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Seroprevalence of Brucellosis Disease and Lumpy Skin Disease on Cattles at Svay Rieng and Prey Veng Province, Cambodia

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Abstract: LSD (Lumpy Skin Disease) is a poxviral disease with significant morbidity in cattle and belongs to the family Poxviridae and the genus Capripoxvirus and is transmitted by hematophagous arthropod vectors. Despite the typically low mortality rate, economic costs arise from deterioration in health, decreased milk production, miscarriages, infertility and harmed hides. Brucellosis disease is one of the most common contagious and communicable zoonotic diseases with high rates of morbidity and lifetime sterility. Serological tests with ELISA (Enzyme-Linked Immunosorbent Assays) indicate exposure to Brucella and LSD in cattle. To identify the presence of those diseases, the research was conducted in two provinces, Svay Rieng and Prey Veng, in Cambodia, starting from July 2021 to January 2022. In the study, the 2018 Thusfield method was adopted, and two cattle were selected from 216 households in the two provinces (112 in Svay Rieng and 104 in Prey Veng). However, not all the families had two cattle, so the total sample size was 300 cattle (227 in Svay Rieng and 73 in Prey Veng). As a result, there was only one brucellosis disease case in Svay Rieng Province, while that disease was not found at all in Prey Veng. Meanwhile, LSD was higher in Prey Veng (80% of the tested cattle) than in Svay Rieng (69%). Among all of the tested cattle, 66.7% had the highest BS (Body Score = 4). The finding suggests that LSD was prevalent in the studied areas, which may cause economic losses. Thus, preventive measures should be taken properly to tackle this issue. Although Brucellosis was a rare case in the studied areas, it may spread faster, causing abortion in cattle and women. Biosecurity is needed to ensure a strict control over this disease.

Key words: Zoonosis, Brucellosis, LSD, antibody, ELISA.

1. Introduction

In the rural areas of Cambodia, livestock husbandry is crucial. Most farmers have expressed dissatisfaction with the decreased income from raising livestock, which provides for their livelihood [1]. Common livestock are raised in Cambodia because they are source of draught power and income [2]. So far, the number of cows has increased to about 15 thousand heads [3]. However, raising systems are still implemented in a traditional way, making cows susceptible to many kinds of diseases such as FMD (Foot and Mouth Disease) [4]. Lumpy Skin Disease (LSD) is an endangered banteng in Cambodia, followed by the launch of a domestic cow vaccination campaign to protect wild bovids from disease transmission along the wildlife-livestock interface. In June 2021, the LSDV (Lumpy Skin Disease Virus) was discovered in domestic cattle in Cambodia and quickly spread throughout the country. A banteng with LSD was spotted in the Phnom Tnout Phnom Pok Wildlife Reserve in September 2021 [5].

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LSD is rated as a serious threat to cattle in Southeast Asia. Its signs include lumps that appear on the external skin and mucous membrane, while the transmission is caused by insects such as flies and mosquitos and touching between cattle. The sign appears in 1-4 weeks, depending on immune systems. This problem really affects socio-economic aspects of cattle farmers [6].

The clinical signs of LSD are nodules sized 5-50 mm on the skin and rounding head, neck, perineum, udder the limbs to became necrotic lesions [7]. For the gross lesions that are ulcerative in the mouth, trachea and gall bladder [8], control and prevention of LSD are used the vaccination on the cattle before infecting the disease, movements of cattle inside the country and across the borders should be strictly controlled by government. Furthermore, all cattle should be protected from vector transmission of the disease [8].

LSD cannot be treated with antibiotics, although supportive care may be offered. The main LSD prevention and control measures include quarantine, movement restrictions, and insect vector management [9]. Abortion and infertility in cows are caused by the bacterial zoonoses brucellosis, which is caused by the genus Brucella [10].

Worldwide, brucellosis causes substantial morbidity in humans and economic losses in livestock [11]. The main ways that brucellosis is transferred from animals to people include direct contact with sick animals, consumption of raw dairy products from animals and consuming contaminated domestic livestock meat [12]. The clinical sign manifestations of human brucellosis include fever, sweat, chills, headache, malaise, myalgia, and even arthralgia of the major joints [13].

Symptoms on cattle in relation to an abortion are challenging for the herd owner and veterinarian. The majority of diagnosed abortions are attributed to infections with the viruses IBR (Infectious Bovine Rhinotracheitis) or BHV (Bovine Herpesvirus) and Bovine Viral Diarrhea, as well as the bacteria *Neospora caninum*, *Brucella abortus*, and *Leptospira interrogans* [14]. Commercial antibody ELISA kits were used for serological testing of zoonotic and highly contagious cattle brucellosis [15].

ELISA (Enzyme-Linked Immunosorbent Assay) test is used to check for antibodies to Brucellosis, Q fever, or Neosporosis, and we investigated the connection to abortion [16]. Effective preventative measures, such as the use of protective clothing and footwear, must be taken to prevent the spread of brucellosis among professions who work with livestock or products from animals [17]. The objective of this study was to identify the presence of LSD and brucellosis disease in cattle using the ELISA test.

2. Material and Methods

2.1 Site Selection

Table 1 shows the number of selected samples in Svay Rieng and Prey Veng provinces, Cambodia. Both provinces are located in southeast of Cambodia and border Vietnam. Svay Rieng province had 7 districts including Svay Rieng, Svay Teab, Romdul, Chan Trea,

Table 1 Site selection and sample size calculation.

Province	District	Commune	Village	Number of cattle
Svay Rieng	Romeas Haek	Koki	Tropeang Skoun	36
			Prey Kdoey	38
		Ampil	Romeas Haek	38
			Prey Ousey	38
Prey Veng	Kamchay Mear	Krabau	Chou thnoul	38
			Troupoung Romeas	37
		Cheach	Kanleang Chrouv	38
			Tbougn Wat	37
Total	2	4	8	300

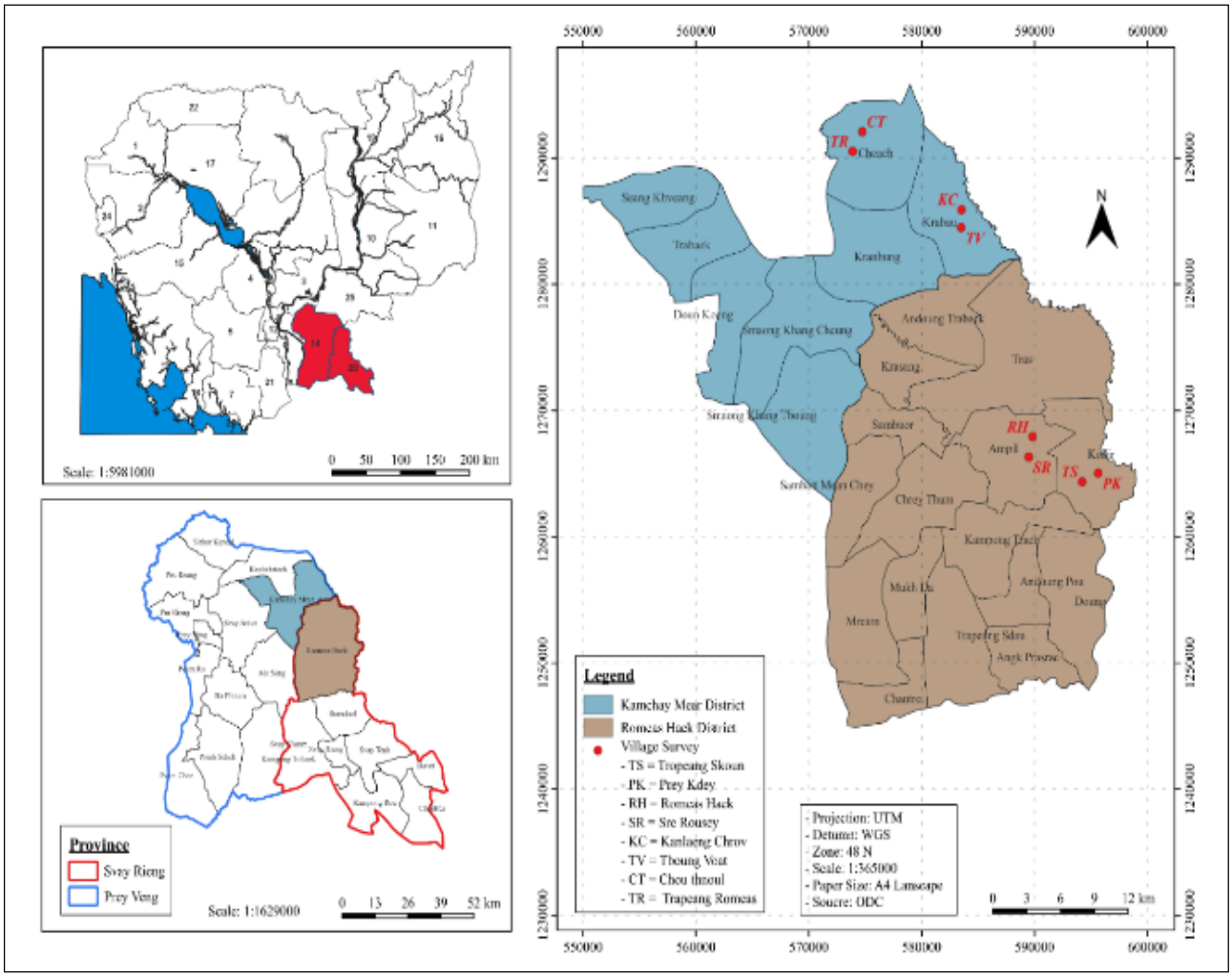

Fig. 1 Map of the cattle sampling sites in Prey Veng and Svay Rieng Provinces, Cambodia. The blue color area represents the Kamchay Mear District, Prey Veng, and gray color represents Romeas Haek District, Svay Rieng Province, Cambodia.

Svay Chrum, Romeas Heak, and Kampong Rou district. Prey Veng province had 13 districts such as Ba Phnum, Kamchay Mear, Kampong Trabaek, Kamhchriech, Me Sang, Peam Chor, Peam Ro, Pea Reang, Preah Sdach, Krong Prey, Kampong Leav, Sithor Kandal and Svay Antor districts (Fig. 1).

In the study, the 2018 Thrusfield method was adopted, and the criteria were that two cattle were selected from 216 farmers that raised cattle in Svay Rieng (112 farmers) and Prey Veng Provinces (104 farmers). However, some of them had a lot of cow, so after the selection, a total sample size for this study was 300 cattle, of them 223 in Svay Rieng and 73 in Prey Veng. The study was conducted from November 2022 to December 2023.

2.2 Sample Preparation and Storage

All blood samples were collected from the jocular vein of cattle, and the body condition score of the cattle was also measured. The processing of collected samples for serology and bacteriological procedures of ELISA test was conducted at NAHPRI (National Animal health and Production Research Institute), General Directorate of Animal Health and Production, Phnom Penh, Cambodia, Geographic Information System (GIS), (487258.82 E, 1275360.17 N).

2.2.1 Laboratory Diagnostic Test

The ELISA kits manufactured by IDvet, France, were used to detect antibodies against *Brucella abortus*, melitensis or suis ID Screen® Brucellosis serum

indirect multi-species and ID Screen® Capripox Double Antigen Multi-species. ELISAs were done according to the manufacturer's protocols provided with the kits.

The sample to positive ratio (S/P%) and competition percentage (S/N%) were calculated using IDSoftTM software provided with the ID Screen® ELISA kits [18]. The S/P% cut off points and Dse (Diagnostic Sensitivities) and Dsp (Diagnostic Specificities) for the brucellosis kits were previously described [18].

2.2.2 Statistical, Relative Risk Analyses and Quantum Geographic Information System (QGIS 3.4 Madeira).

Data analyses were conducted using Microsoft Excel to perform descriptive statistics, including frequency and probability distribution, in order to describe the dataset. Chi-square was used to compare the presence of the diseases by province, including degrees of freedom, *p*-value, risk factor analyses and Odds Ratio (OR) and Relative Risk (RR), with 95% CI (Confidence Intervals). The (QGIS) method was used to indicate the map of the sampling sites in Cambodia.

3. Result and Discussion

3.1 Brucellosis Disease

Based on the result of ELISA Brucellosis disease, it can be seen that only one sample was affected by this disease at Romeas Haek village, Ampil commune, Romeas Haek district, Svay Rieng province. But Brucellosis disease was not present at Kamchay Mear district, Prey Veng province.

3.2 Lumpy Skin Disease

Seroprevalence of LSD was present in both provinces. The presence of LSD was higher in Prey Veng province 120 (80%) than in Svay Rieng province 104 (69%) (Table 3).

According to Table 2, seroprevalence of LSD was compared by province and revealed an overall apparent seroprevalence by 80% of positive finding in Prey Veng province and 69% of positive result in Svay Rieng province and *p*-value 0.046 was not significant.

Table 4 shows significant difference in positive results compared in eight villages. Tboung Wat and Kanleang Chrouv village have higher positive result of LSD than six villages which have higher 94.59% and 92.11%, respectively. Romeas Haek village was seroprevalences 78.95%, Prey Kdoey 76.32%, Chou Thnoul 73.68%, Troupoung Romeas 59.46%, Troeang Skoun 58.33% and Prey Ousey village 63.16% in Svay Rieng and Prey Veng province. The reason that LSD positive case was high in each village was outbreak through vectors.

Table 2 Comparison of brucellosis disease by province.

Province	*Positive*	*Negative*
Prey Veng	1 (0.67%)	149 (99.33%)
Svay Rieng	0 (0%)	150 (100%)
Chi-square	3.97	
***p*-value**	0.046	

Table 3 Comparison of LSD by province.

Province	*Positive*	*Negative*
Prey Veng	120 (80%)	30 (20%)
Svay Rieng	104 (69%)	46 (31%)
Chi-square	3.97	
***p*-value**	0.046	

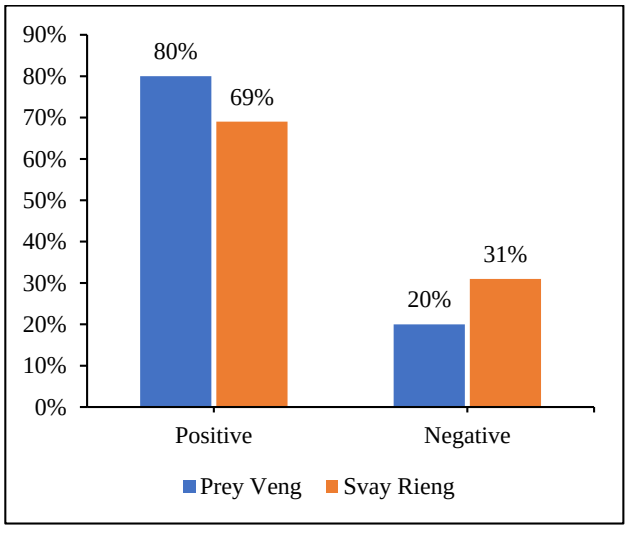

Fig. 2 Comparison of LSD disease by province.

Table 4 Comparison of villages with 95% CI for LSD.

Village	*Total*	*Positive*		*CI 95%*	
		Number	Percentage	Lower	Upper
Chou Thnoul	38	28	73.68	46.39	100.98
Kanleang Chrouv	38	35	92.11	83.53	100.68
Prey Kdoey	38	29	76.32	62.80	89.83
Prey Ousey	38	24	63.16	47.82	78.50
Romeas Haek	38	30	78.95	65.98	91.91
Tboung Wat	37	35	94.59	87.31	101.88
Troeang Skoun	36	21	58.33	42.23	74.44
Troupoung Romeas	37	22	59.46	43.64	75.28
Chi_Square			26.58		
***p*_value**			< 0.001		

Table 5 Comparison of sex and 95% CI for LSD in both provinces.

Gender	*Total*	*Yes*	*No*	*Risk*	*RR*	*CI 95%*	p_*value*
F	187	140	47	0.7487	1.01	0.875-1.15	0.919
M	113	84	29	0.7434			
Total	300	224	76				

Table 6 Comparison of sex and 95% CI for LSD in Svay Rieng Province.

Gender	*Total*	*Yes*	*No*	*Risk*	*RR*	*CI95%*	p_*value*
F	77	53	24	0.6883	0.985	0.875-1.15	0.891
M	73	51	22	0.6986			
Total	150	104	46				

Table 7 Comparison of sex and 95% CI for LSD in Prey Veng Province.

Gender	*Total*	*Yes*	*No*	*Risk*	*RR*	*CI 95%*	p_*value*
F	110	87	23	0.7909	0.959	0.875-1.15	0.631
M	40	33	7	0.8250			
Total	150	120	30				

Tables 5-7 compare LSD that occurred in two different provinces based on the cow sex. It can be seen that in Svay Rieng Province, the percentage of cows that were infected with LSD was two times higher than those tested negative, regardless of the sex. The infected cows account for about 70%, while about 30% were free of the disease. Similar results were observed in Prey Veng Province, where a high percentage of LSD-infected cows were observed in comparison with the cows that had no disease. Regardless of the provinces, similar findings were obtained, which shows that LSD is a common disease that breaks out in those areas. In terms of risk and relative risk, male and female cows have the same chance of contracting LSD. Thus, this disease attacks cows in general.

The cases of LSD prevalence were studied and compared between the two provinces. It is observed that there is a significant difference in the positive LSD cases between Prey Veng and Svay Rieng (p = 0.046). More positive cases were seen in Prey Veng, accounting for 80% of the cows tested, while only 69% of cows were tested positive in Svay Rieng Province.

Table 8 shows the comparison of body condition score of positive result of LSD; two body condition scores have higher positive values. Body condition score (BCS) #1 and BCS #4 were 66.67%, 66.67%, respectively. BCS #3 was 75.45% and BCS #2 75.73%. The body condition score was not significantly different and p-value 0.76.

Table 8 Comparison of body condition score and 95% CI for LSD.

BSC	*Total*	*Positive*		*CI 95%*	
		Frequency	%	Lower	Upper
1	6	4	66.67	20.47	112.86
2	103	78	75.73	61.87	89.58
3	167	126	75.45	64.63	86.27
4	24	16	66.67	43.57	89.77
Chi_square			1.13		
***p*_value**			0.769		

Table 9 Comparison of age and 95% CI for LSD.

Age	*Total*	*Positive*		*CI95*	
		Frequency	%	Lower	Upper
< 1year	101	73	72.28	63.55	81.01
1-2 years	89	74	83.15	75.37	90.92
> 2 years	110	77	70.00	61.44	78.56
Chi_square			4.95		
***p*_value**			0.08		

For comparing, the BSC showed that there was no association with LSD. It ranged from 66.67% to 75.73% (Table 8).

According to Table 9, age 1-2 year has higher positive result of LSD 83.15%, respectively; > 1 year has seroprevalence 72.28% and > 2 year 70.00%. Thus, the LSD did not depend on the age of cattle.

4. Discussion

Planting forage will help to improve cattle BCS in general; however, season, gender, and age of cattle all have an impact on cow BCS [19]. However, our study shows a significant burden associated with brucellosis disease and LSD on the cattle at Prey Veng and Svay Rieng Provinces border. Given the increased movement of livestock and human activity linked with changes in animal production, brucellosis and LSD have the potential to pose a public health danger across the border between Prey Veng and Svay Rieng Provinces. The most common pathogens associated with LAI (Laboratory-Acquired Infection) were Brucella spp. [20]. Serological testing for zoonotic and high-impact animal diseases, including brucellosis, was conducted using commercial antibody ELISA kits; two cow samples were tested positive for Brucella antibodies (0.2%, 95% CI 0.4-0.6, n = 1,141) [21]. i-ELISA test Brucellosis has been identified in 2.25% of dairy cattle. Brucellosis was found in 8.3% of abortions and 2.8% of repeated breeding, respectively, while the frequency of brucellosis in indigenous and cross-bred cattle was 3.6% and 1.7% [10]. Therefore, Lao People's Democratic Republic (PDR) government is using abattoir samples as a trial approach to obtain information on contagious zoonoses, particularly brucellosis, in the huge ruminant population. The Brucella antibody ELISA test revealed one positive sample (out of 683; 0.2%, 95% CI 0.0, 0.8) [18]. Livestock prevalence of brucellosis disease in herd heeds of beef and dairy cattle was respectively 2.6% (0.7-7.9) and 13.3% (2.3-41.6) [11]. Furthermore, Bovine brucellosis seroprevalence was at 3.40% (n = 590; 3.4% for Rose Bengal Plate Test (RBPT), 5.93% for Ii-ELISA) [22]. Anyway, the prevalence of lumpy skin antibodies was determined using an ELISA, which found high exposure rates of 57% and 51.42% in Beni Suef, Egypt [23]. The first outbreak of LSD in Thailand was found in March and the first week of April 2021, with 293 LSD outbreak farms located in four distinct districts. The affected animals had an overall disease and death rate of 40.5% and 1.2% [24]. LSD is an acute

viral disease in cattle that has recently emerged as a major economic loss in Bangladesh. The overall prevalence of LSD in cattle was 41.06%, with the local breed (75%) and young cattle less than one year (64%) being significantly ($p < 0.001$) higher for LSD with significant ($p < 0.001$) skin lesions in whole body (44%) and herd level information having the odd ratio of local breed (95% CI: 0.244-0.553), skin lesions [25].

Overall, the result compared LSD that occurred in two different provinces based on the cow. It is observed that there is a significant difference in the positive LSD cases between Prey Veng and Svay Rieng ($p = 0.046$). The body condition score evaluated in the two provinces was not significantly different ($p = 0.76$). In addition, LSD did not depend on the cattle age ($p = 0.084$), which means that cattle of different age may have a chance of infection when contacting the disease. Comparing to age and 95% CI, > 2 year seroprevalence was 70.00% had lower. For compared of was not significantly different and *p*-value was 0.084.

5. Conclusion

The research of Seroprevalence of Brucellosis Disease and Lumpy skin Disease on Cattles at Svay Rieng and Prey Veng province, Cambodia, by Laboratory diagnostic test used the ELISA test.

The research focused on comparison of LSD by province, communes 95%, sex and 95%, body condition score 95% and age 95%. In addition, LSD has a non-correlation between sex, age and body condition score. However, Brucellosis disease was positive one sample, but it risks infecting cattle and humans also, because Brucellosis disease is zoonosis disease that can kill the baby of pregnant humans to be abort.

The future study will be conducted to identify clinical signs that are indicated on the cattle tested positive with Brucellosis and LSD by serology test.

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