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Research article

HEMATOLOGICAL, BIOCHEMICAL AND CYTOKINE PROFILES IN CATTLE: EFFECTS OF LUMPY SKIN DISEASE VIRUS INFECTION AND FARM SIZE

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Lumpy skin disease (LSD) is a contagious viral disease of cattle. This experiment aimed to study the influence of lumpy skin disease virus (LSDV) infection and farm size on hematological, biochemical parameters and cytokines in 55 beef cattle. The groups included LSDV–infected cattle from large farms (Group 1), LSDV–infected cattle from small farms (Group 2), uninfected cattle from large farms (Group 3), and uninfected cattle from small farms (Group 4). The hematological, biochemical values and cytokine profiles were measured. The results showed that red blood cells, hemoglobin, platelets, white blood cells, neutrophils, and lymphocytes were higher in infected cattle than in the uninfected cattle. The level of eosinophils of the cattle from the large farm was higher than that of the small farm. Gamma glutamyl transferase in the infected cattle was higher than in the uninfected cattle. Albumin of the cattle from the small farm was higher than that from the large farm. IFN–γ in infected cattle was higher than in uninfected cattle, while TNF–α in the infected cattle was lower than in the uninfected cattle. Our study indicated that LSDV infection altered hematological and biochemical parameters, including cytokine profiles, with farm size potentially influencing these alterations.

Keywords: biochemistry; cattle; cytokines; hematology; lumpy skin disease virus, LSDV

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INTRODUCTION

Lumpy skin disease (LSD) is a vector–borne viral disease of cattle and buffalo caused by the lumpy skin disease virus (LSDV), which is a double–stranded DNA virus of the genus Capripoxvirus of the family Poxviridae [1]. LSDV is transmitted by the bite of arthropods, particularly blood–sucking insects [1,2]. LSDV can be transmitted through direct contact and contaminated feed and water. So far, several studies have been conducted on the pathogenesis of LSDV [2]. After infection, the virus replicates primarily in the macrophages, monocytes, and endothelial cells lining lymphatic and blood vessels, resulting in lymphadenitis and vasculitis [3].

After LSDV infection, clinical signs are as follows: fever, skin nodule lesions, which cover different parts of the body, and nasal discharge [2]. Infection results in low mortality but high morbidity in cattle. Although LSD does not cause high mortality, it causes a significant economic impact due to decreased milk production, infertility, and trade restrictions [1]. Recently, outbreaks of LSDV have been reported in different parts of the world [4–6]. In Thailand, LSDV infection was first reported in Roi–Et province, the northeastern part of the country in March 2021. Since then, the disease has spread across the country. It is likely to have serious consequences [7].

Assessment of hematological and biochemical profiles can provide the indicators to evaluate animal health and, consequently, help understand the pathogenesis and diagnosis of viral infections [4]. Sample assessments are usually performed on the blood because the blood is the primary transport vehicle in the body, and any harmful deviations from the normal are detectable in the blood parameters [8]. Furthermore, LSDV also causes changes in biochemical profiles when cellular damage occurs [9]. A few studies have been conducted on hematological, biochemical abnormalities, and immunological alterations during LSDV infection in the vital organs of infected cattle [10].

Cytokines are produced by numerous cells with innate and adaptive immunity and respond to harmful microbes and the induction of inflammation, including inflammatory skin disease [11]. Tumor necrosis factor–α (TNF–α) and interleukin–4 (IL–4) levels were found to be high in LSDV–infected cattle because of the activation of macrophages and lymphocytes in early inflammation during the viremic phase of infection [12]. Furthermore, investigations have revealed that interferon–γ (IFN–γ) and IL–4 are crucial against virus infections via cell–mediated immunity [13]. Peripheral blood mononuclear cells (PBMCs) are involved in innate and adaptive immunity and play a role in preventing pathogen invasion [14]. However, there is yet no evidence available on the impact of cytokines produced by PBMC on LSDV–infected cattle.

The detection of LSDV in cattle herds in LSDV–free areas is of concern, particularly in the absence of antibodies, which may increase the LSD severity [2]. Farm size is one of the main risk factors for viral infections. Several studies suggested that larger herds are at higher risk of bovine viral diarrhea virus infection [15,16]. The source of virus

infection in larger farms could be through the introduction of infected animals into the farms, shared environment with other farms, and high cattle population densities in the area [16]. However, some studies show that the association between LSDV infection and herd size was not statistically significant [17]. Although much research is concerned with farm size and risk factors for outbreaks of LSDV, there are no studies to show the association of farm size with clinicopathological parameters and inflammatory cytokines of LSDV–infected cattle.

Therefore, this study aimed to investigate the influence of LSDV infection and farm size on hematological, biochemical parameters and inflammatory cytokines in cattle infected with LSDV. Our study will provide a further insight into the hematological, biochemical and immunological features of LSDV and an understanding of the relationship between LSDV infection and farm size in an outbreak area.

MATERIAL AND METHODS

Experimental procedures were approved by the Animal Ethics Committee of Mahasarakham University (IACUC–MSU–1/2023).

Animals

This study was conducted between May and July 2021, on a total of 55 beef cattle from farms located in Khon Kaen and Mahasarakham provinces in the northeast of Thailand. For farm classification criteria, small farms were farms with between one and ten cattle, while large farms were farms with more than twenty cattle. All cattle aged four months or older were used in this study; there was no history of LSDV vaccination and animals had only received anthelmintic and foot and mouth disease vaccine every six months regularly. All cattle received feed and water ad libitum. Before starting the experiment, cattle were thoroughly physically examined, including body temperature and ruminal contraction.

Experimental design

This study was divided into two parts:

Part 1: studied the influence of LSDV infection and farm size on hematological and biochemical values. Cattle were divided into four groups: Group 1, 8 LSDV–infected cattle from large farms; Group 2, 25 LSDV–infected cattle from small farms; Group 3, 12 uninfected cattle from large farms and Group 4, 10 uninfected cattle from small farms.

Part 2: the 41 cattle were divided into LSDV– infected (24 animals) and uninfected (17 animals) to specifically analyze cytokine responses, including IFN–γ, TNF–α, and IL–4. The selection ensured a representative sample of varying infection levels, enabling a balanced comparison with the uninfected control group.

Experimental procedure

Sample collection

Blood samples were collected by puncture of the jugular vein of clinically affected and healthy animals and then transported to the Faculty of Veterinary Sciences, Mahasarakham University. Blood samples with anticoagulant EDTA were used for hematological analysis and peripheral blood mononuclear cell (PBMC) isolation. Blood samples without EDTA were centrifuged at 3000 xg for 15 min at room temperature for serum separation for biochemical analysis.

Viral DNA extraction and virus detection

Viral DNA was extracted from EDTA blood using the QIAamp DNA Blood Kits (QIAGEN) according to the manufacturer's instructions. The DNA concentration was measured by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). Detection of the viral genome was performed as previously described [18]. The specific primers (CaPV–074F1 5'–AAAACG GTA TAT GGA ATA GAG TTG GAA–3' and CaPV–074R1 5'–AAA TGA AAC CAA TGG ATG GGA TA–3') and TaqMan probe (CaPV–074P1 5'–6FAM–TGG CTC ATA GAT TTC CT–MGBNFQ–3') were used. The PCR reaction employed the FastStart Essential DNA Probes Master (Roche Life Science) and QuantStudio™ 3 Real–Time PCR System (Applied Biosystems).

Hematology and biochemistry analysis

Hematocrit, red blood cell (RBC) count, hemoglobin, platelets, and white blood cell count (WBC) were measured in an automatic cell counter (IDEXX Procyte DX, Hematology Analyzer, IDEXX Laboratories, Inc., Maine, USA.). Creatinine, aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, and gamma glutamyl transferase (GGT) were performed using an automated biochemistry analyzer (IDEXX Catalyte One Analyzer, IDEXX Laboratories, Inc).

PBMC isolation

PBMCs were isolated from the whole blood samples. Briefly, the blood was first diluted with phosphate buffered saline (PBS), overlaid on the Ficoll–paque plus (Cytiva^{TM}) and centrifuged at 800 xg for 25 min at room temperature. PBMCs at the interphase were washed in PBS at 1,400 xg for 10 min. PBMCs were then collected, and lysing erythrocytes with RBC lysis solution (Sigma–Aldrich, St. Louis, MO). Finally, the PBMCs were washed with PBS and stored at – 20°C for RNA extraction.

RNA extraction and RT–qPCR

Total RNA from the PBMCs was extracted using a Nucleospin RNA kit (Macherey–Nagel, Germany), according to the manufacturer's protocol. The cDNA was

synthesized using the ReverTra Ace qPCR RT Kit (TOYOBO, Japan), according to the manufacturer's protocol. The quantity and quality of the purified RNA were assessed by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). The qRT–PCR was carried out using a QuantStudio™ 3 Real–Time PCR System (Applied Biosystems) and Maxima Sybr Green qPCR Mastermix (Thermo Inc, USA). The relative mRNA in gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method having GAPDH as the housekeeping gene. The primer sequences are listed in Table 1.

Table 1. Primers for the real–time quantitative PCR

Gene (GenBank accession No.)	Primer sequences (5' to 3')	Reference
IFNG (FJ263670)	F: TTGAATGGCAGCTCTGAGAAAC R: TCTCTTCCGCTTTCTGAGGTTAGA	[19]
TNFA (NM_173966)	F: TGACGGGCTTTACCTCATCT R: TGATGGCAGACAGGATGTTG	[19]
IL–4 (M77120)	F: CAA AGA ACA CAA CTG AGA AG R: AGG TCT TTC AGC GTA CTT GT	[20]
GAPDH (U22385)	F: GGC GTG AAC CAC GAG AAG TAT AA R: CCC TCC ACG ATG CCA AAG T	[20]

Statistical analysis

Normality was checked using the Shapiro–Wilk test.

1. The hematological and biochemical parameters were analyzed by ANOVA (Proc GLM, SAS® Software version 9.3, Inc., Cary, NC, USA), followed by the *Tukey post–hoc test*. Data were presented as mean ± standard error. *P*–value < 0.05 was considered statistically significant.

2. IFN–γ, TNF–α, and IL–4 in the infected and uninfected groups were compared using *t*–test. *P*–value < 0.05 was considered statistically significant.

RESULTS

As shown in Table 2, the RBC, hemoglobin, platelets, neutrophils, and lymphocytes of the uninfected group were significantly lower than in the infected group ($P<0.05$), but hematocrit, eosinophil, and monocyte counts were not significantly different ($P>0.05$) between both groups. The eosinophil count for the large farm was significantly higher than for the small farm ($P<0.05$). However, RBC, hemoglobin, hematocrit, platelet, WBC, neutrophil, lymphocyte, and monocyte values were not significantly different ($P>0.05$) between small and large farms.

Table 2. Effect of group (uninfected and LSDV–infected) and farm size on hematological parameters of cattle in northeastern Thailand.

Parameters	Group (G)		Farm size (FS)		*P*–value		
	Uninfected	Infected	Small	Large	G	FS	G*FS
RBC (x10^6/μL)	6.42±0.37b	7.72±0.35a	6.86±0.33	7.28±0.4	*	ns	ns
Hb (g/dL)	9.38±0.47b	11.08±0.45a	10.33±0.41	10.14±0.50	*	ns	ns
HCT (%)	30.18±1.43	32.99±1.36	31.46±1.25	31.71±1.53	ns	ns	ns
PLT (x10^3/μL)	281.30±28.35b	413.17±26.9a	328.59±24.77	365.88±30.22	*	ns	ns
WBC (x10^3/μL)	8.26±1.13b	16.99±1.07a	11.63±0.99	13.61±1.20	*	ns	*
NEU (x10^3/μL)	5.06±0.53b	6.83±0.50a	5.36±0.46	6.53±0.57	*	ns	ns
LYM (x10^3/μL)	2.62±0.82b	9.62±0.78a	5.80±0.72	6.44±0.88	*	ns	ns
EOS (x10^3/μL)	0.46±0.06	0.44±0.05	0.37±0.05b	0.53±0.06a	ns	*	ns
MON (x10^3/μL)	0.1±0.03	0.19±0.03	0.13±0.03	0.16±0.04	ns	ns	ns

Means±SE with different letters are significantly different among groups (P<0.05); *$P<0.05$*; **ns**=non–significant; **RBC**=Red blood cells; **Hb**= Hemoglobin; **HCT**= Hematocrit; **PLT**= Platelets; **WBC**= White blood cells; **NEU**=Neutrophils; **LYM**=Lymphocytes; **EOS**= Eosinophils; **MON**= Monocytes

It was found that the WBC of the infected group was higher than that of the uninfected group (P<0.05). In addition, we found an interaction between the group (uninfected and infected) and farm size (small and large) on WBC (Table 3).

Table 3. Effect of the interaction between group (uninfected and LSDV–infected) and farm size on hematological parameters of cattle in northeastern Thailand.

Parameters	Uninfected		Infected		Interaction (Group* Farm size)
	Small farm	Large farm	Small farm	Large farm	
RBC (x10^6/μL)	5.85±0.55	6.98±0.50	7.86±0.35	7.58±0.62	0.1740
Hb (g/dL)	8.93±0.7	9.83±0.64	11.72±0.44	10.44±0.78	0.10
HCT (%)	28.20±2.12	32.17±1.93	34.72±1.34	31.25±2.37	0.0655
PLT (x10^3/μL)	276.10±41.88	286.50±38.23	381.08±26.48	445.25±46.82	0.4946
WBC (x10^3/μL)	8.86±1.67 ac	7.64±1.52 c	14.40±1.05 ab	19.57±1.86 b	0.0453*
NEU (x10^3/μL)	5.07±0.78	5.05±0.72	5.65±0.50	8.01±0.88	0.1102
LYM (x10^3/μL)	3.29±1.21	1.95±1.11	8.32±0.77	10.93±1.36	0.0880
EOS (x10^3/μL)	0.41±0.08	0.5±0.08	0.32±0.05	0.56±0.09	0.3316
MON (x10^3/μL)	0.09±0.05	0.10±0.05	0.16±0.03	0.22±0.06	0.6742

Means±SE with different letters are significantly different among groups (P<0.05); *$P<0.05$*; **RBC**=Red blood cell; **Hb**= Hemoglobin; **HCT**= Hematocrit; **PLT**= Platelets; **WBC**= White blood cells; **NEU**=Neutrophils; **LYM**=Lymphocytes; **EOS**= Eosinophils; **MON**= Monocytes

As shown in Table 4 and 5, the GGT of the infected group was significantly higher than that of the uninfected group (*P<0.05*). Total protein, albumin, AST, ALP, and creatinine between both groups were not significantly different (*P>0.05*). Albumin of the small farm was significantly higher than for the large farm (*P<0.05*). However, total protein, AST, ALP, GGT, and creatinine were not significantly different (*P>0.05*) between small and large farms.

Table 4. Effect of group (uninfected and LSDV–infected) and farm size on biochemical parameters of cattle in northeastern Thailand.

Parameters	Group		Farm size		*P*–value		
	Uninfected	Infected	Small	Large	G	FS	G*FS
TP (g/dL)	7.71±1.79	8.55±1.69	8.30±1.56	7.96±1.90	ns	ns	ns
ALB (g/dL)	3.28±0.14	3.18±0.13	3.45±0.12a	3.01±0.14b	ns	*	ns
AST (U/L)	110.81±16.70	132.50±15.84	113.04±14.60	130.27±17.80	ns	ns	ns
ALP (U/L)	165.38±22.34	126.86±21.19	159.78±19.52	132.46±23.81	ns	ns	ns
GGT (U/L)	8.18±5.36b	26.51±5.08a	20.43±4.68	14.25±5.71	*	ns	ns
Creatinine (mg/dL)	1.47±0.10	1.62±0.10	1.45±0.09	1.63±0.11	ns	ns	ns

Means±SE with different letters are significantly different among groups (*P*<0.05); **P<0.05*; **ns**=non–significant; **TP**=Total protein; **ALB**=Albumin; **AST**=Aspartate aminotransferase; **ALP**=Alkaline phosphatase; **GGT**=Gamma glutamyl transferase.

Table 5. Effect of interaction between group (uninfected and LSDV–infected) and farm size on biochemical parameters of cattle in northeastern Thailand.

Parameters	Uninfected		Infected		Interaction (Group* Farm size)
	Small farm	Large farm	Small farm	Large farm	
TP (g/dL)	7.52±2.64	7.90±2.41	9.09±1.67	8.01±2.95	0.7686
ALB (g/dL)	3.34±0.20	3.22±0.18	3.56±0.13	2.81±0.22	0.1020
AST (U/L)	120.20±24.67	101.42±22.52	105.88±15.60	159.13±27.58	0.1239
ALP (U/L)	158.60±33.00	172.17±30.12	160.96±20.87	92.75±36.89	0.1901
GGT (U/L)	11.10±7.91	5.25±7.22	29.76±5.00	23.25±8.84	0.9645
Creatinine (mg/dL)	1.49±0.15	1.44±0.14	1.42±0.1	1.82±0.17	0.1201

Means±SE with different letters are significantly different among groups (*P*<0.05); **P<0.05*; **TP**=Total protein; **ALB**=Albumin; **AST**=Aspartate aminotransferase; **ALP**=Alkaline phosphatase; **GGT**=Gamma glutamyl transferase.

IFN–γ of the infected group was significantly higher than the uninfected group (*P*<0.05). TNF–α of the infected group was significantly lower than the uninfected group (*P*<0.05). IL–4 of both groups was not significantly different (*P*>0.05) (Figure 1).

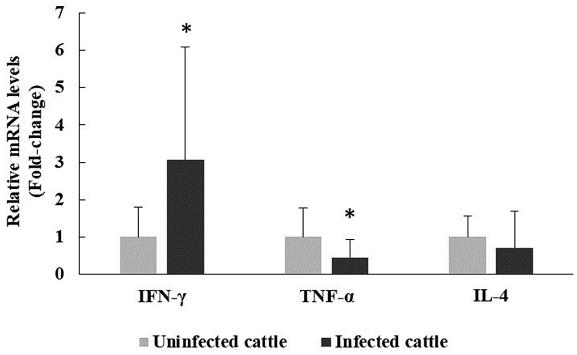

Figure 1. Comparison of IFN-γ, TNF-α, and IL-4 levels between uninfected and LSDV-infected cattle.

Real–time PCR confirmed the presence of viral DNA in blood samples of infected cattle (Figure 2). The cycle threshold (C^t) values were in the range of 20.1–37.5 for LSDV–positive blood samples.

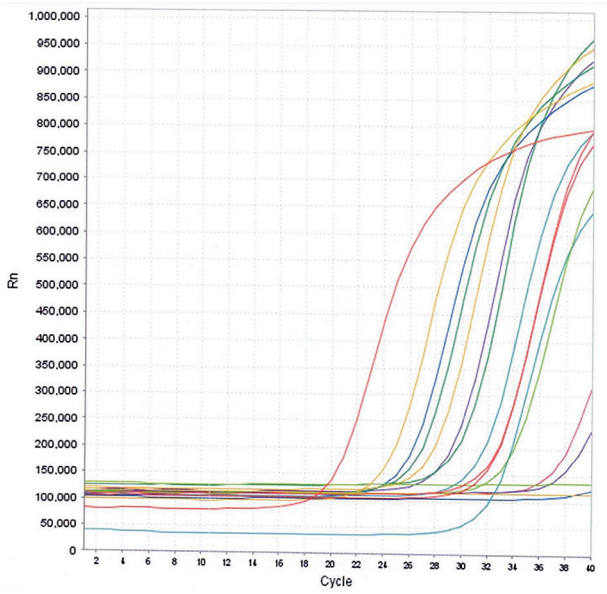

Figure 2. The amplification plots of the real-time PCR assay. Amplification curves of LSDV positive and negative samples result in C^t value.

DISCUSSION

Hematological and biochemical parameters can be used as indicators of health status, not only in individual cattle but also in routine herd monitoring [21,22]. In the present study, we designed the study in order to observe the change of hematological and

biochemical parameters attributed to infection with LSDV in cattle. LSDV infection in cattle did affect RBC profiles. Our study showed that LSDV–infected cattle had higher RBC counts and hemoglobin levels than uninfected cattle, which was in agreement with an earlier study by El–Mandrawy and Alam (2018) [12]. The increased RBC counts could be attributed to absolute erythrocytosis, a condition in which the bone marrow increases RBC production in response to factors such as hypoxia or stress [23]. Another possibility is dehydration, which causes hemoconcentration, thereby artificially raising RBC counts. This is consistent with the findings of El–Mandrawy and Alam (2018) [12], who reported that cattle infected with LSDV frequently suffer from dehydration, accompanied by symptoms such as anorexia, fever, and lethargy, which can increase the amount of RBC in the bloodstream. In contrast, some studies have reported that the RBC counts, hemoglobin concentrations, and hematocrit of LSDV–infected cattle decreased, probably caused by the chronic inflammatory response [24]. In the case of chronic anemia, it is usually mild and progresses gradually [22]. While Allam et al. (2021) [25] found that LSDV caused macrocytic hypochromic anemia in infected cattle. They hypothesized that LSDV may cause the destruction of RBCs. This variation might be due to the infection stage and the cattle's immunological reaction [13,26].

Generally, LSDV infection would alter the leukogram, although the response to LSDV infection may be divergent. A natural LSDV outbreak in Jordan caused leukocytosis [24]. However, natural LSDV infection of cattle in Egypt caused leukopenia [25]. In the present study, it was found that LSDV–infected cattle had a tendency toward leukocytosis with neutrophilia, which was similar to the study by Rouby et al. (2021) [27] and Ahmad et al. (2023) [28]. It is well known that neutrophilia has been seen in chronic inflammation or secondary acute bacterial infections in ruminants [12,22]. The results indicated that the LSDV–infected cattle responded to infection and inflammation. Moreover, in the present study, the number of lymphocytes was significantly increased in the LSDV–infected cattle, which is consistent with earlier reports [29]. This phenomenon is in accordance with the study of Neamat–Allah et al. (2015) [10], who reported that infection of LSDV caused leukopenia and lymphopenia during 1^{st}–2^{nd} days post–infection, then followed by lymphocytosis, monocytosis, and granulocytic leukocytosis during 10–14^{th} days post–infection. These results may be associated with the stage and severity of infection [24]. It was considered possible that lymphocytosis can occur during chronic antigenic stimulation due to LSDV [22]. To date, however, there are no specific criteria that can reliably distinguish the stage of infection, and it needs to be further investigated.

In addition, LSDV infection also altered platelet count. We found that the platelet count in the infected cattle increased when compared with uninfected cattle. This occurrence was in opposition to previous studies, which reported that thrombocytopenia is one of the hematological parameters in natural LSDV infection [12,25]. These results could be related to the destruction of platelets during the chronic inflammatory response due to the LSDV infection [22]. Infestation with arthropod ectoparasites

typically results in elevated eosinophils as well as hypersensitive responses [30,31]. The presence of LSDV DNA in blood–feeding insects like *Stomoxys calcitrans* and *Tabanidae* during outbreaks suggests that these vectors might influence the observed differences in hematological and biochemical parameters in infected cattle, potentially intensifying the spread and impact of the disease [32]. This is most likely due to the inflammatory response brought on by an insect bite, which causes an increase in eosinophils [30]. The present study was performed in the rainy season when there was a high frequency of insect bites. In addition, the crowded situation on the farm may be associated with infections by many ectoparasites. As a result, cattle on large farms may have higher eosinophils levels than those on small farms. Therefore, the eosinophil count of cattle on the large farm was higher than that on the small farm. This is in line with the previous studies showing a higher percentage of eosinophils in tick–infested cattle than in non–infested cattle [31,33].

The white blood cell count of the LSDV–infected cattle in the large farm was higher than that of the other three groups. This result is consistent with the findings of Omer et al. (2000) [34], who found a high seroprevalence of brucellosis in a commercial dairy farm compared with a traditional cattle farm. Kasem et al. (2016) [35] also reported that the morbidity and mortality rate of LSDV of infected cattle in intensive farms was higher than in smallholder farms. Moreover, Omer et al. (2000) [34] and Meadows et al. (2018) [36] considered support for the view that animal density, farm size, husbandry, and environment–based factors are a main factor that influences the spread of disease. It is possible that if the pathogens invade a larger farm, a high cattle density in the farm becomes more susceptible to infection, which is rapidly transmitted among animals. This suggests that the density of animal populations plays a role in determining the severity of an outbreak of an infectious disease [36].

The response of plasma biochemistry during LSDV infection was variable. This variation in biochemistry profiles might be due to the disease process, including the difference of parts in the body. In addition, the difference within the same parameter may be caused by the stage of infection and the complexity of the LSD cases [24].

Plasma liver enzyme activities indicate hepatocellular dysfunction or hepatic damage [21]. The activity of AST, GGT, and ALP is usually used to determine liver function and hepatobiliary disease [37]. In this study, GGT levels of LSDV–infected cattle were higher than in uninfected cattle, while AST and ALP levels were not different between both groups. These findings are similar to the report on the effect of naturally LSDV–infected cattle in Egypt and Jordan [4,24]. Increasing GGT activity indicated it to be a specific and sensitive indicator for liver disease, even if the liver tissue only suffers minimal or clinically unremarkable damage. But the increase in the level of AST indicates severe liver tissue injury [38]. Therefore, the elevation of serum GGT in LSDV–infected cattle may be associated with subclinical hepatocyte injury.

Albumin is a useful indicator of liver function [21]. In the present study, the large farm had lower albumin levels in its cattle than the small farm. This phenomenon is similar

to the study of Ul-Rahman et al. (2023) [9], who reported decreased albumin levels in cattle infected with LSDV in Pakistan and cattle infected with foot–and–mouth disease virus [39]. Our results confirmed that LSDV infection causes a decrease in serum albumin, which is another indicator showing infection–induced hepatic damage. The difference in the decrease of serum albumin between different size farms may result from the severity of the disease in large farms, as the reason mentioned above.

Inflammatory cytokines are used to indicate pathogenesis and the impact of inflammatory disorders from virus infection [40]. Generally, Th1 cells secrete IFN–γ and TNF–α against intracellular pathogens. IFN–γ is involved in the inflammatory response and promotes immune responses to infections [41]. Additionally, it has been reported that IFN–γ is essential for the clearance of poxvirus during infections [42]. These observations may indicate IFN–γ involvement in the host's inflammatory response to LSDV infection. In the present study, the IFN–γ of LSDV–infected cattle was higher than in uninfected cattle. Similar results were reported by Norian et al. (2016, 2017) [19,43], who found that IFN–γ levels in live attenuated goat and sheep poxvirus–vaccinated cows increased within week 3 post–vaccinations.

Besides, TNF–α of LSDV–infected cattle was lower than in the uninfected group. This occurrence was in line with the study of Kels et al. (2020) [44], who reported that TNF deficiency caused severe lung pathology but had no impact on viral load in mice infected with poxvirus. Furthermore, TNF deficiency increases the production of IFN–γ, IL–6, and IL–10 and transforming growth factor–beta [44]. Taken together, these findings indicate that TNF–α had an indirect antiviral effect against LSDV in cattle. Nevertheless, this phenomenon contradicts the previous report of El–Mandrawy and Alam (2018) [12], who found those elevated levels of TNF–α in naturally infected cattle in Egypt. However, the difference between our findings and the previous study might be due to the difference in the study method, virus strain, and stage of infection.

Generally, molecular techniques via DNA amplification by PCR are specific, sensitive, and fast for laboratory confirmation of viral infections, including LSDV [45]. Detecting LSDV DNA by conventional and real–time PCR are widely applied methods of detecting LSD in blood and skin samples [3,5]. In this study, real–time PCR was used for the detection of LSDV DNA in blood samples from cattle. Real–time PCR was shown to be highly sensitive to LSDV. This is consistent with previous studies showing that real–time PCR provided a highly sensitive and rapid assay for the diagnosis of LSDV for monitoring and controlling the spread of the disease [2,3,5]. In the present study, we used this technique to confirm the presence of viral DNA in infected cattle. The cycle threshold values were in the range of 20.1–37.5 for LSDV–positive blood samples.

In conclusion, the present study revealed the influence of LSDV infection and farm size on hematology, biochemistry, and inflammatory cytokine values in LSDV–infected beef cattle in Thailand. LSDV infection was found to affect hematological parameters,

including, i.e., RBC, hemoglobin, platelets, WBC, neutrophil, and lymphocyte, while farm size influenced eosinophil levels. For blood biochemistry, LSDV infection influenced GGT levels, and farm size affected albumin levels. LSDV infection also influenced IFN–γ and TNF–α levels. In addition, our study indicated that real–time PCR is an applicable method for detecting LSDV in blood samples.

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Authors' Contributions

All authors contributed to the study conception and design. Data analysis and the fieldwork were performed by TN and PS. PS, PT and DO carried out the experiment. Data interpretations were performed by PS, WA and ZW. The first draft of the manuscript was written by PS, WA and ZW and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare no conflict of interest.

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UTICAJ INFEKCIJE VIRUSOM BOLESTI KVRGAVE KOŽE I VELIČINE FARME NA HEMATOLOŠKI, BIOHEMIJSKI I CITOKINSKI PROFIL KOD GOVEDA

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Bolest kvrgave kože (eng. *Lumpy skin disease* - LSD) je zarazna virusna bolest goveda. Ovaj eksperiment je imao za cilj proučavanje uticaja infekcije virusom bolesti kvrgave kože (LSDV) i veličine farme na hematološke i biohemijske parametre, kao i citokine kod 55 goveda. Grupe su uključivale goveda sa velikih farmi zaražena LSDV (Grupa 1), LSDV zaražena goveda sa malih farmi (Grupa 2), neinficirana goveda sa velikih farmi (Grupa 3) i neinficirana goveda sa malih farmi (Grupa 4). Mereni su hematološki parametri, biohemijske vrednosti i profili citokina. Rezultati su pokazali veće vrednosti za eritrocite, hemoglobin, trombocite, ukupne leukocite, neutrofilne granulocite i limfocite, kod inficiranih nego kod neinficiranih goveda. Nivo eozinofilnih granulocita kod goveda sa velikim farmama bio je veći nego na malim farmama. Gama glutamil transferaza kod inficiranih goveda bila je veća nego kod neinficiranih. Albumin goveda sa malih farmi bio je veći od onog sa velikih farmi. IFN-γ kod inficiranih goveda je bio veći nego kod neinficiranih goveda, dok je TNF-α kod inficiranih goveda bio niži nego kod neinficiranih goveda. Naša studija je pokazala da je LSDV infekcija menjala hematološke i biohemijske parametre, uključujući profile citokina, u zavisnosti od veličine farme koja potencijalno utiče na ove promene.