



## Correlation of Spleen Weight with Body Temperature and Cycle Threshold Value of Bali Cattle Given Jembrana Virus Inoculum

Yoga Cahya Mahardika<sup>1</sup>, Pungky Slamet Wisnu Kusuma<sup>2</sup>, and Tatang Sopandi<sup>3\*</sup>

<sup>1-3</sup> Study Program of Biology, Faculty of Science and Technology, Universitas PGRI Adi Buana Surabaya, Indonesia

[yoga.cahya@mail.ugm.ac.id](mailto:yoga.cahya@mail.ugm.ac.id)<sup>1</sup>, [pungky@unipasby.ac.id](mailto:pungky@unipasby.ac.id)<sup>2</sup>, [tatang.sopandi@unipasby.ac.id](mailto:tatang.sopandi@unipasby.ac.id)<sup>3</sup>

\*Penulis Korespondensi [tatang.sopandi@unipasby.ac.id](mailto:tatang.sopandi@unipasby.ac.id)

**Abstract.** Beef demand in Indonesia continues to increase, particularly during religious holidays. Bali cattle, one of the main sources of beef, face a serious threat from Jembrana disease, a viral infection that compromises the immune system and causes significant economic losses. This study aimed to determine the correlation between spleen weight, body temperature, and cycle threshold (Ct) values in Bali cattle following Jembrana virus inoculation. A correlational method was applied to eight female Bali cattle injected with Jembrana virus inoculum. Physiological changes in body temperature were monitored, followed by necropsy to assess spleen weight. In addition, Real-Time Polymerase Chain Reaction (RT-PCR) was performed before and after inoculation to detect viral presence and determine Ct values. Data were analyzed using SPSS with Pearson correlation tests. The results revealed a very strong positive correlation between spleen weight and body temperature, while a very strong negative correlation was observed between spleen weight and Ct values. These findings indicate that Jembrana virus inoculation leads to increased body temperature and splenic enlargement in Bali cattle, accompanied by a decrease in Ct values, reflecting higher viral loads. Therefore, the administration of antipyretics, anti-inflammatory drugs, analgesics, vitamins, and antibiotics prior to vaccination is recommended to reduce the risk of hyperthermia, splenic swelling, decreased Ct values, and secondary infections.

**Keywords:** Bali Cattle; Body Temperature; Cycle Threshold Value; Jembrana Virus; Spleen Weight.

### 1. INTRODUCTION

Beef is an important animal food source in Indonesia, alongside chicken and fish. Although considered a luxury item, demand for beef can increase on certain occasions, such as religious holidays. Retail beef prices can increase by 10-20% in the lead-up to national religious holidays (Priyanti & Inounu, 2016). Beef cattle are largely raised traditionally in Indonesia, providing a source of income and savings for rural communities on a limited scale (Ishak et al., 2020). One type of beef cattle widely raised by breeders is the Bali cattle. Bali cattle are distributed throughout almost all provinces in Indonesia and are growing rapidly in various regions due to their several advantages. Bali cattle are native to Indonesia, a superior commodity, and have great potential for maximum meat production (Hafid, 2015). Bali cattle are a leading commodity as a good supplier of animal protein. According to Guntoro et al. (2018) found that the fertility rate of Bali cattle is relatively high compared to other cattle, reaching 83%, unaffected by feed quality. Furthermore, considering their compact and harmonious carcass characteristics and body shape, Bali cattle are classified as ideal beef cattle, even surpassing European beef cattle such as Hereford and Shorthorn. Bali cattle are a local breed with significant potential for development in Indonesia, having adapted well to the local environment. However, Bali cattle breeders often face challenges in their livestock farming, such as Jembrana disease (Guntoro et al., 2018).

Jembrana disease can cause a mortality rate of approximately 20% in Bali cattle (Su et al., 2018). Jembrana disease began to emerge and become an epidemic in Indonesia in 1964 (Soyi & Kusumawati, 2019). Jembrana disease attacks the immune system of Bali cattle and is caused by a Lentovirus from the Retroviridae family. The main clinical signs of this disease are fever, enlarged lymph nodes in the shoulders, in front of the knees, and below the ears, and blood sweating. Jembrana disease can be transmitted directly through interaction between sick and healthy cattle, or indirectly through intermediaries (Firison et al., 2022).

To prevent pathogen infection, Bali cattle are equipped with a defense system, one of which is the spleen. This complex organ with many functions. Its role in the defense system is related to the immunological response to antigens derived from the blood, where it functions as a secondary lymphoid organ. The spleen is the organ that contains the most Jembrana virus-infected cells (Berata, 2010).

The presence of Jembrana virus can be detected using several tests. One way to diagnose the disease is by identifying it through isolation of the causative agent. One frequently used molecular-based technology is the Polymerase Chain Reaction (PCR) method. This method is expected to enable rapid and accurate detection of Jembrana virus. Polymerase Chain Reaction (PCR) is a relatively frequently used method (Irwanto et al., 2021). Jembrana virus-infected cells in the spleens of Bali cattle are approximately 9.5% (Berata, 2010). Detection of Jembrana virus antigen is closely related to increased body temperature, total leukocyte count, and the number of infected lymphocytes (Astawa et al., 2016). However, studies on the correlation between spleen weight inoculated with Jembrana virus and body temperature and cycle threshold (Ct) values in Bali cattle have not been widely published. This study aims to determine the close relationship between spleen weight inoculated with Jembrana virus and body temperature and Ct values in Bali cattle.

## **2. RESEARCH METHODS**

The study was conducted using a correlational research method between the spleen weight of Bali cattle that had been given Jembrana virus inoculum with body temperature and Ct Value.

### **Preparation of Experimental Animals**

Sixteen healthy female Bali cattle, aged 1-1.5 years, were obtained from breeders on Nusa Penida Island. All cattle were sprayed with insecticide (Butox 1 ml/liter), dewormed, multivitamins, and antibiotics seven days prior to vaccination.

### **Making virus inoculum**

The spleen stock infected with Jembrana virus isolate Tabanan strain 87 stored in a minus 80°C freezer was left in a Biosafety Cabinet until the texture was soft. Next, the spleen was ground in a sterile mortar, then the ground result was put into a sterile tube. A 10% spleen suspension was made by adding RPMI (Roswell Park Memorial Institute) media. The suspension was centrifuged at 3000 rpm for 15 minutes at 4°C, the supernatant was taken with a 3 ml syringe and placed in a container containing ice gel.

### **Inoculation and Clinical Observation of Cattle**

Cattle declared clinically healthy were injected intravenously with the inoculum at a dose of 1 ml per head. Clinical signs, such as elevated body temperature and swollen superficial lymph nodes, were observed daily. Necropsies were performed on day 7, after which the spleens were aseptically removed.

### **Body Temperature Measurement**

Body temperature is measured by inserting a thermometer into the anus (rectum) for approximately 1 minute. The measurement is stopped after the digital thermometer gives a maximum body temperature signal. Measurements were carried out three times each time the sample was taken, then the values from the results of the three measurements were averaged.

### **Spleen Removal**

The necropsy room and equipment were prepared in a sterile condition. Prior to the necropsy, the donor cow was bathed in clean water. Next, the blood was removed by a bloodletting procedure. The incision area was disinfected with 70% alcohol. After the abdominal cavity was dissected, the spleen was aseptically removed and wrapped in sterile aluminum foil. The wrapped spleen was taken to a sterile room for weighing.

### **Cycle Threshold Value**

The Cycle Threshold value (Ct) measurements were performed using the TaqMan Real-Time Reverse Transcription-PCR and JDVp26 Antigen Capture Enzyme-Linked Immunosorbent Assay methods (Stewart *et al.*, 2007). Blood samples were taken from cows through the jugular vein. 2.5 mL of each cow's blood was taken and mixed with EDTA. Total DNA isolation from the sample collection using a kit followed the procedure recommended by the QIAamp RNA Kit. The total DNA collected was then used in the PCR master mix process. The initial stage of DNA extraction aims to obtain a DNA solution that can be used for RT-PCR analysis. The second stage, after obtaining the DNA solution, RT-PCR analysis was carried out using MIX solution, DNase-free water (negative control), EPC solution (positive solution) with a temperature of 95°C at the initial denaturation and denaturation stages and at

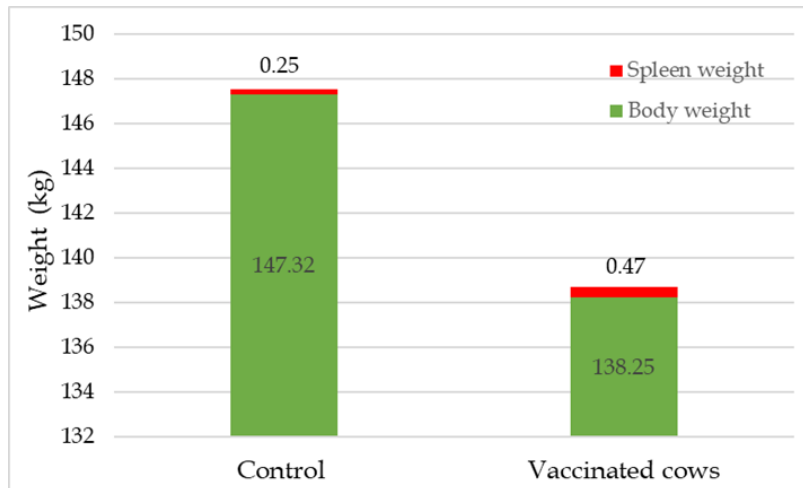
the annealing stage at a temperature of 60°C with 40 repetitions.

### Analysis Data

Data from observations of wet weight of the spleen, body temperature and Ct value will be analyzed using Pearson correlation analysis using SPSS software.

## 3. RESULTS AND DISCUSSION

### Spleen Weight



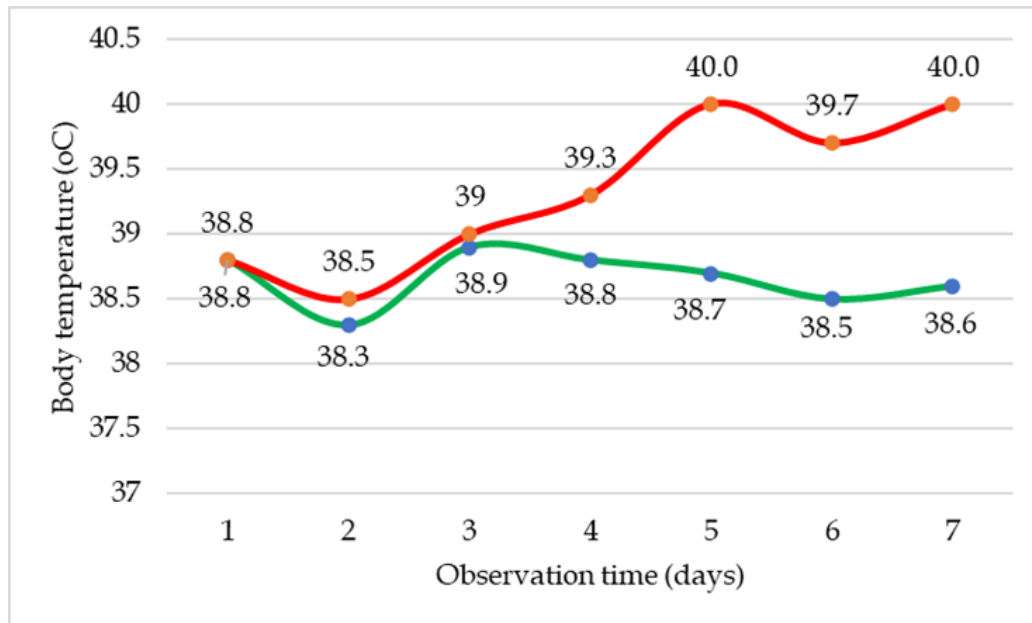
**Figure 1.** Average spleen weight and body weight of Balinese cattle inoculated with Jembrana virus.

The spleen weight of Bali cattle inoculated with Jembrana virus is shown in Figure 1, which shows that the average spleen weight of Bali cattle inoculated with Jembrana virus ( $0.46 \pm 0.12$  kg/spleen) was significantly ( $P < 0.05$ ) greater than that of the control ( $0.25 \pm 0.16$  kg/spleen). The results of this study also showed that the average body weight of Bali cattle inoculated with Jembrana virus ( $138.25 \pm 17.09$  kg/head) was significantly ( $P < 0.05$ ) lower than that of the control ( $147.32 \pm 6.32$  kg/head).

This study indicates that the percentage of spleen weight per body weight of Balinese cattle inoculated with Jembrana virus was higher than that of cattle not inoculated with Jembrana virus. The percentage of spleen weight of Balinese cattle inoculated with Jembrana virus was around 0.34% of body weight, while that of uninoculated cattle was 0.17%. According to Lestari (2017), the average normal spleen weight of Balinese cattle is around 0.17% of body weight. The increase in spleen weight inoculated with Jembrana virus is thought to be due to the Jembrana virus attacking the cattle's immune system, especially lymphocyte cells. According to Etriwati et al. (2017), Jembrana virus predilections the spleen, an organ that produces lymphocyte cells. Jembrana virus infection triggers activation of the innate and

adaptive immune systems, including the release of pro-inflammatory cytokines and the recruitment of immune cells such as macrophages and lymphocytes to fight the virus, but an excessive immune response can exacerbate clinical symptoms and tissue damage (Desport and Lewis, 2010). Jembrana Disease Virus infection triggers the activation and accumulation of immune cells in the spleen and increased blood flow (congestion), which causes an enlarged spleen (splenomegaly) in infected cattle (Wilcox et al., 1992; Soesanto & Wilcox, 1999).

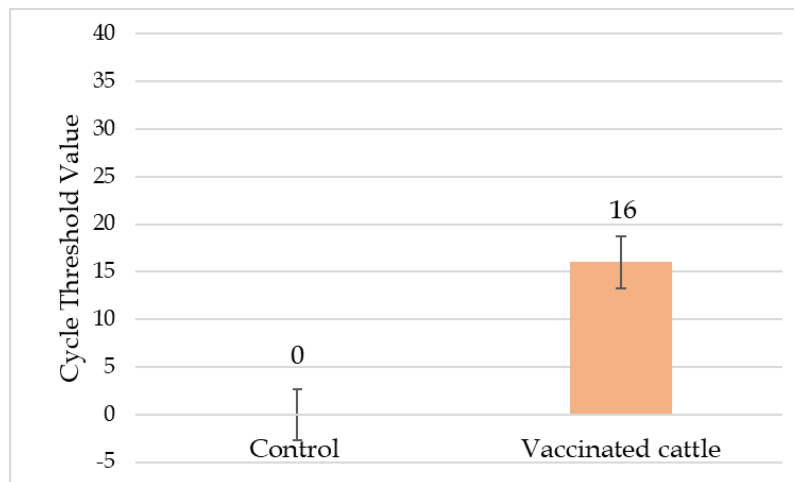
### Body Temperature



**Figure 2.** Average body temperature of Balinese cattle inoculated with Jembrana virus.

The results of the study (Figure 2) showed that the average body temperature of Bali cattle inoculated with Jembrana virus fluctuated daily, but higher than that of Bali cattle not inoculated with Jembrana virus. This study indicates an increase in body temperature in cattle inoculated with Jembrana virus beyond normal limits. According to Sari et al. (2013), the normal rectal temperature of Bali cattle over one year old is around 37.6°C – 39.0°C. This increase in body temperature is thought to be due to a viral infection, which triggers immune cells to respond by releasing cytokines to fight the viral infection. Cytokines also function as pyrogens, substances that can increase body temperature by influencing the hypothalamus to increase body temperature (Conti et al., 2004; Eskilsson et al., 2014). Cattle inoculated with Jembrana virus experienced fever and changes in the immune cell population (CD4, CD8, and B-cells) and cell distribution in lymphoid organs (Tenaya et al., 2012). Suherman et al. (2013) suggested that fever is part of the systemic response to infection, where the body attempts to create an environment less conducive to the virus and increases immune cell activity.

### Cycle threshold Value



**Figure 3.** Average CT Value of Balinese cattle inoculated and not inoculated with Jembrana virus.

The measurement results (Figure 3) showed that Bali cattle inoculated with Jembrana virus had a Ct value of  $16.01 \pm 2.71$ , which was considered a strong positive value, while uninoculated cattle had a Ct value of 0. A Ct value of 16 indicates a very high viral load, which indicates that Jembrana virus replication is actively taking place in the sample. Jose et al. (2024) classify a Ct value of 10-20 as high, indicating a large number of viruses. The increase in the Ct value is thought to be due to the presence of a large number of Jembrana viruses (Sala et al., 2023). The Ct value in RT-qPCR is understood as the number of amplification cycles until the fluorescence signal passes the detection threshold; therefore, a low Ct value indicates a relatively high initial nucleic acid target (high viral load) (Dahdouh et al., 2020). However, interpretation of Ct as an absolute viral load or predictor of infection severity should be done with caution because variations in kits, protocols, and amplification efficiency can affect results (Dutta et al., 2022).

### Correlation of Spleen Weight with Body Temperature and CT Value

**Table 1.** Correlation of spleen weight with body temperature and CT value of Balinese cattle inoculated with Jembrana virus.

Parameter	Correlation	Parameter		
		Spleen weight	Body temperature	CT value
Spleen weight (kg)	Pearson correlation	1	0.987**	-0.837**
	Sig. (2-tailed)		<0.001	0.010
	N	8	8	8
Body temperature (°C)	Pearson correlation	0.987**	1	-0.786*
	Sig. (2-tailed)	<0.001		0.021
	N	8	8	8
CT value	Pearson correlation	-0.837**	-0.786*	1
	Sig. (2-tailed)	0.010	0.021	
	N	8	8	8

The results of the correlation analysis (Table 1) show that the spleen weight of Balinese cattle inoculated with Jembrana virus has a very strong positive correlation (0.987) with body temperature but a very strong negative correlation (-0.837) with Ct value. The very strong positive correlation between spleen weight and body temperature indicates that the increase in spleen weight in Balinese cattle inoculated with Jembrana virus is accompanied by an increase in body temperature. The increase in body temperature is suspected to be due to infection or systemic inflammation through the release of cytokines or inflammatory mediators (Garami et al., 2018). According to Mardiatmi et al. (2015), during fever, there will be a decrease in lymphocytes, especially B lymphocytes and platelets. Suherman et al. (2013) reported that the increase in spleen weight and body temperature of Balinese cattle infected with Jembrana virus occurs due to a series of immune responses against viral infection by releasing cytokines as pyrogens.

The very strong negative correlation between spleen weight and Ct values indicates that the lower the Ct value (indicating a high viral load), the larger the spleen weight thought to be related to increased immune activity in the spleen, including lymphoid cell proliferation and immune cell infiltration in response to infection. Irwanto et al. (2021) reported that the presence of Jembrana virus in Bali cattle can be sensitively detected using RT-PCR, which is still considered the standard method for detecting viral RNA. Several studies have also shown that Jembrana virus or antigen is abundantly detected in the spleen, suggesting that the spleen is a crucial target organ during infection. In this study, the Ct value of 16 is considered very low, indicating a very high viral nucleic acid target (high viral load) in the sample. This low Ct value aligns with the finding of increased spleen weight, which is thought to be related to the activation of an intense immune response, including lymphoid cell proliferation and immune cell infiltration within the spleen tissue as the body attempts to inhibit viral replication. These findings support the hypothesis that a higher viral load (indicated by a lower Ct value) increases the immune response in the spleen, reflected in increased spleen weight. However, it should be emphasized that this relationship is correlational, and a direct causal mechanism requires further confirmation through histopathological and immunohistochemical examinations.

#### 4. CONCLUSION

This study concluded that Jembrana virus inoculation increased spleen weight and body temperature in Bali cattle. Spleen weight in Bali cattle inoculated with Jembrana virus had a very strong positive correlation with Cycle Threshold (CT) Value but a very strong negative correlation with the weight of the spleen. Administration of antipyretics, anti-inflammatories, analgesics, vitamins and antibiotics is necessary before vaccination to prevent an increase in body temperature, an increase in spleen weight, a decrease in cycle threshold value, and the presence of secondary infections.

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