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Lumpy Skin Disease in Bangladesh: Seroprevalence and Risk Factors of LSD in Cattle

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ABSTRACT

Lumpy skin disease (LSD) is currently one of the most economically significant growing livestock diseases in Bangladesh due to its widespread occurrence and considerable cattle population. The goal of this study was to provide animal-level seroprevalence and risk factors for seropositivity without a history of immunization against LSDV in Bangladesh. Between October 2021 and March 2022, cross-sectional research was carried out-throughout Bangladesh. Seven hundred thirty-six (736) cattle serum samples were collected, and each sample was examined using a commercial ELISA kit. The overall seroprevalence of LSD among cattle in Bangladesh was estimated as 24.59% (181/736) (95% CI: 21.62-27.83). The study found a significant variation in seroprevalence rates across different geographic areas studied. Barisal had the lowest prevalence (16.3%), while Khulna and Mymensingh had the highest prevalence (30.4%). Additionally, young animals (OR=4.97 95% CI: 2.12 - 8.50) and crossbred cattle (OR=2.71, 95% CI: 1.90-3.80) had a higher risk of contracting LSD infection. Furthermore, there was a strong correlation ((P<0.05)) found between the sex of the animals (male 16.57% (55/332), 95% CI: 12.95-20.94 and female 31.2%, 95% CI: 1.58-3.26) and the incidence of LSDV in cattle. However, there were no discernible (P>0.05) variations in the prevalence between lactation and LSD infection. The study suggests that LSD prevalence in Bangladesh is moderate and highlights the need for effective control measures.

Key words: Seroprevalence, Risk factors, Lumpy skin disease, Cattle, Bangladesh

INTRODUCTION

Globally, a billion people depend on the livestock sector for their livelihood and food security, which contributes on average 40% of the value of the world's agricultural output (Abera et al. 2015). Like many other developing countries, Bangladesh is also contingent on a multifunctional livestock sector. Apart from a major play in producing food and income, livestock are a treasure for insurance of credit, a stock of wealth, and a vital security net during times of disaster (Abera et al. 2015). Livestock production is an essential component of Bangladesh's agricultural system, accounting for 13.44% of the country's Gross Domestic Products (GDP) on a statistical basis (DLS, 2022). In Bangladesh, work related to producing cattle and poultry provides a living for about 20% of the population (Banglapedia 2022). In forthcoming days, livestock production will be decreased because of the race for natural resources like land and water, competition between food and feed, and constant rise of raw feed materials price (Abera et al. 2015). Currently, this sector in Bangladesh is profoundly affected by the shortage of feed, livestock diseases (transboundary, zoonotic), hereditary problems of indigenous livestock, frequent natural disasters, and the nonexistence of selling setup.

Cross-border cattle movements may expose them to the infectious illness caused by lumpy skin disease (LSD). The LSD virus (LSDV), a double-stranded DNA *Capripoxvirus* in the *Poxviridae* family, is the cause of

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LSD infection. Typical symptoms of the disease include mild to severe fever, the appearance of widespread skin nodules, lymphadenitis, edema, and about 1-5% of fatal cases (WOAH 2022). A core of necrotic tissue known as sit-fasts is being formed by the skin nodules that range in size from 1 to 7 cm in diameter (Tuppurainen and Oura 2012; Kiplagat et al. 2020). LSDV is closely related to two other viruses in the genus *Capripoxvirus*, sheeppox and goatpox viruses (Das et al. 2021). The host range of LSDV is constrained, and it doesn't infect non-ruminant hosts. Both male and female cattle of indigenous and crossbreds are of all ages susceptible to LSDV. However, younger animals may be more susceptible to severe sickness (Badhy et al. 2021). The primary mechanical mechanism of transmission for LSDV is by several likely arthropod vectors, including biting flies, mosquitoes, and three hard ticks (*Rhipicephalus decoloratus, Amblyomma hebraeum, and Rhipicephalus appendiculatus*) (Chihota et al. 2003; Tuppurainen et al. 2011; Abera et al. 2015; Alkhamis and VanderWaal 2016; Tuppurainen et al. 2017). In addition, the stable fly (*Stomoxys calcitrans*) is recognized as the most likely vector for transmission of LSDV due to its prevalence and association with outbreaks (Kahana-Sutin et al. 2017). Although the role of various vectors in the spread of LSDV has not yet been researched in Bangladesh the disease's potential vectors (mosquitoes, flies, and ticks) are all present there and are quite likely to be to blame. Due to its negative effects on milk production, weight loss, irreversible damage to hides, miscarriage, infertility, and death, LSD is an economically important viral illness of cattle. Although the death rate is frequently less than 10%, the morbidity rate might reach 100% (Kumar et al. 2021). Farmers suffer enormous financial losses, significantly hindering global trade (Khan et al. 2022). Nevertheless, the disease was initially identified in 1929 in Northern Rhodesia (Zambia) and remained indigenous to sub-Saharan Africa until 1990, when it began to spread to North Africa and later the Middle East (Ochwo et al. 2019). The World Organization for Animal Health (WOAH) categorized LSD as a notifiable disease because of its potential rapid spread and substantial economic importance.

For LSD to be successfully controlled, accurate, timely diagnostic methods are required to confirm the clinical diagnosis. LSDV has been examined using a variety of serological methods, such as the virus neutralization test (VNT), indirect fluorescent antibody test (IFAT), and ELISA (Gari et al. 2008, 2012). Although most serological assays are trustworthy and provide throughput screening, they are unable to differentiate between Parapoxvirus and *Capripoxvirus.* The LSDV, sheeppox virus, and goatpox virus are all *Capripoxvirus*es, and a commercial ID Screen® Capripox double antigen multi-species ELISA kit that is now available can detect antibodies against these viruses in serum without reacting with Parapoxvirus (Tian et al. 2010; Ochwo et al. 2019; WOAH 2022). On September 15, 2019, WOAH received word of Bangladesh's first LSD epidemic. In July 2019, after starting in the Southeast (Chattogram district), the disease started to spread across the country. In Bangladesh, LSD is currently one of the most economically significant growing livestock diseases due to its widespread occurrence and considerable cattle population. Apart from a few outbreak reports (Badhy et al. 2021). Bangladesh's epidemiological trends for transboundary illnesses, in particular LSD, are lacking. As far as we are aware, Bangladesh does not have any information on the seroprevalence of the LSDV. Hence, LSD prevention and control programs may be hampered. Therefore, this study aimed to provide animal-level seroprevalence and risk factors for seropositivity without a history of immunization against LSDV in Bangladesh. This research will enhance our understanding of the epidemiology of LSD and potentially lead to improved strategies for managing the disease in Bangladesh.

MATERIALS AND METHODS

Ethical approval

The study was carried out according to the ethical norms of the Sylhet Agricultural University's Faculty of Veterinary, Animal, and Biomedical Sciences ethical committee (permission No. #AUP2020020). Every safety measure was implemented to reduce animal stress during sample collection.

Study area

A nationwide cross-sectional study was conducted from October 2021 to March 2022. The selected areas were eight divisions of Bangladesh: Barisal, Chattogram, Dhaka, Khulna, Mymensingh, Rajshahi, Rangpur, and Sylhet divisions (Fig. 1). From these eight divisions, different sampling areas were selected based on the abundance of animal and land species by observation.

Sample size calculation

Cross-sectional surveys are typically used in Low- and Middle-Income countries (LMIC) like Bangladesh to get information on disease prevalence. It’s mainly because of the lack of a surveillance system, resource limitation, and scarcity of samples. There has never been a study done on the seroprevalence of the LSD virus in Bangladesh. So, as a 50% prevalence with a 95% confidence interval and 0.05% precision level using online epitools Ausvet the sample size is 385 (Sergeant 2018).

However, we randomly selected 736 individual cattle, and blood samples were collected. The animal was chosen since it was not previously immunized but appeared/suspected to be infected with the LSDV virus. Additionally, complete relevant data on gender (male and female), age, vaccination history, breed (native and cross), lactation status, etc. were collected with a pre-structured questionnaire. Age was calculated in months, and there were four age categories: 0–12 (calf), >13 (bull), 12–24 (heifer), and >24 (cow). Simple vacuum tubes were used to draw blood samples from cattle's jugular veins, and the serum was separated by centrifuging the samples blood at 3000g for 10min and for serological analysis, the serum samples were stored at -20°C.

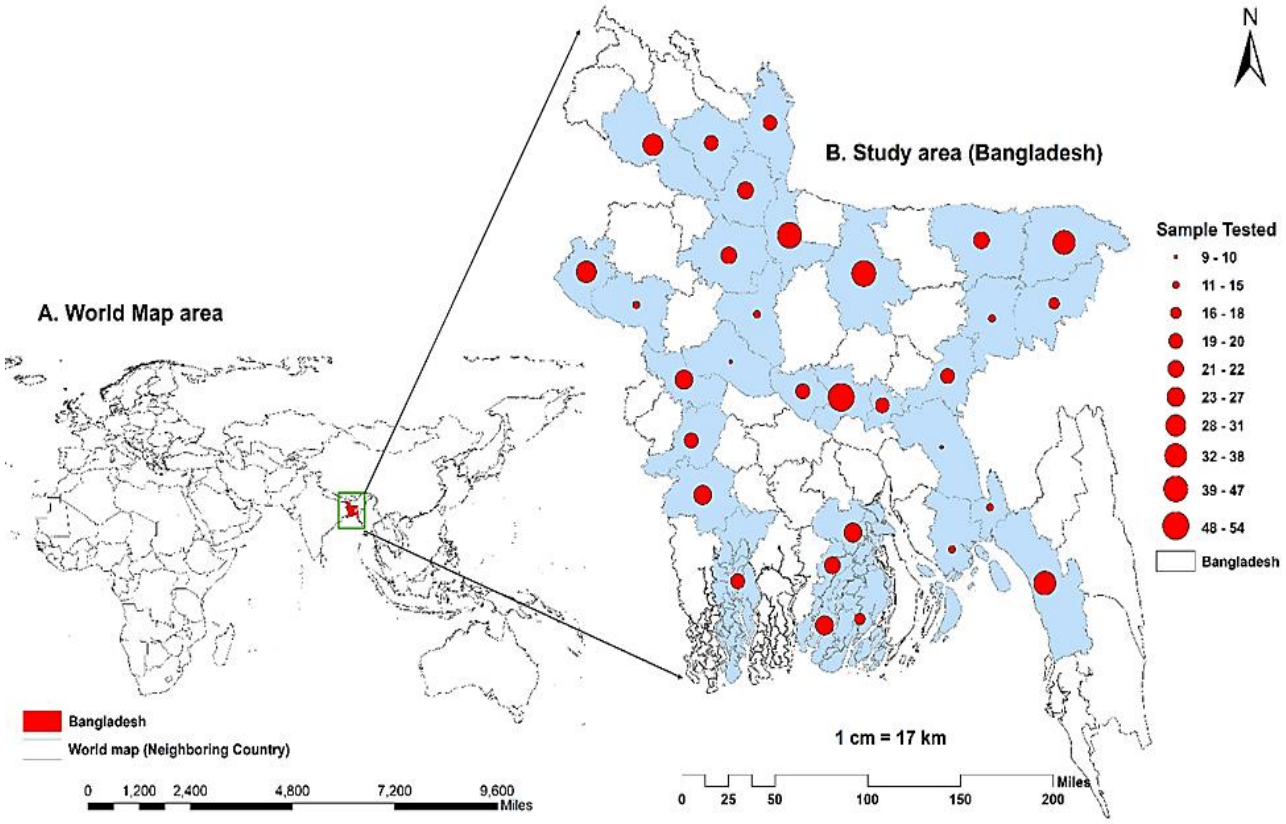

Fig. 1: Map of Bangladesh showing study location and sampling areas. The map was created using ArcGIS 7.0.

Serological examination using ELISA

In accordance with the manufacturer's recommendations, the serum samples were tested using ID Screen® Capripox double antigen multi-species (ID.vet, Grabels, France) to look for antibodies against LSDV. ELISA microplate was used to test the optical density at 450nm. For each sample, the sample/positive (S/P) ratio was calculated by the following formula: S/P% = (OD of each sample – OD of negative control) / (OD of positive control – OD of negative control) × 100. The samples with OD <30% were considered as negative, while those ≥30% were considered as positive.

Statistical analysis

Laboratory results and collected data were initially coded for analysis in a Microsoft Excel spreadsheet. All statistical analysis was completed using SPSS version 25. Prevalence was calculated as the proportion of infected individuals among the sampled individuals. In univariable analysis, the connections between putative determinants and LSD were examined using the Chi-square ($\chi 2$) test and regression analysis. The multivariable logistic regression analysis included explanatory variables with p-values less than 0.20 in univariate analyses. Backward elimination was employed in multivariable analysis. ArcMap 10.8 was used to construct the research area map.

RESULTS

Overall seroprevalence of LSD in cattle

The overall seroprevalence of LSD was estimated at 24.59% (95% CI: 21.62-27.83). The highest prevalence was 30.4% in Khulna and Mymensingh, whereas the lowest prevalence recorded in Barisal was 16.3%. Yet, the same seroprevalence of 25% was shown in both the Chattogram and Sylhet divisions. The clinical prevalence ranges from 18.5% to 29.4% in Rajshahi, Rangpur, and Dhaka divisions (Table 1). The seroprevalence was observed lowest in the male animals, 16.6% (95% CI: 12.95-20.94), than in females (Table 2). The prevalence was estimated higher for heifers (52.1%) and crossbred animals (39.2%) in the current study (Table 3).

Risk factors of LSD occurrence in cattle

According to the univariable analysis, the odds ratio for calves and heifers to have the disease was 1.04 (CI: 0.61-1.79) and 4.97 (CI: 2.12-8.50) times greater than for cows (Table 3). So far, crossbred animals (OR-2.71, CI: 1.90-3.80) are significantly ($P \leq 0.001$) higher in risk of having LSD than deshi (indigenous) cattle (Table 2). As all the variables in the current study gained $P<0.20$, we included all of them for the multivariable analysis. In the final multivariate logistic regression model with backward elimination, two variables, namely breed and animal category, were retained as significant variables with $P<0.05$ (Table 4). The study suggested that animals that frequent graze with other animals in communal practice (92.27%) have a higher risk than those that stall feed (7.73%) (Table 5). However, the wet season has a higher (64.09%) incidence compared to the dry season (35.91%) (Table 5). Compared to the fourth lactation stage (3.85%), the first lactation stage (73.08%) has a greater prevalence of LSD. However, the second and third results were comparable (Table 6).

Table 1: District-wise prevalence of Lumpy Skin Disease of Cattle in Bangladesh

Division	District	n/N	Prevalence% (95% CI)	Fisher's Exact Test/ χ^2	p-value
Mymensingh	Jamalpur	9/45	20.00% (9.58-34.60)	4.53	0.03
	Mymensingh	19/47	40.43% (26.37-55.73)		
	Total	28/92	30.4% (21.97-40.46)		
Sylhet	Habiganj	2/15	13.33% (1.66-40.46)	3.37	0.34
	Moulavibazar	4/17	23.53% (6.81-49.90)		
	Sunamganj	4/22	18.18% (5.19-40.28)		
	Sylhet	13/38	34.21% (19.63-51.35)		
	Total	23/92	25.00% (17.28-34.73)		
Dhaka				1.75	0.42
	Manikganj	7/19	36.84% (16.29-61.64)		
	Narayanganj	7/19	36.84% (16.29-61.64)		
	Dhaka	13/54	24.07% (13.49-37.64)		
	Total	27/92	29.3% (21.02-39.33)		
Chattogram	Brahmanbaria	6/19	31.58% (12.58-56.55)	11.38*	0.02
	Cumilla	1/10	10.00% (0.25-44.50)		
	Feni	2/14	14.29% (1.78-42.81)		
	Noakhali	0/14	0.00% (0.00-23.16)[a]		
	Chattogram	14/35	40.00% (23.87-57.89)		
	Total	23/92	25.00% (17.28-34.73)		
Khulna	Jashore	8/27	29.63% (13.75-50.18)	2.36	0.50
	Jhenaidhah	7/20	35.00% (15.39-59.22)		
	Kushtia	5/25	20.00% (6.83-40.70)		
	Khulna	8/20	40.00% (19.12-63.95)		
	Total	28/92	30.4% (21.97-40.46)		
Rajshahi	Bogura	2/22	9.09% (1.12-29.16)	2.66*	0.66
	Chapainawabganj	7/31	22.58% (9.59-41.10)		
	Pabna	1/9	11.11% (0.28-48.25)		
	Sirajganj	3/15	20.00% (4.33-48.09)		
	Rajshahi	4/15	26.67% (7.79-55.10)		
	Total	17/92	18.5% (11.87-27.61)		
Rangpur	Dinajpur	6/31	19.35% (7.45-37.47)	6.85*	0.07
	Gaibandha	5/22	22.73% (7.82-45.37)		
	Kurigram	1/19	5.26% (0.13-26.03)		
	Rangpur	8/20	40.00% (19.12-63.95)		
	Total	20/92	21.7% (14.54-31.21)		
Barishal	Barguna	6/26	23.08% (8.97-43.65)	8.18*	0.03
	Jhalokhati	2/22	9.09% (1.12-29.16)		
	Patuakhali	6/18	33.33% (13.34-59.01)		
	Barishal	1/26	3.85% (0.10-19.64)		
	Total	15/92	16.3 (10.14-25.17)		

Superscript Means One-sided 97.5% Confidence Interval; N = Total tested, n = positive case, CI = Confidence interval; *Superscript means Fisher's Exact Test, where at least 20% of cells have an expected count less than 5.

Table 2: Association of Lumpy Skin Disease with breed and sex of cattle from the univariable logistic regression analysis

Variables	No of animal tested	No of animal positive	Prevalence (%)	Odds Ratio (95% Confidence Interval)	P-value
Animal type					
Crossbred	199	78	39.2	2.71 (1.90 - 3.80)	<0.001
Deshi/Indigenous	537	103	19.2	Ref	
Sex					
Female	404	126	31.2	2.283 (1.58-3.26)	<0.001
Male	332	55	16.57	Ref	

Table 3: Risk factors associated with Lumpy Skin Disease and category of the animals from the univariable logistic regression analysis

Animal Category	No of animal tested	No of animal positive	Prevalence (%)	Odds Ratio (95% Confidence Interval)	P-value
Calf	236	44	18.6	1.04 (0.61-1.79)	<0.001
Bull	211	36	17.1	0.94 (0.54-1.64)	
Heifer	144	75	52.1	4.97 (2.12-8.50)	
Cow	145	26	17.9	Ref	

Table 4: Risk factors associated with Lumpy Skin Disease from the Multivariable logistic regression analysis

Variables/Animal Type	Odds Ratio	95% Confidence Interval	P-value
Crossbred	2.67	1.82 - 3.93	<0.001
Deshi/Indigenous	Ref		
Calf	1.16	0.67 - 2.00	<0.001
Bull	1.20	0.67 - 2.14	
Heifer	5.50	3.16 - 9.56	
Cow	Ref		

Table 5: Effects of grazing practice and seasons on Lumpy Skin Disease occurrence in cattle

Variables	Percentage (%)	95% Confidence Interval (CI)
Grazing Practice		
Communal	92.27	87.44-95.34
Stall	7.73	4.12-12.99
Seasons		
Wet	64.09	56.87-70.72
Dry	35.91	29.28-43.13

Table 6: Seroprevalence of Lumpy Skin Disease in different lactation stage

Lactation stage	No of Lactating Animal	Prevalence (%)	95% Confidence Interval (CI)
1st	19	73.08	53.92-86.3
2nd	3	11.54	4.0-28.98
3rd	3	11.54	4.0-28.98
4th	1	3.85	0.68-18.89

DISCUSSION

LSD is a transboundary infectious viral illness of cattle that results in significant monetary losses. Underestimating the vector and route of transmission is a major problem for LSDV spreading swiftly in a disease-free nation. They could be used as tools of economic bioterrorism and present a threat to international trade. Following its initial outbreak in 2019, the LSDV expanded quickly throughout Bangladesh due to a significant amount of animal mobility. It used Eid ul-Azha, the largest festival for animal sacrifice in Islam, as a transmitting opportunity. In 2019, the virus began to spread to other regions of Asia, including China, India, and Pakistan (DLS 2019). An important source of LSDV transmission in Bangladesh is animal transportation from neighboring afflicted nations.

Bangladesh is surrounded by India and Myanmar and shares international trade with them. Uncontrolled livestock trade from neighboring country borders has a high chance for the distribution of transboundary disease. A large number of cattle populations enter through land ports during the Eid ul Azha festival, mainly from India. The agricultural nature of studies areas contains a high concentration of transmission vectors. This study used the first antibody ELISA test kit for Capripoxviruses that was commercially available to examine the serological prevalence and factors associated with seropositivity of the virus that causes lumpy skin disease in the eight divisions of Bangladesh. This study is the first to ever document the seroprevalence of LSDV in cattle in Bangladesh. The author's observation during the collection of samples and data found most Bangladeshi farmers kept their cattle in herds that shared a water source and grazing area with non-bovine animals. There are flies or mosquitoes everywhere on the farm. There is absence of vaccination history against lumpy skin disease virus. All samples were taken from animals with clinical suspicions (most of these animals contained fever, edema, skin nodules, or lameness). Despite the possibility of clinical signs being mistaken for ephemeral fever and hypoproteinemia.

We found an overall animal-level seroprevalence of 24.59%. This is comparable with the African region. Other investigations confirmed similar animal-level prevalence and estimated a seroprevalence of 23-31% in various agroecological zones in Ethiopia (Gari et al. 2012) and Molla et al. reported a seroprevalence of 25.4% in Ethiopia (Molla et al. 2018). One study in Egypt reported a lower seroprevalence of 19.5% (Selim et al. 2021) and another study from Uganda showed a much lower frequency (8.7%) (Ochwo et al. 2019). Then again, the highest seroprevalence was recorded in Khulna and Mymensingh (30.4%) division, followed by Dhaka (29.3%) and Sylhet and Chattogram (each 25%). A previous outbreak study in Chattogram found a 10% prevalence in the cattle population, which is lower than our findings in Chattogram (Hasib et al. 2021) . One study reported that 71% of the sera from vaccinated livestock tested positive in an ELISA analysis based on the recombinant P32 antigen (Tursunov et al. 2024). This variation may be due to population density, sampling period, abundance of vector and dissimilarity of climate. Nevertheless, now it's look like LSD is widespread in Bangladesh.

Among the risk factors analyzed, breed, sex, and age have a significant relation with LSD sero status in Bangladesh. Indigenous cattle are less susceptible compared to crossbred (OR=2.71). This statement goes with Albera et al., who stated crossbred animals are more susceptible than indigenous ones. Some other studies also suggested that *Bos taurus* is more prone to LSD (Abera et al. 2015). A descriptive investigation involving four types of cattle in this study found a statistically significant correlation between the serostatus of LSD. However, the incidence of young animals is larger than that of calves. This may indicate uncommon exposure and the existence of passive maternal immunity. According to our study, the rate of attacks on nursing calves was lower than that of young animals (Abera et al. 2015). A different explanation for the lower seroprevalence found in calves in this investigation could be that they are less vulnerable to fly bites, as previously noted (Troyo et al. 2008). The fact that the calves, who were reared in a homestead with fewer insect vector activities, had the lowest prevalence was another factor that may be put up. The study found that adults have a high seroprevalence (52.1%), and this may be due to exposure to field viruses. The presence of a significant association between the sex of the animals and the seropositivity of LSD was found in the current investigation. It seems that female animals (31.2%) are at higher risk for LSD compared to male animals (16.6%). A similar finding, like the females being twice more likely to be seropositive to LSD, was reported from Uganda (Ochwo et al. 2019). Contrarily, male cattle had a larger cumulative incidence than female cattle, which could be attributed to stress-related tiredness and fatigue rather than a biological cause (Gari et al. 2012). Another explanation was the fact that many male animals, particularly those utilized for heavy labor, were draft oxen, which would increase

sensitivity. The same investigators also noted that draft oxen cannot effectively defend themselves against biting flies while chained in the yolk, and the beat scratches on their skin caused by tilling may lure biting flies that could potentially spread the LSDV infection. The absence of a statistically significant association between lactation and seropositivity to LSD was observed in the current investigation. Regarding lactation, a decline in prevalence was seen as the number of lactations increased. Some studies reported that animals that have frequent contact with other animals in grazing land or water sources have a higher risk for spreading disease (Gari et al. 2010; Hailu et al. 2014; Rehman et al. 2024). In contrast, another study also stated frequent movement of animals has two times lower odds of being infected by LSDV than animals herded separately (Molla et al. 2018). According to the current study, LSD risk is highest during wet seasons. Epidemiologic findings also revealed that LSD incidence varies with the vector population density, peaking in warm weather and falling off in the dry season (FAO 2013). However, our study finding is consistence with previous findings by Hasib et al. (2021).

Conclusion

This study is the first to document seroprevalence in Bangladesh, and this cross-sectional study shows that lumpy skin disease significantly impacts the livestock sector. This research finding will be useful for introductory knowledge about LSDV in Bangladesh. According to overall LSD prevalence, LSD is presently thought to be endemic in Bangladesh. Additionally, it can be useful for monitoring the condition and reducing its risk factors. Before establishing a controlling and preventative plan, more research is required to determine the disease's state.

Author's contribution

Ruhena Begum: Data curation, formal analysis, methodology, writing-original draft, writing review & editing. Md Bashir Uddin: Conceptualization, formal analysis, methodology, investigation, writing-original draft, supervision, writing review & editing. Md. Mahfujur Rahman: Formal analysis, methodology, supervision, writing-original draft, supervision, writing review & editing. Milton Roy: Data curation, formal analysis. Asikur Rahman: Data curation, formal analysis. Saad Muhammad Rafe-Ush-Shan: Data curation, formal analysis. Md. Shahidur Rahman Chowdhury: Formal analysis, software. Hemayet Hossain: Formal analysis, software, writing-original draft. Jahangir Alam: Methodology, writing-original draft, writing review & editing. Ho-Seong Cho: Formal analysis, writing-original draft, writing review & editing. Md. Mukter Hossain: Conceptualization, formal analysis, fund acquisition, investigation, methodology, project administration, supervision, writing-original draft, writing review & editing. All authors have read and approved the final version of the manuscript.

Conflicts of interest

The researchers declare that no financial or commercial relationships that might be seen as having a conflict of interest existed while the research was being conducted.

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