarrhea. Organs, especially the spleen, are scraped with mortar, and PBS is added. This sample will later be tested using the PCR method to detect the presence of genetic material for the jembrana virus. All samples tested showed that there was 1 sample infected with *Jembrana Disease*, namely in the sample with agenda number AR 0908, while the other samples showed negative results for *Jembrana Disease*.

Fifty-three samples were obtained from survey activities on December 27, 2023 - February 4, 2024 to be tested for jembrana disease (Table 1) with 52 samples tested showing negative *for jembrana disease virus.* This test is intended for the identification *of jembrana disease virus* in Balinese cattle both symptomatic and asymptomatic.

Table 1. Results of JD sample test on pig blood at the Biotechnology Laboratory of the Lampung Veterinary Center on December 27, 2023 – February 4, 2024

No. Agenda	Number of samples	Sample type		Test results	
		Buffycoat	**Organ**	**Positive**	**Negative**
AR 0864, 0907, 0908	3	1	2	1	2
PR 1856	2	2	-	-	2
PR 1858, 1859	29	29	-	-	29
PR 0067, 0069, 0079	14	14	-	-	14
PR 0101	4	4	-	-	4
AR 0050	1	-	1	-	1
Sum	**53**	**50**	**3**	**1**	**52**

In the PCR process, negative controls are included in the form of DNA templates that are negative *for the disease virus* and positive controls in the form of DNA templates that are positive *for the virus and the virus is positive*. The results were considered valid if DNA bands appeared in the positive control with a DNA molecular length of 360 base pairs, and conversely, no DNA bands appeared in the negative control, indicating the absence of contamination. In sample wells 1 and 2, there are no DNA bands as in the negative control well, meaning that samples 1 and 2 are considered negative (Figure 1). The result is considered positive when DNA bands appear from the sample well with the same molecular length as the positive control. In the well, sample 1 and the negative control do not show DNA bands. Therefore, sample 1 is considered negative. Meanwhile, sample well 2 shows the presence of the same DNA bands with positive control, meaning that sample 2 is positive *for Jembrana Disease* (Figure 2).

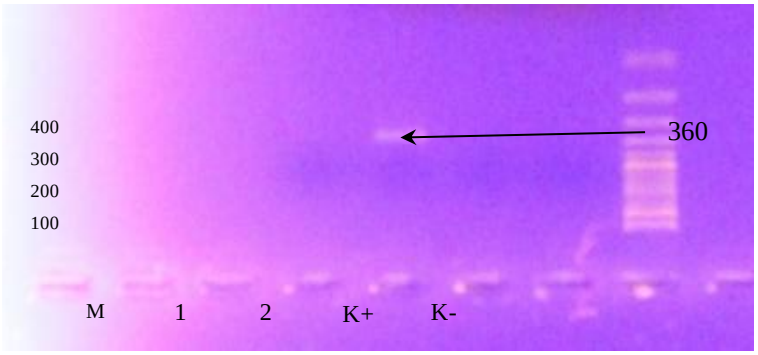

Figure 1. PCR results of Balinese cattle samples negative *for Jembrana Disease*

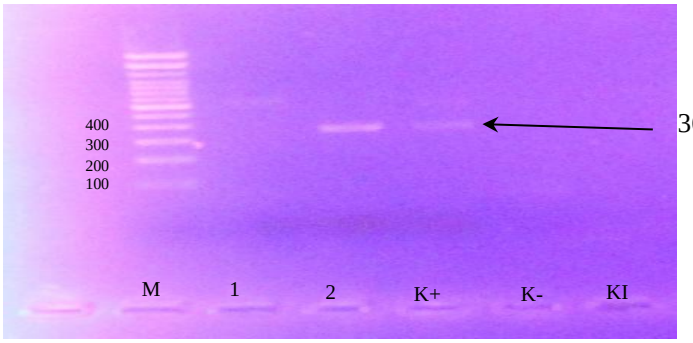

Figure 2. PCR results of Balinese cattle samples positive for *Jembrana Disease*

Monitoring by the PCR method was also carried out in 2013 by taking samples from all over Bali and the Bali Superior Cattle Farm Center (BPTU) Pulukan, showing that the presence of the jembrana virus was not detected (N=4,276) (Agustini et al., 2014). Using the same technique in 2015, the results of surveillance on samples from all over Bali also showed negative results for the presence of the jembrana virus (Agustini et al., 2016).

A number of samples that have been amplified in the PCR test but are below the detection limit, but can be clearly distinguished from the negative DNA control *of Jembrana Disease Virus* (JDV), were re-analyzed using conventional PCR. A JDV 1 *primary forward* pair with a sequence (5-GCAGCGGAGGTGGCAATTTTGATAGGA-3) and a *JDV 3 reverse primer* with a sequence (5-CGGGTGGTGGTGTCCACCCCATG-3) were used to amplify the 360 bp fragment within the open reading frame (Lewis *et al*., 2009). The number of these base pairs is referred to as the length of the DNA on the chromatin, abbreviated with bp (base pair). DNA bands of the same length were obtained at positive controls.

Testing for *Jembrana Disease* in Balinese cattle in Indonesia is important to consider because 27% of the total cattle population from Indonesia are Balinese cattle, making a major contribution to beef production in Indonesia (Desport *and* Lewis, 2010). The Directorate General of Livestock and Health (2007) stated that the strengthening of the Animal Disease Detection and Control System was carried out as an effort to deal with the threat of the entry of infectious animal diseases that have emerged and have the potential to cause losses to the Indonesian community and livestock. Molecular testing *of Jembrana Disease* with the *Polymerace Chain Reaction* method is important to ensure that the beef to be consumed is free *of Jembrana Disease.*

The ability of PCR in identifying the presence of the jembrana virus can be used to check health to speed up the identification process. *Polymerace Chain Reaction* (PCR) is one of the methods that is relatively often used in testing the identification of the presence of the jembrana virus. Testing for the detection of Jembrana disease can also use other methods such as *Indirect*

Enzyme Linked immunosorbent Assay (ELISA), *Western Immunoblotting* (WIB), and *Immunohistochemistry* (CPI) (Directorate of Animal Health, 2015).

CONCLUSION

The molecular detection technique *of jembrana disease* in 53 samples was identified in 1 sample, which can be seen through the results of sample visualization, which showed the presence of DNA bands aligned with the positive control.

BIBLIOGRAPHY

Agustini, N. L. P., I N. Dibia, and D. Mustikawati. 2014. Surveillance of Jembrana Disease in Bali Province in 2013. Veterinary Bulletin, BBVet Denpasar. 26 (84)

Agustini, N. L. P., I W. M. Tenaya, and I K. E. Supartika. 2015. Jembrana Vaccine Effectiveness Test. Veterinary Bulletin, BBVet Denpasar. 27 (86)

Agustini, N. L. P., D. K. Pradana and S. E. Melyantono. 2016. Surveillance of Jembrana Disease in Bali Province in 2015. Technical Report on the Results of Surveillance, Monitoring and Development of Test Methods

Desport, M., & Lewis, J. (2010). Jembrana disease virus: host responses, viral dynamics and disease control. *Current HIV research*, *8*(1), 53-65.

Fatimah, F., & Rahim, R. (2019). Development of Bali Cattle Farming Business through the Implementation of the Agribusiness System in Gowa Regency. *COMPETITIVENESS*, 8(1), 107-125.

Firison, J., Salim, A., Sudarmansyah, S., Alfayanti, A., Fauzi, E., Afrizon, A., & Ishak, A. (2022). Analysis of the Causes of Jembrana Disease in Balinese Cows and Prevention Efforts. *AGRITEPA: Journal of Agricultural Science and Technology*, *9*(2), 403-410.

Indriawati, Margawati, E.T. and Ridwan, M., (2013). Identification of the Jembrana virus in Balinese cattle using the molecular marker of the env SU gene. *Biology News*, 12(2), pp.211–216.

Lewis, J., McNab, T., Tenaya, M., Hartaningsih, N., Wilcox, G., & Desport, M. (2009). Comparison of immunoassay and real-time PCR methods for the detection of Jembrana disease virus infection in Bali cattle. *Journal of virological methods*, *159*(1), 81-86.

Mardiatmi. (2015). Guidelines for the Control and Control of Jembrana Disease. Directorate of Animal Health, Director General of Livestock and Animal Health, Ministry of Agriculture.

QIAGEN. 2020. QIAamp® Fast DNA Stool Mini Handbook. https://www.qiagen.com/ch/resources/download.aspx?id=2a3f2c0b-2e8a-49fd-b442-829108ae1a4a&lang=en, downloaded on Tuesday, March 19, 2024 at 22.00 WIB.

Sutarno and A. D. Setyawan. (2015). Genetic diversity of local and exotic cattle and heir cross breeding impact on the quality of Indonesian cattle. *Biodiversity* 16 (2): 327-354.

Su, X., Wang, H., Zhou, X., Li, Z., Zheng, B., & Zhang, W. (2018). Jembrana disease virus Vif antagonizes the inhibition of bovine APOBEC3 proteins through ubiquitin-mediate protein degradation. Virology, 519, 53–63. https://doi.org/10.1016/j.virol.2018.03.028