# Pathological Investigation of Lumpy Skin Disease in Cattle from Sleman, Indonesia

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### **Abstract**

In early 2023, there was an outbreak of lumpy skin disease (LSD) in cattle in Indonesia, with particularly high prevalence in Sleman, Special Region of Yogyakarta, Indonesia. Since then, cases of this disease have been increasing, causing significant economic losses to cattle and buffalo farmers. This study aimed to investigate the pathological changes caused by LSD virus infection in various organs of Sleman cattle. We investigated 15 animals from 10 farms. Skin samples were taken by biopsy. ELISA testing was performed on serum samples. We also performed necropsies on two LSD infected cow carcasses to observe the macroscopic and microscopic effects of the virus. During the necropsies, samples were obtained from skin nodules, skeletal muscle, and internal organs (lung, liver, kidney, lymphatic nodes, spleen, and digestive organs). These were subjected to histopathological examination using hematoxylin and eosin staining. Among the live animals, fever, lethargy, hypersalivation, and lacrimation were the most common clinical signs. Our qualitative descriptive analysis of the pathological changes, clinical signs, and ELISA results showed that LSD infection in cattle causes mild to severe damage to various organs. Our macroscopic examinations found that affected animals had skin nodules of varying sizes over the entire body. We observed mild to severe inflammation and hemorrhage in the internal organs, including the skeletal muscles, spleen, liver, heart, rumen, reticulum, abomasum, and small intestine. Based on this investigation, we conclude that, in addition to its effects on the skin, LSD causes pathological changes in various internal organs.

Keywords: dermatitis, hemorrhage, lumpy skin disease, skin nodules

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### INTRODUCTION

Lumpy skin disease (LSD) is caused by lumpy skin disease virus (LSDV) of the *Capripoxvirus* genus and Poxviridae family (Abutarbush, 2017). LSD affects livestock such as ruminants and buffalo (Arjkumpa, 2022). It is characterized by skin nodules, fever, and lesions on mucous membranes (Liang *et al.*, 2022). The transmission rate of this virus typically exceeds 10%, and the mortality rate is between 1–5%. Morbidity rates can reach 27% in affected regions (Ince and Turk, 2019). A study of a 2022 outbreak in dairy cattle in India reported a morbidity rate of 31.7%, a mortality rate of 2.97%, and a case fatality rate of 9.37% (Manjunathareddy *et al.*, 2024).

In early 2023, there was an outbreak of LSD among cattle in Indonesia, with a particularly high prevalence in Sleman, Special Region of Yogyakarta. Data from the Indonesian Animal Health Information System (ISIKHNAS) showed that, by November 28th, 2023, there had been 56,889 documented LSD cases across the 27 provinces of Indonesia (ISIKHNAS, 2023). The LSD in Sleman had spread to 14 sub-districts of Kapanewon, with 2,124 infected animals in the region (Razak, 2023). Although the Sleman Agricultural, Food, and Fisheries Office has tightened regulations on animal supervision in animal markets, it has not closed these markets or restricted livestock mobility. As a result, the disease is likely to spread to other regions (Subarkah, 2023). The central government of



Indonesia has implemented vaccination programs in affected regions (Nugroho *et al.*, 2024).

The pathological effects of the LSD virus include round, uniformly-sized skin nodules, which may merge into larger nodules that lack clear boundaries. The virus replicates in host cells such as macrophages, fibroblasts, pericytes, and endothelial cells. When cells in the lymphatic ducts and blood vessel walls are breached, vasculitis and lymphangitis develop. In severe cases, thrombosis and infarction may occur. Viremia occurs after the first reaction and persists for 1-10 days (Kumar et al., 2021). The incubation period of the disease ranges between 7–35 days. **Symptoms** include pyrexia, circumpapular lesions, skin nodules, and edema of the limbs and abdomen (OIE, 2024). The virus takes up residence in the blood; the oral, nasal, and ocular fluids; and the skin lesions and scabs that develop (Reddy et al., 2024). Edema and local cellulitis may cause regional lymph nodes to swell to 3-5 times their normal size. Kidney lesions measuring about 10-30 mm in diameter have been reported, while interstitial bronchial lesions measuring 10-20 mm. These may spread across the entire surface of the lung (El-Neweshy et al., 2013).

A histopathological study has found that the lesions caused by LSDV differ depending on the phase of disease development. Some of the specific lesion types that were observed include vasculitis, thrombosis, infarction, and perivascular fibroplasia. In infected parts of the body, there was infiltration of inflammatory cells, including macrophages, lymphocytes, eosinophils. Eosinophilic intracytoplasmic inclusion bodies were found in the keratinocyte macrophages, epithelial, follicular cells, epithelium, and endothelial cells of infected animals, and in the sebaceous glands. The epidermal and dermal layers of the skin were subject to edema and infiltration by large epithelioid macrophages. The study found endothelial cell proliferation and lymphocytic inflammatory cell infiltration in the blood vessels of the dermis and subcutaneous tissue, which, in some cases, led to thrombosis and necrosis (Manjunathareddy et al., 2024).

To our knowledge, there have been no pathological studies to date, either macroscopic or microscopic, of the LSD virus affecting cattle in Sleman, Special Region of Yogyakarta. Despite the rapid spread of the disease since its outbreak, there have been few pathological studies in Indonesia. This study aimed to explore the pathological changes to the organs of cattle in Sleman, Special Region of Yogyakarta, Indonesia, resulting from LSDV infection.

### MATERIALS AND METHODS

### **Ethical Approval**

The research protocol for this study was approved by the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia (approval no. 058/EC-FKH/Int. /2023).

### **Study Period and Location**

This study was conducted at the Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Special Region of Yogyakarta. The collection of samples from animals infected with LSDV was carried out at community farms in Sleman, Special Region of Yogyakarta, between March and November 2023.

### **Sample Collection**

Blood samples and skin and organ tissue samples were obtained from LSD infected animals in the Sleman Regency, Special Region of Yogyakarta. Farms were selected based on the outbreak severity. At each farm, we began by gathering anamnesis from the farmer and identifying visible clinical signs and symptoms in the cattle. These included skin nodules, fever, lethargy, dyspnea, hypersalivation, lacrimation. For the histopathology process, 15 skin biopsies were performed on 15 infected animals to obtain samples for histopathological analysis. Necropsies were performed on two dead animals with severe clinical signs, and internal organ samples (lung, liver, spleen, kidney, lymph nodes, rumen, reticulum, omasum, abomasum, and small intestine) were taken. The samples were

stored in containers with 10% neutral buffered formalin.

### **ELISA Methods**

Sera sample testing was carried out at the Wates Veterinary Center, Yogyakarta, using an ELISA ID Screen® Capripox Double Antigen Multi-Species kit. The ELISA test is determined to be valid if two conditions for the optical density (OD 450 nm value) are fulfilled. The first of these is that the average OD value of the positive control (ODPC) must be greater than 0.350 (ODPC > 0.350). The second is that the ratio of the average values of the positive and negative controls (ODPC and ODNC) must be greater than 3 (ODPC/ODNC > 3). If the sample to positive ratio (where s = optical density of the sample optical density of the negative control, and p = optical density of the positive control - optical density of the negative control) percentage (S/P %) is <30%, the test result is negative; if the S/P% value is  $\ge 30\%$ , the result is positive.

## Macroscopic and Histopathological Examination

Post-mortem examinations of the gross lesions were performed, and the results were recorded. Tissue samples were collected from skin biopsies of the 15 live and two dead animals. These were placed in 10% neutral buffer formalin for 24 hours, then cut into 3–5 mm slices, each of which was put in a tissue cassette. The samples were automatically processed, embedded in paraffin, cut into 4–5 µm thick sections with microtomes, and then stained with hematoxylin and eosin for microscopic examination (Wick, 2019).

### **Data Analysis**

The results of both the macroscopic and histopathological examinations of the organ samples were obtained through qualitative descriptive analysis. Microscopic observations were carried out using a binocular microscope and documented using ImageJ software. The slides were evaluated by a pathologist from the Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

### **RESULTS AND DISCUSSION**

Based on the anamnesis from farmers, the clinical signs in animals infected with the LSD virus are weakness (collapse), anorexia, nasal discharge, subcutaneous skin nodules, and circular scabs on the skin surface. As a result of not eating or drinking, the animals also tend to be emaciated. Our observations of the live cattle confirmed that the clinical signs and symptoms in infected animals include skin lesions, swelling, enlarged lymph nodes, lethargy, fever, dyspnea, hypersalivation, lesions on the mucous membranes, and nasal discharge (Table 1).

All of the animals had cutaneous nodules and ulcers. Parvin et al. (2022) have shown that the skin lesions showed the duration of LSDV infection can be determined based on the skin lesions, with nodules indicating an acute infection and ulcers indicating chronic infection. The distribution of nodules and ulcers over the head, legs, neck, and abdomen was recorded for each animal. The body temperature of the LSDaffected animals ranged between 38°C-41°C. Information on the durations since the onset of skin lesions was gathered from the farmers. We found that the onset of the skin lesions was observed between 5 and 20 days after an animal first showed signs of infection. The average time between onset and recovery was 5 weeks.

Samples were obtained from those live animals with only mild clinical signs of infection through biopsies of the skin nodules. These animals were selected for biopsy to minimize risk. The procedure was performed carefully so the animals did not feel any pain. The biopsy wound was then sutured. In addition to our investigation of the 15 live animals, we also performed necropsies on two animals that had died after LSDV infection. These two animals were aged 3 and 5 months at death. The farmers reported that they had shown severe clinical signs, including weakness, lethargy, dyspnea, hypersalivation, nasal discharge, and anorexia. The data from our cattle sample is summarized in Table 2.

As can be seen in Table 2, the skin nodules of the infected animals densely covered the entire body. They were most common on the head, neck,



<b>Table 1.</b> Clinical signs of lumpy skin disease in affected animal	Table 1.	. Clinical	signs of lumpy	skin disease	in a	affected animals
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No	Clinical signs	Total	Result		
1	Fever	13	76%		
2	Lethargy	12	70%		
3	Dyspnea	7	41%		
4	Hypersalivation	5	29%		
5	Nasolacrimal discharge	4	23%		
6	Skin Nodule	17	100%		
7	Lesions of the mucous membrane	4	23.5%		

Table 2. Macroscopic changes in the organ samples of cattle with lumpy skin disease

Total of	Sample	Macroscopic changes of organs							
sample		Skin	Heart	Spleen	Lung	Small intestine	Rumen	LN	Skeletal muscle
15	Skin biopsy	N	NA	NA	NA	NA	NA	NA	NA
1	Cow No. 1	N	NA	NA	NA	NA	NA	I, H	NA
1	Cow No. 2	N	Н	Н	A	Н	Н	I, H	Н

NA = not available, N = nodule, H = hemorrhages, I = inflammation, A = anemic, LN = lymphnodes.



**Figure 1.** Skin nodules on cattle with lumpy skin disease. (A) Skin nodules on the hind legs of an affected animal (arrows); (B) Skin nodules on the abdominal surface of an affected animal (arrows).

abdomen, and front and back extremities. Some nodules had been sloughed away by necrosis, leaving crusts and deep ulcers. The diameter of the skin nodules varied in size, with most measuring 0.5–2 cm. In our investigation of macroscopic changes to the internal organs, we identified hemorrhages in the muscle, heart, spleen, intestine, and mesentery of the cattle. These included petechiae, ecchymosis, and diffuse hemorrhages.

Macroscopic images of the skin nodules in LSDV-infected animals are provided in Figure 1. Many of the nodules were subcutaneous,

particularly those on the legs, abdomen, neck, and head. The results of the ELISA validation test based on the average ODPC and ODNC values and the ratio of these two averages yielded results of 0.896 and 17.51, respectively. As the ODPC/ODNC ratio was >3, the test was validated. The sample OD result was 0.996, so it was declared seropositive.

We found macroscopic changes in the heart, subcutaneous tissue, skeletal muscle, spleen, lungs, kidneys, small intestine, rumen, reticulum, omasum, and abomasum of animals with LSD (Figure 2). We found petechiae hemorrhages in



**Figure 2.** Macroscopic changes to the internal organs of cattle with lumpy skin disease. (A) A petechial hemorrhages on the surface of the heart (arrow); (B) Diffuse hemorrhages in the mesentery (arrow); (C) Diffuse hemorrhages in the serous tunica of the rumen and reticulum (arrow); (D) Diffuse hemorrhages in the subcutaneous tissue of the leg (arrow); (E) Diffuse hemorrhages in the spleen; (F) Petechial hemorrhages in the serous tunica of the small intestine (arrow).

the ventricles of the heart muscle and the serous tunica of the small intestine; diffuse hemorrhages in the mesentery, rumen-reticulum, spleen, and subcutaneous tissue of the legs; and lymph node swelling and hemorrhage. Histopathological analysis of the samples taken from skin nodule lesions showed vacuolar degeneration, erosion or rupture of vesicles, acanthosis, hyperplasia, hyperkeratosis, infiltration of mononuclear inflammatory cells, and hemorrhage in the epidermal layer (Figure 3). Inflammation and hemorrhage were apparent in the analysis of the other internal organs. These histopathological results indicated that, in severe cases, LSD

infection can cause inflammation and hemorrhaging in the lungs, heart, spleen, lymph nodes, and liver. We also found emphysema in the lungs and damage to the interalveolar septa, causing dilatation of several alveoli (Figure 4).

The spread of LSDV throughout Indonesia began in early 2023, and Sleman was particularly affected. Since that time, vaccination programs have been introduced in the region to prevent more widespread infection. However, there are still many cases of LSD in cattle. This study examined the macroscopic and microscopic pathology of LSD. We found that LSD skin nodules are characterized by severe

inflammation. We also observed the classic clinical signs of LSD, which include anorexia, fever, enlarged lymph nodes, nodules covering some or all of the body, weakness, and swelling of the joints (Ochwo *et al.*, 2020; Kumar *et al.*, 2021). Indonesian farmers and veterinarians have

now learned to identify the clinical signs of LSD. We found that the persistence of the skin nodules and the duration of illness were almost identical, averaging 5–15 days.

The duration of the illness and the visible skin nodules is 2–4 weeks (Parvin *et al.*, 2022).

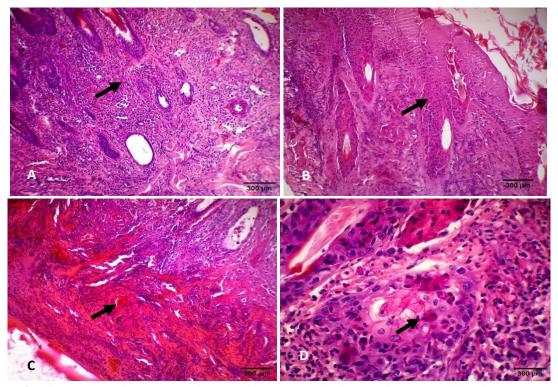


Figure 3. Histopathological changes in the skin nodules of animals with lumpy skin disease.

(A) Acanthosis of the epidermal layer of the skin, infiltration of mononuclear inflammatory cells in the dermis and surrounding blood vessels (arrows) (HE, 100×); (B) Hyperkeratosis of epidermal cells (arrows show acanthosis) (HE, 100×); (C) Diffuse hemorrhages and infiltration of mononuclear inflammatory cells in the dermis (arrows) (HE, 100×); (D) Eosinophilic intracytoplasmic inclusion bodies in the epithelial cells of the epidermis (arrows) (HE, 400×).

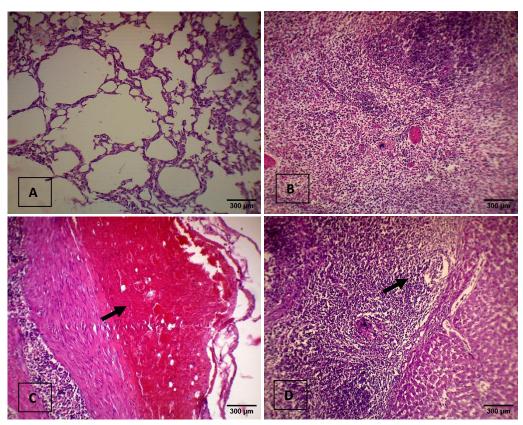
The morbidity rate in our study population was 33.8%, which was slightly higher than previously reported (Gupta *et al.*, 2020). We observed more severe clinical signs in the two animals necropsied than in our sample of live animals. The animals in this study were all aged 3–4 months. Previous research has found higher mortality rates among young animals with immature immune system development and those with high production stress (Limon *et al.*, 2020).

The spread of LSD across the Sleman Regency of Yogyakarta was very fast. In under 3 months, there were 2,214 documented cases. This was likely due to the cattle-rearing system in the

area, that involves the use of communal pastures, allowing contact between different cattle herds. The usual distance between cages is also relatively close, and livestock traffic between regions was not halted following the outbreak. Furthermore, cattle in Indonesia, generally, and in Sleman, particularly, are not vaccinated against LSD. This combination of factors has facilitated the transmission of LSD between animals in this area. Parvin et al. (2022) have reported that new outbreaks are more common within a 4-5 km radius of an infected cage. This is again a consequence of backyard farmers a communal system that allows herds to mix.

The diagnosis of LSD is generally based on the clinical signs followed by molecular diagnosis. The macroscopic and histopathological examinations of skin nodules and organ tissue samples, and the necropsy of infected carcasses performed in this study, aimed to identify the effects of LSDV infection on organs other than the skin. The histopathology changes observed in included hemorrhage, the skin necrosis, vasculitis, dermatitis, and infiltration lymphocytic inflammatory cells in the dermis and around the blood vessels. This effect on the blood

vessels appears to indicate that the virus spreads through the body via the blood vessels, traveling in the cells that it infects. A previous study has reported multiple infarctions in the same parts of the dermis and subcutaneous tissue as observed in prior study (Sanz-Bernado *et al.*, 2020). Further microscopic observations included vacuolar degeneration; swelling caused by eosinophilic intracytoplasmic inclusion bodies; and transmural, hemorrhagic, necrotic, proliferative, and ulcerative dermatitis.



**Figure 4.** Histopathological changes in the internal organs of animals with lumpy skin disease. (A) Emphysema of the lungs (HE, 100×); (B) Depletion of lymphocyte cells and megakaryocyte formation (HE, 100×); (C) Diffuse hemorrhages around the heart and infiltration of inflammatory lymphocyte cells of the heart muscle (arrows) (HE, 100×); (D) Severe inflammation in the liver parenchyma and surrounding blood vessels, predominantly induced by inflammatory lymphocytes (arrows) (HE, 200×).

We observed severe hemorrhaging around the heart, which was accompanied by infiltration of mononuclear inflammatory cells in the interstitial spaces between the heart muscle. Similar infiltration of mononuclear inflammatory cells was also apparent in the parenchyma of the liver. The lesions observed histopathologically

occurred during the developmental phase of the infection. We found eosinophilic intracytoplasmic inclusion bodies in the epithelial cells of the epidermis. This indicated that the LSD virus had replicated in these cells. Other histopathological observations included vasculitis, thrombosis, vascular infarction,

perivascular fibroplasia, and inflammatory cell infiltration of the infected areas, including macrophages, lymphocytes, and eosinophils. Eosinophilic intracytoplasmic inclusion bodies also found in keratinocyte macrophages, endothelial cells, and pericytes. Edema and infiltration of large epithelioid macrophage-type cells were seen in the dermis and epidermis. There was endothelial cell proliferation in the blood vessels of the dermis and subcutaneous tissue, and infiltration of lymphocytic inflammatory cells into the blood vessels had led to thrombosis and necrosis. A previous study has also reported the formation of specific intracytoplasmic inclusion bodies in the cells epithelial and various organs (Manjunathareddy et al., 2024).

The most common and apparent macroscopic pathology of LSD was the presence of round, uniformly-sized skin nodules, some of which had merged into larger, firm, undefined nodules. The LSD virus can replicate in host cells such as macrophages, fibroblasts, pericytes, and endothelial cells of the lymphatic ducts and blood vessel walls. This leads to vasculitis and lymphangitis, and, in severe cases, thrombosis and infarction. The virus can live and replicate in the skin lesions and scabs, the blood, and the oral and nasal cavities (Reddy et al., 2024).

In some of our biopsy samples, we found inflammation around the blood vessels (vasculitis with thrombus formation). The virus was distributed throughout the blood vessels and spread through infected cells. However, the cause of the observed changes in the blood vessels remains unclear (Parvin et al., 2022). We detected the presence of the LSD virus in 5-25-day-old skin nodules. Scab formation is known to occur at a more advanced stage, typically 20-35 days after infection. The formation of intracytoplasmic body inclusions in skin nodule lesions seen in the present study has been seen in a previous study (Gharban et al., 2019). Vasculitis, congestion, edema, and necrosis in the skin layers have also been seen in another study (Sanz-Bernardo et al., 2020).

Circular necrotic lesions have been observed in the respiratory organs, gastrointestinal tract,

breast, lungs, bladder, kidneys, uterus, and testicles of infected animals (Ali et al., 2021). Lymph nodes in the head, neck, genitals, mucous membranes, and nasal and oral cavities can swell to 3-5 times their normal size. The number of nodules varies, and they range between 0.5-5 cm in diameter. After 1–2 days, the nodules rupture, causing the virus to spread. Lymphangitis and vasculitis lead to edema (Ratyotha et al., 2022). We observed hemorrhaging in the kidneys that ranged between 10-30 mm in diameter. Lesions in the interstitial or bronchial tissue of the lungs measure 10-20 mm in diameter and spread over the entire surface of the lungs (Kumar, 2021). Viraemia occurs after the first reaction and persists for two weeks (Purnama et al., 2019). In young stock, lactating animals, and malnourished animals, the disease is more severe due to low humoral immunity, with an incubation period of 28-35 days (Roche et al., 2020). The animal becomes vulnerable to other bacterial and viral infections, which gain entry through skin lesions and respiratory inhalation, which can lead to complicated infections (Abdulga et al., 2016; Namazi and Tafti, 2021). Differential diagnoses for this disease include pseudo-LSD (caused by bovine herpes virus type 2), pseudo-cowpox, bovine papular stomatitis (caused insect Parapoxvirus), bites, urticaria, and demodicosis (Tuppurainen et al., 2017).

Further studies are needed to determine the genomic structure of the LSD virus in Indonesia generally, and in Yogyakarta, particularly. This will facilitate the molecular diagnosis of LSD. Efforts are also needed to prevent the spread of the LSD virus through vaccination programs.

### **CONCLUSION**

The macroscopic clinical signs observed in animals from the Sleman Regency in the Special Region of Yogyakarta, Indonesia infected with LSD were subcutaneous skin nodules measuring 0.5–2 cm, scabs from ruptured nodules, and diffuse petechial hemorrhages in the internal organs, including the heart, spleen, rumen, reticulum, omasum, skeletal muscles, lymph nodes, and liver. The microscopic manifestations

of the disease were severe inflammation and hemorrhaging in the skin and internal organs, including the muscles, spleen, liver, heart, and digestive organs. Further studies are needed to investigate the efficacy of vaccines for the control of LSD.

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### **AUTHORS' CONTRIBUTIONS**

YPK, SW, BST, SGY, and TU contributed to the study design and execution. YPK and ST were responsible for drafting and revising the manuscript. BST and SGY handled sample collection, while TU conducted the ELISA analysis. All authors reviewed and approved the final version of the manuscript.

### **COMPETING INTERESTS**

The authors declare that they have no competing interests.

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