The Detection and Analysis of Factors Associated with the Incidence of Lumpy Skin Disease in Cattle Transhipped at Merak Port, Indonesia

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Abstract

Lumpy Skin Disease (LSD) is caused by the Lumpy Skin Disease Virus (LSDV) that affects cattle and buffalo. The symptoms include the development of lumps or nodules on the skin of infected animals. Therefore, this study aimed to detect the presence of LSD and determine factors associated with the incidence in cattle transhipped through Merak Port. Samples were collected in the form of oral and nasal swabs, then tested with quantitative real-time PCR (qPCR). The results showed that there were two positive LSD samples confirmed by molecular testing using qPCR. The positive cattle did not show clinical signs or were suspected to be sub-clinically infected, while the Ct values obtained were 27.71 and 28.88. The use of molecular methods with qPCR showed relatively good results for the detection of LSD. Cattle that did not show clinical signs were detected as positive by the test. This is because qPCR can detect viruses more quickly and accurately, even at very low viral load levels. Factors associated with the incidence of LSD in the Merak Port ($p < 0.05$) were farm origin and biosecurity measures, knowledge of livestock handlers, vaccination status, and disinfection practices.

Keywords: animal quarantine, factors associated, lumpy skin disease, PCR, transhipped cattle

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INTRODUCTION

Lumpy Skin Disease (LSD) is a viral infection of cattle and buffalo caused by the LSD virus, a member of the genus Capripoxvirus. Disease is characterized by symptoms in the form of lumps or nodules on the skin of infected animals (Gupta *et al.*, 2020). Lumpy skin disease virus (LSDV) is a double-stranded DNA genome, with approximately 150 kilobase pairs (kbp) in length. Furthermore, the dimensions are substantial, ranging from 230 to 260 nm, and enveloped within a lipid membrane (Liang *et al.*, 2022). It is considered a developing viral disease (Sikkema, 2021) comprising recent infections, or those in new geographical areas, as well as previously unrecognized ones. The typical clinical signs of LSD include firm, slightly raised, circumscribed skin nodules, ranging from 2 to 7 centimeters in diameter most commonly found on the neck, legs, tail, and back, which starts with the presence of fever (Sevik, 2017). The economic loss caused by LSD is considerable, as it leads to damaged skin quality, infertility or sterility in bulls, abortion, and decreased body weight growth (Leliso *et al.*, 2021). The financial losses incurred by cattle farmers can reach about 45–65% (Namazi, 2021).

The first case of LSD in Indonesia was identified in 2022 in Riau Province (Barantan, 2022). Before the incident, the country had been declared free from LSD, with no previous report. Disease is classified as Quarantine Animal Pests and Disease Group I, according to the Decree of the Minister of Agriculture, the Republic of Indonesia No. 3238/Kpts/PD.630/9/2009. This group includes animal disease not yet present in the territory of the Republic of Indonesia,

potential for serious and rapid spread, no control measures known, endanger human health, has social consequences capable of disrupting the community, and may potentially lead to high economic losses.

The transmission of LSD can occur through direct contact with infected animals, the ingestion of contaminated food and water, iatrogenic means, and vectors (WOAH, 2022[a]). Ectoparasites are dangerous not only due to the ability to irritate the host with bites but also to transmit disease agents. Mosquitoes and flies are vectors for various disease, including LSD in livestock (Nugroho *et al*., 2021). Furthermore, movement represents a significant risk factor for the spread of LSD, particularly in instances where infected cattle are transported from one region to another. In this instance, it can be classified as a transboundary animal disease (TAD) (Sendow *et al*., 2021). The number of cattle imported from Sumatra to Java Island through the Port of Merak, Banten in 2021 and 2022 was estimated at 83.942 heads with a frequency of 5.554 times and 84.507 heads with a frequency of 5,640 times, respectively. A total of 41,654 cattle were transhipped between January and May 2023, with a frequency of 2.749 entries.

This study was conducted at Banten Animals, Fish, and, Plants Quarantine Center, Indonesia with the objective of performing polymerase chain reaction (PCR) tests on cattle transhipped through Merak Port. Furthermore, data were collected from cattle service users through the use of a questionnaire to identify factors associated with the incidence of LSD in transhipped cattle. The results are expected to offer insights that can be used as a basis for control measures against disease.

MATERIALS AND METHODS

Ethical Approval

This study was approved by Animal Ethics Committee School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia (Agreement Number 57/KEH/SKE/I/2024).

Study Period and Location

The study was carried out from December 2023 to March 2024. Sample collection was performed at the Merak Port which is the working area of Banten Animals, Fish, and, Plants Quarantine Center, Indonesia, then testing was conducted at the Center for Diagnostic Standards of Animal, Fish, Plant Quarantine, East Jakarta, Indonesia. The processing and analysis of data was carried out at the Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University.

Samples

Samples were obtained from oral and nasal secretion swabs of cattle that were in transit through Merak Port in Cilegon City, Banten Province, Indonesia (Figure 1). The collection period was the time of cattle entering or exiting the port, as well as during transportation. The swab samples were placed into a viral transfer medium using Biocomma Classic VTM, which contains antibiotics, BSA, cryoprotectant, biological buffer, and amino acid in Hank's solution. Sampling was conducted randomly using simple random selection (WOAH, 2022[b]). The calculation of the sample size was based on the formula: $n = 4pq/L^2$, where n is the sample size, p is the estimated prevalence rate, q= 1-p, and L = the maximum acceptable error value. The formula is used to estimate the sample size in prevalence surveys through simple random sampling with a 95% confidence level (Ditjen PKH and AIP-EID, 2014). The calculation of the study sample is as follows: $n = 4 \times 0.893 \times 0.107 / 0.0025 = 152$ samples. The qPCR test was then carried out at the Center for Diagnostic Standards of Animal, Fish, Plant Quarantine, East Jakarta, Indonesia.

DNA Extraction

Oral and nasal swab samples were extracted for DNA using the QIAamp Viral DNA Mini Kit. About 100 µL of oral and nasal swab samples were transferred to a microtube and combined with 100 µL of ATL. The samples were homogenized using a vortex, then a total of 20 µL proteinase K was added to the microtube,

followed by vortexing for 15 seconds and incubation at 56°C for 1 hour. Subsequently, precipitation was achieved through the addition of a spindown. About 200 µL of AL was added followed by vortexing, incubation at 70°C for 10 minutes, and spindown. In the subsequent step, 200 µL of absolute ethanol was added followed by vortexing and spindown. The solution was transferred to a mini spin column and centrifuged at 8000 rpm (TOMY High-Speed Refrigerated Micro-Centrifuge MDX-310) for 1 minute. The collection tube was replaced with a new one and then 500 µL of Buffer AW1 was added and centrifuged at 8000 rpm for 1 minute. The collection tube was replaced with a new one and 500 µL of Buffer AW2 was added, followed by centrifugation at 14.000 rpm for 3 minutes. A similar procedure was carried out for 1 minute, then the column was placed in a 1.5 mL microtube, followed by the addition of 50 µL of Buffer AE. The sample was incubated at room temperature for 5 minutes and centrifuged at 8000 rpm for 1 minute. The spin column was discarded and the DNA was stored at -20°C or used immediately.

Quantitative Real-Time Polymerase Chain Reaction (qPCR) Evaluation

In this study, the extracted DNA was stored at -20°C for immediate use. Virus detection by PCR amplifies the P32 gene. The test was performed in a qPCR machine using a Mastermix SensiFAST Probe Lo-ROX Kit with a forward primer (5′-AAAACGGTATATGGAATA GAGTTGGAA-3′) and a reverse primer (5′-AAATGAAACCAA TGGATGGGATA-3′). PCR testing was conducted with enzyme activation at 95°C for 10 minutes, denaturation at 95°C for 15 seconds, and extension at 60°C for 60 seconds, with a total of 45 cycles. The result is considered positive when the Ct value is < 35 and negative when > 45 (Bowden *et al*., 2008).

Data Collection by Questionnaire

Data were collected using a questionnaire for cattle service users and the questions contained were used to determine factors associated with the incidence of LSD in cattle transhipped through Merak Port. The categories of questions were farm origin and biosecurity, clinical signs during transit, knowledge of LSD, vaccination status, proximity to other farms, addition of livestock during transit, and disinfection.

Data Analysis

Data from the PCR test were analyzed descriptively, while questionnaire data from 63 cattle service users were analyzed using the Chi-Square test to determine whether there was a relationship with the incidence of LSD in cattle transhipped through Merak Port.

RESULTS AND DISCUSSION

qPCR testing

A qPCR test on 152 samples in this study showed two positive samples for LSD. The positive samples were numbered 28 and 152, with cycle threshold (Ct) values of 27.71 and 28.88, respectively (Figure 2). Sample 28 originated from Sumbawa Regency and was transported to South Jakarta through Merak Port, while 152 originated from Central Lampung Regency and was destined for Jambi City. However, none of the 152 oral and nasal swab samples showed any clinical signs of LSD.

Based on Figure 1, the Bali cattle from Sumbawa transited through Merak Port, and the Limousin cattle from Central Lampung, Indonesia, which tested positive in the qPCR test for LSD, did not show clinical signs. Examination of cattle on the truck did not find clinical signs of LSD, including those typical of the acute phase (phase 1) of lacrimation, increased nasal secretions, salivary secretions, as well as multinodular lesions around the skin and mucous membranes. In Phase 2, clinical signs included markedly enlarged subscapular and precrural lymph nodes, which are three to five times the normal. There was also an increase in multinodules on the head, neck, limbs, genitals, udder, mucous membranes, nasal cavity, and mouth. Nodule lesions, 0.5 to 5 cm in diameter, were found in varying numbers and sizes. In Phase 3, clinical signs included nodule lesions that ulcerated and became necrotic. Serum

exudates were present, causing pain and lameness. Ulcerative lesions were observed in the mucous membranes of the eye and nasal cavities. After one month, complete healing of the ulcerations and thickening of the skin, as well as hyperpigmentation of the lesions, was observed in phase 4, suggesting cattle had a subclinical infection. Generally, in subclinical conditions, the proliferation of the virus may be inhibited to the extent that the viral load is below the threshold required to cause clinical signs. This inhibited viral proliferation is presumably due to increased levels of IFN-γ in subclinically infected cattle (Suwankitwat *et al*., 2023). The presence of IFN-γ can reduce the HMI response by inhibiting the development of Th2 cells and IL-4. In cases with clinical signs of LSD, the HMI response is significantly higher than the CMI, possibly due to lower levels of IFN-γ compared to subclinical animals (Suwankitwat *et al*., 2023). Conversely, cattle with clinical signs may have a high sufficient viral load to cause visible clinical signs of LSD.

Figure 1. Location of swab sampling for LSD PCR test and number of LSD incidences in Java Island and Lampung Province, Indonesia (iSIKHNAS, 2023).

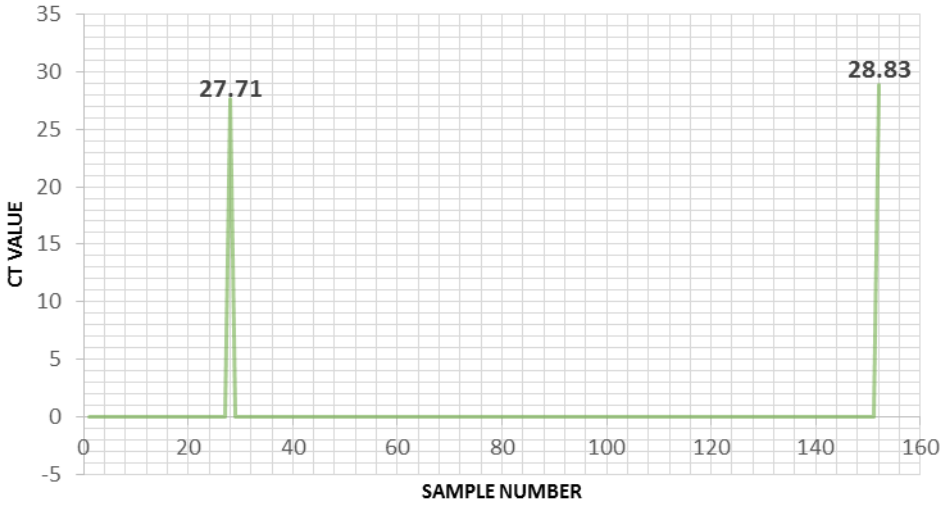

Figure 2. Ct value of 152 cattle oral and nasal swab samples. Positive samples number 28 and 152 with Ct values of 27.71 and 28.83 respectively.

Table 1. Percentage of questionnaire results of cattle service users through Merak Port

Variables	Factor Level	Percentage (%)
Farm origin	Feedlot	85.7
	Not a Feedlot	14.3
Farm biosecurity	Done	85.7
	Not done	14.3
Clinical signs/illness while on transport	Available	0
	Not available	100
Knowledge of LSD	Know	3.2
	Not knowing	96.8
LSD vaccination	Yes	90.5
	No	9.5
Adjacent to other farms	Yes	20.6
	No	79.4
Addition of livestock while on transport	Yes	0
	No	100
Disinfection	Yes	68.3
	No	31.7

Table 2. Chi-Square test on questionnaires to cattle service users transhipped through Merak Port

Variables	Chi-Square	95% CI
Farm origin	0.000	0.907–1.823
Farm biosecurity	0.000	0.907–1.823
Clinical signs/illness while on transport	-	-
Knowledge of LSD	0.000	0.127–2.033
LSD vaccination	0.048	0.677–133.3
Adjacent to other farms	0.464	0.907–1.016
Addition of livestock while on transport	-	-
Disinfection	0.035	0.960–1.286

Cattle infected with LSD showed a range of clinical manifestations, including those without any observable signs. These clinical signs may be influenced by the virulence level of the infecting cattle. For instance, cattle infected with a high virus concentration may show more severe clinical signs than those infected with a low virus concentration. Another study reported that when cattle consumed food contaminated with LSDV at relatively low virus concentrations, the only clinical signs observed were mild fever with no signs typical of LSD, such as nodular lesions on the skin (Dietze *et al*., 2018).

Based on the results, the Ct values obtained were 27.71 and 28.88. Another study reported that Ct values for subclinically infected animals with LSD ranged between 30.1 and 36.1 (Suwankitwat *et al*., 2023). The Ct value serves as a reference to determine the viral load present in the collected samples, including oral and nasal swabs. The use of molecular methods, specifically qPCR, is efficacious for the detection of LSD, particularly in cattle that do not manifest clinical signs. This is because qPCR can detect the virus more quickly and accurately, even at very low viral load levels compared to conventional PCR. The capability is of great importance for the detection of LSDV, as the virus is often challenging to identify in the early stages of infection. Comparative studies have shown that the detection rate of qPCR is 39.13% higher compared to conventional PCR (Zeedan *et al*., 2019). The ability of qPCR to detect the virus even at low concentrations makes it a more reliable tool for the detection and diagnosis of LSD. In addition, the selection of detection

through mouth and nose samples is quite effective when tested with qPCR. This is because LSDV is most likely to be found in droplets and aerosols formed by infected cattle. Oral and nasal swabs may be an easier sampling method to detect disease (Dietze *et al*., 2018). The results showed that cattle with no clinical signs of LSD yielded positive results following the appearance of Ct values (Figure 2). In another study, LSDV was detected through a range of samples, including nasal swabs, which yielded positive results following PCR testing. An outbreak of LSD was found in small and commercial farms in Russia (Kononov *et al*., 2019).

Other studies have shown that sub-clinically animals excrete the virus through the saliva, mucus, and eye discharge (Shumilova *et al.*, 2022). This shed virus can attach to objects around the cage, such as equipment, feed, and drinking water. Other healthy animals can contract the virus when in contact with contaminated objects. The potential for the transfer of pathogenic microorganisms and toxic chemicals into the surrounding environment through the wastewater of agricultural facilities represents a significant concern. Ineffective waste management practices facilitate the dissemination of harmful substances, thereby increasing the risk of environmental contamination (Zainuddin *et al*., 2019). The virus can be transmitted to other cattle, as LSDV is present in saliva and nasal secretions (Afzal *et al*., 2024). Conversely, animals with clinical signs release significantly larger amounts of the virus, specifically through necrotic nodules (Shumilova *et al.*, 2022). The virus may contaminate the surrounding area of animal and increase the risk of transmission. Efforts to control this disease should not be limited to the clinical animals, but also consider those with subclinical infection.

Questionnaire Data of Cattle Service Users

A total of 63 respondents were obtained from the data collection on cattle service users. The data from cattle service user questionnaire is presented in Table 1.

The results showed that 85.7% of cattle passed during data collection. Swab samples were obtained from feedlots, while 14.3% were collected from traditional farms. Cattle from traditional farms were Limousin and Balinese, while those from feedlots were Brahman cross. Approximately 85.7% of farms applied biosecurity measures, and 14.3% did not. The biosecurity measures implemented included the use of disinfectant sprays, the isolation of sick livestock, and the restriction of human traffic in the feedlot. Other measures include checking or isolating newly introduced livestock species, controlling visitor contact, managing contact between livestock, pets, and wild animals entering the farm, separating sick livestock, cleaning and disinfection practices, disease monitoring and record-keeping, as well as conducting communication, training, and employee assessment (Paramitadevi *et al*., 2023). Based on the results, none of cattle were sick or showed signs of LSD during transportation. The majority of cattle users (96.8%) were unaware of the presence of LSD, with only 3.2% being aware. The majority of cattle (90.5%) had been vaccinated against LSD, while 9.5% had not, including Bali cattle from the Sumbawa District. Upon transportation to the destination, 20.6% passed through or were close to other farms, while 79.4% were not. Based on the results of this questionnaire, there was no increase in livestock entering or exiting through Merak Port. A total of 68.3% of cattle were subjected to disinfection, while 31.7% were not.

The results of the questionnaire were analyzed using the Chi-Square test to determine whether there was a relationship between the incidence of LSD at the Port of Merak entry and exit points with the variables presented in Table 2. The Chi-square test showed a value of < 0.05 and factors associated with the incidence of LSD were farm origin, biosecurity, knowledge of cattle service users, vaccination status, and disinfection. In the category of farm origin, the result showed a value of 0.000 (< 0.05), indicating a significant relationship between the origin of the farm and the incidence of LSD in cattle transported through Merak Port. The origin of cattle farms is categorized into two types namely feedlots and traditional farms. This situation pertains to the

management of care, which is relatively more attentive in terms of nutrition, sanitation, and biosecurity. In feedlot systems, the maintenance standards are generally met, which helps to protect cattle from various disease agents, including LSDV.

Traditional farms often permit cattle to roam on grazing fields or to remain in enclosures without the benefit of calculated nutrition. Sanitation and biosecurity at these farms are less rigorously managed, potentially leading to a higher risk of LSD transmission compared to cattle in feedlots. This is consistent with the results of the qPCR tests (Figure 1), where cattle that tested positive were from traditional farms. Furthermore, the presence of LSD poses a risk of transmission to nearby cattle farms (Susanti *et al*., 2023). A good example of a biosecurity system is Animal Quarantine Installation (AQI), a farming system that has been designed with a high level of biosecurity to prevent the spread of animal disease (Zainuddin *et al*., 2019; Purnama *et al*., 2019). AQI refers to an entire building, including the equipment, land, and supporting facilities. Quarantine measures are given to carrier subjects with the character of a high risk before entry (Zainuddin *et al*., 2019).

In intensive farming systems such as feedlots, there are measures in place to prevent the entry of disease from outside sources. A previous study reported that intensive farming systems could reduce the occurrence of LSD by 0.41 times compared to extensive and semi-intensive. In extensive farming systems or when cattle are grazed on pasture, owners cannot control consumption in terms of food and water, which may lead to infections with LSDV. Transmission through feed and water can occur due to contamination from the saliva and secretions of cattle infected with LSD (WOAH, 2022[a]). Furthermore, extensive farming systems are positively associated with higher incidences of LSD, 8.25 times compared to intensive and semi-intensive systems. The risk of contamination from communal food and water sources is higher in areas with dense animal populations. The more animals that share food and water sources in open fields, the higher the risk of transmission (Selim *et al*., 2021).

In the biosecurity category, the Chi-square value was less than 0.05, showing a significant relationship between the implementation of biosecurity measures on farms and the occurrence of LSD. Cattle that tested positive for qPCR originated from traditional farms maintained extensively or grazed on pastures and were semi-intensive. Therefore, traditional farms that do not implement biosecurity or have low biosecurity measures can increase the risk of disease entry. In a previous study, traditional farms in Indragiri Hulu Regency with low biosecurity measures were associated with the occurrence of LSD (Susanti *et al*., 2023). Measures must be taken concerning water, sanitation, hygiene, and biosecurity. In the livestock sector, biosecurity programs represent the initial line of defense against the introduction and spread of disease (Fikri and Purnama, 2020). These programs are typically implemented within specific geographic areas or production systems at elevated risk (Paramitadevi *et al*., 2023).

The implementation of suboptimal biosecurity measures on rural farms represents a significant risk factor for the occurrence of LSD (Roche *et al*., 2020). The lack of or low biosecurity on traditional farms may be attributed to several factors, including farmers knowledge and available facilities. Farmers are not yet fully aware of disease prevention through biosecurity measures. This condition leads to poor prioritization of biosecurity until a case occurs on farms. A lack of information was the most significant barrier to biosecurity actions, cited by 65.71% of respondents, followed by a lack of time (31.43%) and high costs (2.86%) (Lestari *et al.*, 2022). In addition, poor biosecurity practices also lead to a higher risk of LSDV transmission. This is because the virus can be transmitted through direct contact with sick animals, both on farms and when traveling between regions (Aleksandr *et al*., 2020). LSDV is also transmitted through contaminated medical devices, saliva, and nasal swabs (Dietze *et al*., 2018).

Knowledge of cattle service users showed a significant correlation with the incidence of LSD

in cattle transported through Merak Port (Table 2). Cattle service users who are unaware of LSD, including clinical signs, economic losses, and the importance of prevention, are associated with disease incidence during transport from the origin to the destination. Lack of knowledge potentially leads to delayed responses to livestock health problems. Service users without the knowledge of LSD do not often report to quarantine officials and veterinary authority officials both regionally and nationally. Consequently, the risk of transmission may occur when cattle are transported, specifically on entry to areas already infected. The risk of spreading to other cattle and farms also becomes higher (Gumbe, 2018). Good knowledge among farmers about LSD, transmission methods, and the signs facilitates prompt reporting of cases to animal quarantine officials. This also aids quarantine and veterinary authority officials in taking appropriate measures, thereby reducing the risk of spread.

Based on the results, vaccination showed a significant relationship with the occurrence of LSD in transported cattle. Vaccination is an effective preventative measure against LSD in cattle. Appropriate administration of vaccines can protect cattle from infection and reduce the risk of spread. One of cattle that tested positive in this study was from Sumbawa and had not been vaccinated, posing a risk factor for LSD virus infection both on the farm and during transport to the destination. Vaccination status is among factors associated with the risk of LSD occurrence. In general, vaccination is administered to control disease outbreaks and prevent further spread (Bianchini *et al*., 2023). It is typically given after the presence and spread of the virus in a particular area have been confirmed. Maximum protection from the vaccine is usually achieved at least three weeks after administration (Roche *et al*., 2020). Cattle that have been vaccinated against LSD tend to be more resistant to infection. In cattle transported from Lampung, specifically those from feedlots, vaccination was performed using attenuated vaccines, offering effective protection against LSD infection. Effective protection can occur when vaccination coverage reaches 80% (Nurjanah, 2022). Veterinary authorities stipulate that vaccination strategies must be effective to ensure antibodies prevent LSDV infection. Furthermore, the use of live attenuated vaccines in transhipped cattle necessitates an investigation into the potential for adverse effects, including the risk of acquiring novel disease due to homologous recombination with other viruses. The application of attenuated vaccines also entails other risks, such as clinical side effects and detoxification (Sprygin *et al*., 2018; Krotova *et al*., 2022).

The occurrence of LSD in transported cattle was found to be significantly related to disinfection. Cattle transported from Lampung were subjected to disinfection upon departure towards Merak Port. However, not all transport vehicles could be disinfected, given the large number of cattle transported daily. For Sumbawa, both the transport vehicles and cattle were disinfected upon departure. It is uncertain whether the transport vehicles and cattle were disinfected during the transportation from Sumbawa through regions such as Bali, East Java, Central Java, West Java, and Cilegon. This poses a risk of transmitting LSD from contaminants on the transport vehicles, feed, and human clothing, specifically since cattle pass through areas already infected, such as East Java, Central Java, West Java, and Banten (iSIKHNAS, 2023). The transportation from Sumbawa to Jambi can take approximately 8 days. Transportation over long distances is a risk factor for cattle to contract LSD. These infected cattle also pose a high risk of spreading the LSDV during transportation and upon arrival at the destination farm when disinfection is not performed. Therefore, inadequate disinfection in practice, both on farms and during transport, is a risk for LSD infection (Bianchini *et al*., 2023). The implementation of disinfection practices on cattle transport vehicles can be an effective method of reducing the risk of transmission (Saegerman *et al*., 2018). The practice of disinfecting transport vehicles, equipment, and on-farm areas is an effective control measure for LSD. The successful control and eradication in the Balkans provide evidence regarding the effectiveness of disinfection (Calistri *et al*., 2020).

In the category of the presence or absence of clinical signs during transportation to Merak Port, there were no clinical signs based on the respondents answers. Based on the result, there was no relationship between clinical signs during transportation and the incidence of LSD. In accordance with the qPCR testing, there were two positive results for LSD despite not showing clinical signs (Figure 2). The result showed that no respondent added cattle during the transportation. The category of being adjacent to other farms during transportation also showed no association with the incidence of LSD. This risk factor is related to the distance of transmission from infected cattle. Short-distance transmission can occur at distances < 5 km, which is associated with the role of vectors such as mosquitoes (*Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, and *Culicoides nubeculosus*), ticks, and flies (*Haematopota* spp. and *Stomoxys calcitrans*), while longer distance transmission is likely due to movement (Aerts *et al*., 2021; Bianchini *et al*., 2023).

The analysis results on factors associated with the incidence of LSD show that several options can be implemented to reduce the spread of LSDV as control measures. In particular, management needs to be improved, specifically on traditional farms, where the risk of LSD infection is higher (Susanti *et al.,* 2023). The use of good and nutritious feed is also important for the formation of body resistance. The significance of nutrition in meeting needs, such as forage, is to enhance the immune system, thereby reducing disease susceptibility. Aside from forage, farmers must also provide supplementary feed and supplements to maintain cattle immune system, increasing disease resistance (Hilmiati, 2019). In extensive rearing systems, such as pasture-based, farmers must consider the proximity of grazing areas to water bodies, including lakes and puddles, as well as the seasonality of rainfall. This is because the existence of vectors, which can transmit LSD, is influenced by the presence of humid, wet areas, and puddles. The greater the number of vectors present in an area, the greater the risk of LSD in extensively reared cattle (Molla *et al*., 2018). Farmers should not graze cattle during the rainy season and in wetlands, rivers, and lakes. Other results suggest a significant correlation between LSD prevalence and average annual rainfall. Areas with high annual rainfall exceeding 1000 mm were found to have a higher disease incidence (Ochwo *et al.*, 2019). This suggests that humid and wet climatic conditions may play a role in supporting populations of vector insects transmitting LSDV.

Cattle should have access to clean water, as infection may occur through water contaminated with LSDV (Horigan *et al*., 2018). The next step in controlling LSD on farms is implementing effective biosecurity measures. Factor analysis results showed that there was a significant relationship between biosecurity measures and the incidence of LSD. In Indonesia, where LSD cases still occur, it is essential to improve biosecurity in both feedlots and traditional farms. The implementation of biosecurity measures is of significant importance, particularly in traditional livestock farms. These measures should include good hygiene and sanitation practices, as well as the proper handling of manure waste to prevent accumulation and ensure daily cleaning. Failure to maintain hygiene and cleanliness in the presence of manure waste can lead to an increased number of vectors, which elevates the risk of LSD transmission. In addition, biosecurity measures must be implemented on farms to prevent the introduction of LSDV contamination. This includes limiting the movement of strangers and vehicles on the farm. New cattle should be kept separate in different pens on entry, regardless of the physical health status and routine cleaning of cage equipment is also essential (Sendow *et al*., 2021). Separation of sick cattle from healthy cattle is crucial to minimize transmission, given that LSD spreads very quickly on farms (Wolff *et al*., 2020). It is also important to provide training on biosecurity measures for traditional farmers, traders, and market operators, to minimize the risk of spread (Roche *et al*., 2020).

Another control effort that needs significant attention is to increase public awareness and knowledge about LSD. The most important points that need to be conveyed to the community include clinical symptoms, how to report when a

case occurs, and prevention measures for farmers. Public awareness-raising measures are very necessary, specifically in areas where LSD cases have occurred. Awareness-raising is primarily focused on the clinical signs of LSD and methods for prevention or biosecurity (Pandey *et al*., 2022). Continuous socialization is essential for farmers to receive information. This action is an effort to increase farmers knowledge and concern about the dangers of LSD. Furthermore, the presence or suspected cases of LSD should be reported immediately to minimize the risk at the farm or when being transhipped out of the island. Socialization of cattle service users also needs to be carried out by quarantine officers throughout Indonesia with information materials tailored to the target audience, by using language and media easily understood. This is crucial to ensure that the information disseminated is well conveyed (Ditjen PKH, 2022).

The results showed that control measures can be implemented for cattle on farms and those assigned to be crossed by vaccinating LSD. However, it is crucial to ensure that the vaccination treatment is adequately covered to ensure effective population immunity. The Indonesian government, through the veterinary authority, should establish a minimum coverage target for LSD vaccination of approximately 80% (Nurjanah, 2022). Live attenuated vaccines provide good and efficient results, specifically in LSD-infected areas. Vaccination results with attenuated vaccines provide good protection for livestock, specifically cattle, with clear and recorded annual targets (Tuppurainen *et al*., 2017). Another advantage is that it is relatively affordable and produces a strong and long-lasting immune response (Tuppurainen *et al*., 2020). However, the use of live attenuated vaccines as implemented by the Government of Indonesia necessitates monitoring in the field for effectiveness. Vaccinated cattle may show side effects in the form of skin lesions and local inflammation (Bedekovic *et al*., 2017).

The government should consider the use of inactivated vaccines in LSD control efforts. Inactivated vaccines are generally safe, as the virus used has been killed, reducing the risk of serious side effects. These vaccines are well-suited for use in areas that are still free of LSD, particularly in Indonesia. Furthermore, inactivated vaccines play a crucial role in the final stages of disease eradication. Once animal population is largely protected with live vaccines, inactivated vaccines can be used to eliminate residual circulating viruses (Hamdi *et al*., 2020). Another consideration is that inactivated vaccines are relatively expensive and require multiple administrations. LSD vaccination must be supported by registration of vaccinated animals as well as monitoring and evaluation of vaccination results to determine the effectiveness of control measures. It is also recommended that animal quarantine officers consider implementing a policy of mandatory vaccination requirements for cattle and other livestock susceptible to LSD. This policy is intended to serve as a control measure for transhipped cattle, preventing contraction and transmission of LSDV over long distances during transportation. Furthermore, the documentation requirements must show whether it is necessary to use different types of vaccines depending on the status of the area. For example, the requirement to use live attenuated vaccines in infected areas and inactivated ones in free areas.

Disinfection is a very important control measure, specifically for cattle transhipped over long distances. It must be applied at the time of departure, during the journey at animal health control points, and upon arrival at entry quarantine. This treatment aims to minimize the risk of transmission of LSD before, during, and upon arrival at the destination. Cleaning should be carried out before disinfection to make it more effective in killing the virus, specifically in cages and farm equipment. Furthermore, disinfection must be conducted with active agents that are effective against LSDV, such as chloroform 20%, formalin 1%, phenol 2%, sodium hypochlorite 2–3%, iodine, virkon 2%, and ammonium quaterner 0.5% (Ditjen PKH, 2022). It is also necessary to disinfect trucks, drivers, and equipment carried during the journey to the destination.

Other control measures that can be taken are to require laboratory results declaring the transhipped cattle negative for LSD using

conventional PCR or qPCR tests. However, it is necessary to consider strengthening and adding facilities as well as LSD test materials at each quarantine station. This is to support PCR testing in each region in Indonesia, specifically for cattle movement. In quarantine at destination, the newly arrived cattle must be tested for early detection of infection that does not show clinical symptoms (Yuniarti *et al*., 2024). In the study conducted at the Merak port entry point, certain cattle did not show clinical symptoms positive for LSD after being tested through qPCR. Other studies have shown that early detection is of critical importance to prevent the spread of LSD as well as to reduce the costs associated with infection (Liang *et al*., 2022). The major limitation of this study was that other factors associated with the incidence of LSD in cattle transhipped through the port of Merak were not analyzed. Therefore, other risk factors, such as the knowledge of the person in charge of the transport about the vector and disinfection should be further studied. These risk factors must also be accompanied by risk management options as control measures.

CONCLUSION

Several factors were found to be associated with the incidence of LSD in cattle transhipped through Merak Port. These factors included farm origin and biosecurity, knowledge of LSD, vaccination status, and disinfection practices. Cattle that tested positive by PCR originated from Sumbawa and Central Lampung. More specifically, these cattle were suspected to have subclinical infection. Based on factors associated with the incidence of LSD, control efforts should focus on minimizing the risk, specifically in transhipped cattle.

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AUTHORS' CONTRIBUTIONS

FR, SM: Conceptualized the study, M: Design of study Methodology, MWA, AKD: Validation, FR: Collection, DNA extraction, FR, SM, and M: PCR analysis. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that there are no known competing financial interests or personal relationships capable of influencing the work reported in this paper.

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