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Research article

Sero-prevalence and PCR identification of lumpy skin disease virus in cattle at Mymensingh district of Bangladesh

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Abstract

Lumpy skin disease (LSD) is a transboundary, an infectious viral disease of cattle causing substantial economic losses due to skin damage and decreased animal production. This study aimed to determine the serological prevalence and molecular detection of lumpy skin disease virus (LSDV) from cattle in Mymensingh district, Bangladesh with assessment of risk factors. A total of 200 samples consists of whole blood (184), tissue (10) and pus (6) were collected from three different Upazilas of Mymensingh and tested using indirect enzyme linked immunosorbent assay (ELISA) kit. The DNA was extracted from whole blood using a DNA extraction kit and kept at -20°C until further analysis. Extracted DNA was used in a PCR test to detect lumpy skin disease virus (LSDV). Overall, the sero-prevalence of LSD among cattle in Mymensingh was 28.26% (52/184) (CI: 19.36-38.61). Fulbaria Upazilas showed the highest prevalence of 30% (24/80) compared to Mymensingh Sadar 24% (12/50) and Muktagacha 29.63% (16/54). Moreover, the risk of getting LSD infection was lower among cross breed (OR=0.27, CI: -0.1-0.75) and female animals (OR=0.39, CI: -0.15-1.03). Furthermore, freely grazing animal 29.17% (CI: 12.61-51.09) and young group 72.73% (CI: 39.03-93.98) of animal showed risk in having lumpy skin disease. LSD virus was detected in the sero-positive blood sample (100%), tissue sample (90%), and pus/edema fluid discovered (83.33%) after amplification of a 192 bp DNA fragment. The findings of this study will be helpful in creating efficient methods for identifying and managing LSD in Bangladesh and avoiding the financial losses connected with dairy production.

Keywords: Cattle, Lumpy skin disease, Mymensingh, Prevalence, Risk factors

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INTRODUCTION

Lumpy skin disease (LSD) is a highly infectious viral disease of cattle and buffaloes having significant economic consequences for the livestock industry, first found in Zambia in 1929 which was epidemic during that time (Gari et al., 2012). Initially the disease is characterized by fever, lacrimation, nasal discharge followed by the characteristic nodular lesions on the skin. LSD is a disease with high morbidity and low mortality rate (Kitching and Taylor, 1985) affecting almost all ages and breeds of cattle. The recent outbreaks of lumpy skin disease were discovered in many countries including the People's Republic of China, Georgia, Russia, and Bangladesh (Giasuddin et al., 2020; Badhy et al., 2021). International alarm has been raised by the lumpy skin disease's recent geographic expansion. This transboundary disease was added to the list of notifiable diseases by the World Organization for Animal Health because of its potential for rapid spread and substantial economic losses (Tuppurainen and Oura, 2012).

Lumpy skin disease (LSD) is regarded as a major economically significant illness of cattle with major effects on livelihoods and food security, especially among smallholders. It affects cattle and is brought on by the lumpy skin disease virus (LSDV). The disease's economic costs are linked to reduced milk production, decreased traction power, weight loss, poor growth, abortion, infertility, and skin damage. In animals with mouth and respiratory tract lesions, pneumonia is a common outcome. The indirect economic losses from the LSD are caused by trade restrictions and livestock movement on a national and international scale (Khan et al., 2022).

The disease caused by the DNA virus belongs to the genus Capripoxvirus and family Poxviridae, subfamily Chodopoxvirinae. LSDV Closely resembles and shares an antigenic relationship with the sheeppox and goatpoxin viruses (Quinn et al., 2002; Radostits et al., 2007; Babiuk et al., 2008). The only known serotype of LSDV is highly stable and remains viable in the environment and dried scabs for several weeks at room temperature. The virus can live in dry crust for up to 35 days, necrotic skin nodules for up to 33 days, and air-dried hides for at least 18 days (WOAH, 2021).

Insects that bite are thought to be the main source of LSDV transmission. During certain outbreaks, the virus was found in Aedes, Culex, and Ixodid ticks as well as mosquitoes (Chihota et al., 2003; Tuppurainen et al., 2011). The occurrence of disease is greater during warm, rainy weather. During the dry season, incidence declines, which may be related to a decline in insect vectors (occurrence/numbers). Direct and indirect contact could be minor sources of infection (e.g., through infective saliva contaminated feed and water). Since the LSD virus can remain for long periods of time in both the milk of lactating cows and the semen of infected bulls, these are additional possible transmission channels (Irons et al., 2005; Osuagwuh et al., 2007).

For many years, lumpy skin disease was only found in Africa, where it caused a few severe pandemics that threatened food security and subsequently increased poverty in a number of the affected nations. It first appeared in Turkey in 2000 and has since spread to several other Middle Eastern nations. The sickness was recorded in various European nations between 2015 and 2016, including Bulgaria, Macedonia, Serbia, Kosovo, Montenegro, and Albania. LSD was first identified in Bangladesh between July and September 2019 (DLS, 2019). It first appeared in the Chattogram district and quickly spread to the Dhaka, Gazipur, Naryanganj, Satkhira, and Pabna districts. Although the epidemiology unit data indicated that the Chattogram province had a particularly high incidence of LSD compared to other regions at the beginning of the outbreak, this disease has since been reported intermittently more or less everywhere in the nation (Badhy et al., 2021; Das et al., 2021). Lumpy skin disease is an important vector-born disease of cattle and has recently spread in Mymensingh, Bangladesh. LSD causes considerable

devastating economic losses mainly due reduced losses milk production remarkably, abortion and infertility, emaciation permanent hide damage, and disruption in the trade of cattle and their Products (Das et al., 2021).

There is few research on sero-prevalence of LSD disease and related factors that lead to the spread of the disease, even though the disease has a considerable economic impact, particularly in pastoral areas of the distant region (Dubie et al., 2022). Various methods have been used for serological investigation of LSDV. These tests include a skin hypersensitivity test, virus neutralization test (VNT), immunoperoxidase monolayer assay (IPMA) or indirect fluorescent antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) (Haegeman et al., 2020). In this study the commercially available ELISA test kit was used for the detection of LSD in naturally infected cattle. The recent and unprecedented spread of LSD in Bangladesh and several other countries has highlighted the need for better research efforts into this rapidly emerging pathogen. The present study was conducted to investigate the seroprevalence, molecular detection of LSD virus and identification of risk factors of the disease.

MATERIALS AND METHODS

Study area and period

The Mymensingh region is situated between latitudes 24°15' and 25°12' north and 90°04' and 90°49' east. The average annual temperature is 0.99% higher than Bangladesh's averages at 28.73°C (83.71°F). Mymensingh generally has 114.59 wet days (31.39% of the time) and receives about 70.7 millimeters (2.78 inches) of precipitation yearly, which is favorable for the vector of LSDV. Among the thirteen Upazilas of Mymensingh district, three Upazilas were selected as study areas (Figure 1) because of its animal population and other pre-selected criteria. This area was chosen as an LSD hotspot. From January 2022 to August 2022, this study was carried out.

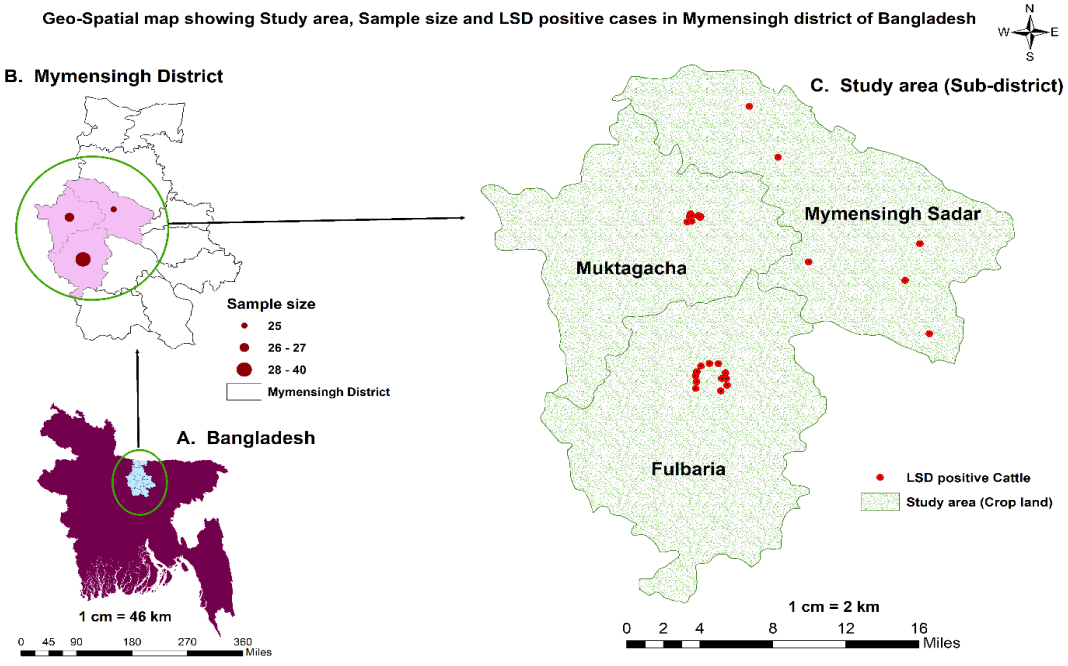

Figure 1 Geo-spatial map showing study area, sample size and LSD positive cases from where individual cattle samples were collected. Shape file was extracted from DIVA-GIS using Geographical information system (GIS) to develop the map with ArcMap 10.8 (ArcGIS, ESRI, USA)

Sample size and sampling technique

A simple random sampling technique was applied to sample the animals from different Upazilas in Mymensingh. The study area was stratified into three Upazilas (Sadar, Fulbaria, and Muktagacha) according to the availability of samples and history of outbreaks. For every stratified zone (Upazilas), samples were randomly selected according to the history of the outbreak, the availability of animals and their restrainers, well equipped restraint tools, working personnel, etc. A field sample collection was chosen to reveal the actual disease prevalence of LSD. As there has never been a study done on the seroprevalence of the LSD virus in the study area. So, as a 50% prevalence with 95% confidence interval and 0.05 precision level using online epitools for sample size calculation (Sergent, 2018). Samples were also collected from the surrounding area where the disease outbreak was found. A total of 200 animals were selected where 184 animals (both from diseased and healthy) were chosen for blood collection. The rest of the samples (16) were skin scrapings, ruptured nodules, pus from affected area, fluids of brisket edema and all of the animals were suffering from LSD or previously affected with the disease leaving the above-mentioned lesions. A pre-structured questionnaire was supplied to the owner of the animal regarding the collection of some information like animal ID, age, breed, sex, body weight, surrounding outbreak history, fly and mosquito controlling system, etc.

Blood Collection and serum separation

A 10-ml blood sample was taken from the jugular vein after the animal had been handled and restrained properly. Five milliliters (5 ml) of blood were kept after collection in the EDTA vacutainer blood collecting tube, while 5 ml of the remaining blood were transferred to the vacutainer blood collecting tube without EDTA. According to the questionnaire form, each tube was assigned a mark. The sample was immediately kept at a temperature of 4°C. Following that, samples were sent to Sylhet Agricultural University's postgraduate laboratory, which is part of the department of medicine, for the separation of serum. The vacutainer blood collecting tube (without EDTA) was placed into a high-speed micro centrifuge (Model D3024R, DLAB Scientific Inc., USA) at 5000 RPM for 10 minutes. The separated serum was collected into Eppendorf tube and stored in -20°C temperature.

DNA extraction from blood samples

The phenol, chloroform, and isoamyl alcohol techniques were used to extract genomic DNA. In brief, the blood samples were homogenized by repeatedly inverting the tubes, and 400 µL of blood samples were added to an Eppendorf tube containing 700 µL of de-ionized water. The mixture was then centrifuged at 10,000 rpm for 10 minutes. After removing the supernatant, again 700 µL of deionized distilled water was added, and it was then homogenized using a vortex machine. Subsequently, after centrifuging the mixture at 10,000 rpm for 10 minutes, the supernatant was discarded. After discarding the supernatants, 200 µL lysis buffer and 2 µL Proteinase k were added to the tubes, homogenized by inverting the tube multiple times, and then incubated overnight at 37°C. After the incubation period was completed 100 µL of 4.5 M sodium chloride (NaCl) was added to the tubes, which were then homogenized by inverting several times. The tubes were then filled with 225 µL chloroform and shaken for 10 minutes. The mixture was then centrifuged for 10 minutes at 14000 rpm. The tube had three layers, therefore around 200 µL of the aqueous phase/ upper phase was moved into another sterile Eppendorf tube. The 200 µL isopropanol was poured into the tube and stirred with a vortex or by inverting it numerous times. Then the samples were centrifuged for 15 minutes at 14000 rpm, removed the supernatant, and added 500 µL of 70% ice-cold ethanol to the tube, maintained at room temperature at 15 minutes and

centrifuged for 15 minutes at 14000 rpm. Removed the supernatant and dried the tube containing the particle for 10 minutes at room temperature. After that, the DNA pellet was re-suspended in 100 µL of TE buffer (1%). The tube was then incubated overnight at 37°C. Pipetting the extracted DNA for homogenization, and the isolated DNA was stored at -20°C.

DNA extraction from tissue samples

Monarch® Genomic DNA Purification Kit, UK, was used to extract DNA from samples in accordance with the manufacturer's instructions. In short, the skin lesions were taken and was cut into little pieces using a sterile blade in a sterile way. They were macerated with a mortar and pestle after being weighed. Then 5 mL of PBS was added and mixed well and added 1 ul proteinase K and 3 µL of RNase A and mixed immediately by vortexing. 100 µL of cell lysis buffer was added, followed by immediate full-speed agitation. 400 µL of gDNA binding buffer were added after 56° C of incubation, and they were mixed for 5–6 seconds. A gDNA purification column that was already put into a collecting tube received the lysate next. Centrifuged the mixture for 3 minutes at 1000 rpm. Later, discard the flow through and collection tube. Transferred column to a new collection tube and added 500 µL gDNA wash buffer. Closed the cap and centrifuged immediately for 1 minute. Repeated this step again. Finally, the DNA was eluted using 50 µL elution buffer and stored at –20 °C until further use.

Serological examination using ELISA

In accordance with the manufacturer's recommendations, the serum samples were tested using ID Screen® Capripox double antigen multi-species (IDVet, Grabels, France) to look for antibodies against LSDV. In a nutshell, each well received 40 µL of dilution buffer (supplied kit). In wells A1 and B1, a positive control (10 µL), a negative control (10 µL), and 10 µL samples were introduced to the remaining wells. The ELISA plate was then incubated for 45 minutes at 37°C. About 300 µL of wash solution, made by diluting the wash concentrate (20X) with double-distilled water (supplied kit), was used to hand wash each well three times. Each well received around 100 µL of previously made single-strength conjugate, which was then incubated for 30 minutes at room temperature. Following another wash, 100 µL of substrate solutions were added to each well, and they were then incubated for 15 minutes in a dimly lit room. The reaction was stopped by adding stop solution (100 µL). The sensitivity and specificity of the ELISA test kits used in this study were 91% and 99.7% respectively. ELISA microplate was used to test the optical density (OD) at 450 nm. For each sample, the percentage of OD of sample/OD of positive control (S/P) was calculated by the following formula:

$$\frac{S}{P}\% = \frac{(\text{OD sample} - \text{ODNC})}{(\text{ODPC} - \text{ODNC})} \times 100$$

The samples with an OD <30 % were considered as negative, while those ≥30% were considered as positive.

PCR amplification of LSDV

Extracted DNA was evaluated by the polymerase chain reaction (PCR) test as previously described (Ireland and Binepal, 1998). In a final volume of 25 µl reaction was conducted using 5 µl of DNA sample, 12.5 µl of 2x master mix (Add Bio Inc, South Korea), 1 µl of each primer (10 pmol), and 5.5 µl of DNase-free water. Primers and thermal profile are presented in Table 1. The generating PCR products of 192 bp and run through 1.5% of agarose gel and visualized in UV trans-illuminator after electrophoresis.

Table 1 Specific primer and thermal profile performed in this study

Primer	Primer Sequence 5'-3'	Amplicon Size	Reference
Forward	5'-TCCGAGCTCTTTCCTGATTTTTCTTACTAT-3'	192 bp	(OIE, 2022)
Reverse	5'-TATGGTACCTAAATTATATACGTAAATAAC-3'		

Thermal profile: 2 minutes at 95°C; then 45 seconds at 95°C, 50 seconds at 50°C and 1 minute at 72°C (34 cycles); 2 minutes at 72°C and hold at 4°C until analysis.

Agar gel Electrophoresis

Agarose powder (ADD BIO INC., Korea) was combined with 1X TAE buffer to make a 100 ml 1.5% agarose solution. The solution was then chilled to between 50 and 55 °C and 5 µl of safe gel stain dye was added. After carefully blending the solution (to prevent water bubbles), gel was cast in a gel plate with a comb put in one of the poles. After the gel had solidified, the gel plate was loaded into the gel electrophoresis tank to the point where the gel was submerged to a depth of 2 to 5 mm. The gel hole was loaded with samples. A 100bp DNA ladder (AddBio Inc. Ltd., Daejeon, Korea) was run concurrently to determine the band sizes. The gel was run for 30 minutes at 100 volts, and the results were recorded using a gel documentation system and UV trans-illumination (Bio-Rad Laboratories Inc., CA. United States).

Statistical Analysis

All the data were collected and sorted from a recorded pre-structured questionnaire form. Then the data were written and recorded to Microsoft Excel 2013, and the analysis was assessed with SPSS 26.0 (IBM SPSS Statistics version 26.0). The precision of these estimates was ensured by calculating a 95% confidence interval for the proportions. Applying 2X2 table along with determined measures of association by using Chi–square test to determine the significance of differences in prevalence among animal species, age, sex, breed, etc., the odds ratio (OR) was also calculated, and P values ($P \leq 0.05$) were regarded as significant. The term "prevalence" refers to the amount of disease in a known population at a designated time, without distinction between old and new disease. The prevalence of different diseases was calculated using the following formula:

$$\text{Prevalence} = \frac{(\text{Number of current cases (new and preexisting) at a specified point in time})}{(\text{Population at the same specified point in time})} \times 100$$

Study area mapping was structured by ArcGIS desktop 107_167519.

RESULTS

Clinical findings

The infected animals initially had a fever (up to 41.5 °C) that continued for 3–4 days, which was followed by leg edema, swollen lymph nodes, lameness, and anorexia. The most noticeable clinical finding occurred just after the febrile period, when skin nodules started to form on the body's surface. The nodules were spherical, slightly elevated, well-circumscribed, firm, painful, and between one and three centimeters in size (Figure 2A and B). A deep wound was made when some of the nodules ruptured. Secondary bacterial infections frequently infiltrated wounds, causing significant suppuration and sloughing. Some of the nodules shrank, while in others, skin necrosis led to hard, elevated patches (sit-fasts) that were obviously separated from the surrounding skin. Only a small percentage of animals showed nasal discharge. Except for a few, most of the animals healed, but others perished from severe lesions, anorexia, and emaciation.

Figure 2 Generalized lumpy skin disease (LSD) nodules (A) and skin lesions (B) in cattle

Sero-prevalence of LSD in cattle

Overall, sero-prevalence of LSD in the cattle from Mymensingh district was 28.26% (52/184) (CI: 19.36-38.61). However, the highest sero-prevalence was found in Fulbaria Upazilas 30% (24/80) (CI: 16.56-46.53), compared to Mymensingh Sadar 24% (12/50) (CI: 9.36-45.13) and Muktagacha 29.63% (16/54) (CI: 13.75-50.18) (Table 2, Figure 3). A chi-square test for independence with α= 0.05 was used to assess whether the study area was related with disease. The chi-square test was statistically insignificant, $\chi 2$ (2, N=92) =0.308, p>0.05, with cramer's V=0.058, (Table 1) indicating very weak relationship. As seen in Table 2, the animals from the Fulbaria were more likely to be affected by disease than the other area.

Table 2 Sero-prevalence of LSD among selected Upazilas of Mymensingh, Bangladesh

Location (Study area)	Test positive (Total tested)	Prevalence % (95% CI)	Chi-square test	P-value
Fulbaria	24 (80)	30% (16.56-46.53)	0.308	0.86
Mymensingh Sadar	12 (50)	24% (9.36-45.13)		
Muktagacha	16 (54)	29.63% (13.75-50.18)		
Total	52 (184)	28.26% (19.36-38.61)		

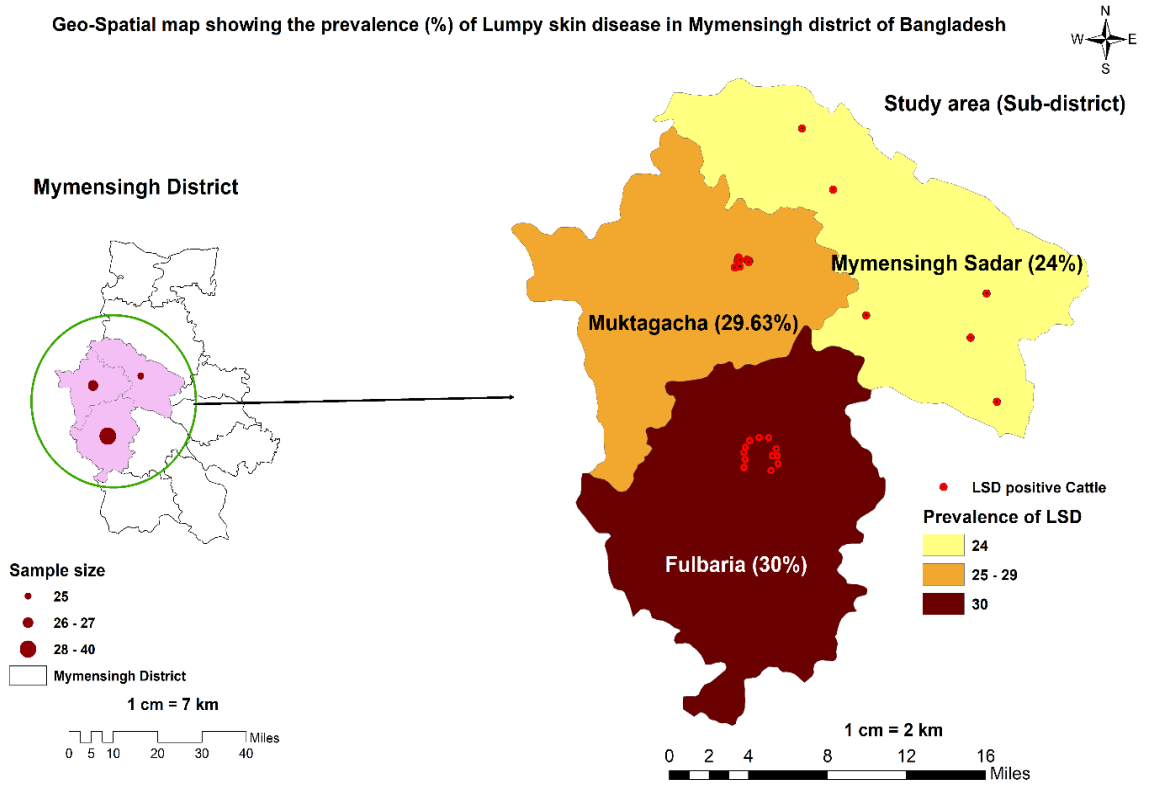

Figure 3 Prevalence of lumpy skin disease in cattle at Mymensingh district of Bangladesh. Shape file was extracted from DIVA-GIS using Geographical information system (GIS) to develop the map with ArcMap 10.8 (ArcGIS, ESRI, USA)

Molecular detection of LSDV

One pair of primers were used in PCR to detect the local LSDV strain in tissue, blood, and pus/edema sample. Due to its excellent specificity, this primer was utilized as a detection tool. The Lumpy skin disease virus was detected in 90% of tissue samples and 100% of sero-positive blood samples. Additionally, 83.33% of the pus/edema fluid tested positive for LSDV (Figure 4, 5).

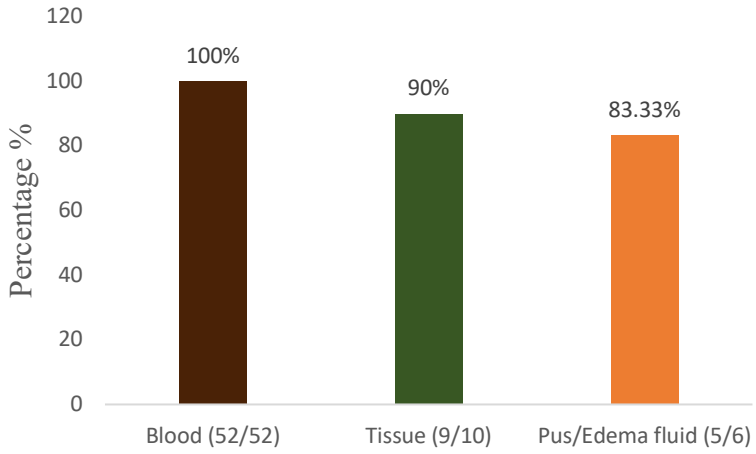

Figure 4 Percentage of PCR positive LSDV in cattle from different types of samples

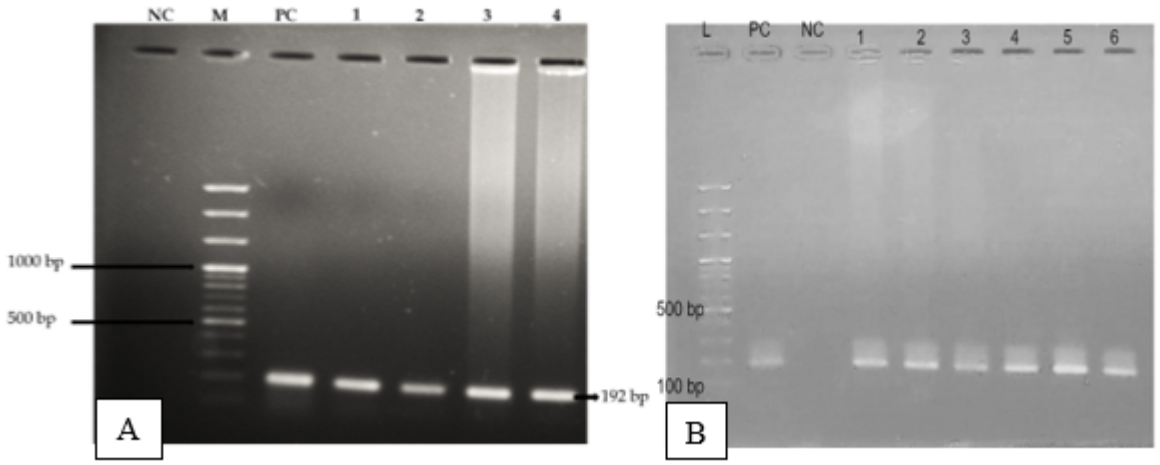

Figure 5 Agarose Gel Electrophoresis showing the 192 bp amplicon of the partial P32 gene, M: 100 bp DNA ladder, PC positive control, NC: negative control, A (1-4): LSD positive from blood samples; B (1-6): LSD positive from tissue and pus samples

Risk Factors associated with LSD

The sero-prevalence was observed in the lowest in male 18.60% (8/43) compared to female 36.73% (OR=0.39, CI-0.15-1.03). Analysis of different age group prevalence of Lumpy Skin Disease indicated that the difference in prevalence among the three age groups were relatively high in young group 72.73% (CI: 39.03-93.98) and lowest in calves 19.23 % (CI: 6.55-39.35). Also, adult showed prevalence was 23.64% (CI: 13.23-37.02). The prevalence of lumpy skin disease in different age group of cattle was highly significantly variant (P<0.05). However, the cross breed has a 0.27 times lower risk of LSDV compare to the local breed (OR=0.27, CI-0.1-0.75) (Table 3). Moreover, prevalence of freely grazing animal 29.17% (CI: 12.61-51.09) was higher than confined animal that was 19.35% (CI: 7.45-37.47) (Table 3).

Table 3 Statistical Analysis of risk factors associated with LSD in cattle

Category (Risk factor)	Animal tested	No. of positive (%)	Chi-square value (95% CI)	OR (95% CI)	P-value
Sex					
Male	86	16 (18.60)	3.713	0.39 (0.15-.03)	0.054
Female	98	36 (36.73)			
Breed					
Local	140	30 (21.43)	6.74	0.27 (0.1-0.75)	0.009
Cross	44	22 (50)			
Age					
Calves	52	10 (19.23)			
Young	22	16 (72.73)	12.354	N/A	0.002
Adult	110	26 (23.64)			
Rearing practice					
Confined	124	24 (19.35)	0.722	1.72 (0.49-6.0)	0.396
Grazing	60	17 (29.16)			

DISCUSSION

Clinically, all classical symptoms of LSD like fever, generalized skin nodules, enlargement of lymph nodes, anorexia, oedema of legs and lameness were observed in most of the cases we observed in the outbreak in India, Middle east, and European countries (Das et al., 2021). Current study found 28.26% seroprevalence of lumpy skin disease. This is close to the seroprevalence of 27% reported by Gari et al. (2012) but considerable higher than the seroprevalence recorded by others in West Wollega zone, Ethiopia (Abera et al., 2015). However, Molla et al. (2015) found similar results in central and north-western parts of Ethiopia 25.4% (Molla et al., 2015). These variations in animal level prevalence might be due to the differences of densities and efficiency of arthropod vectors, environment, cattle population, sampling period, and testing methods employed for the studies. Anyway, there is paucity of information regarding LSD seroprevalence in Bangladesh. The results were compatible with previous reports which concluded that the PCR could be used in the identification of LSDV in biopsy samples (tissue/skin, pus/edema fluid and blood samples).

Ninety percent of tissue samples and all seropositive blood samples contained the Lumpy skin disease virus. Furthermore, LSDV testing revealed that 83.33% of the pus/edema fluid was positive. Skin nodules and scabs provided more obvious bands indicating their high virus concentration. The outcomes were consistent with some previous study stated that the viral concentration in the nodules is significantly higher than the virus that is present in the bloodstream during viremia. As a result, skin nodules can be regarded as more suitable samples for LSDV detection. Scabs are also the best sample material since they are easy to gather, don't require local anesthetic, and endure lengthy transportation at various conditions. Surprisingly in our study, few tissue and pus samples did not show positive bands. This may be due to collection of specimens improperly and/or too late, inadequate samples, packed and shipped without maintaining proper cool chain as well as problems of processing samples in the laboratory. Analysis of the association between age and sero-prevalence for LSD found to be statistically highly significant among the three-age group; still, the sero-prevalence in calves is relatively lower (19.23%) as compared and young (72.73%). This might be due to transfer of passive maternal immunity and low frequency of exposure as well. Yet, all age groups were invariably susceptible to LSD infection in our study which agrees with the previous reports presented by others (Abera et al., 2015). The presence of significant association between sex and sero-positivity to LSD was observed in current study. Although female animal was more Sero-positive when compared to males. Female animals are usually kept long time by farmers while males are called at a younger age. Thus, the effect of sex may be an artifact of duration of exposure. These findings agree with the findings of other investigators (Abera et al., 2015; Dubie et al., 2022). Cross breed has higher prevalence of LSD than in local breed and statistically significant. This is in line with the previous report by Abera et al. (2015) but contrasts the study of Molla et al. (2015). This difference may be due to the immune system and management practices of the animal. Furthermore, grazing system have significant effect on LSD. Confined is less association compared to open grazing with LSD. This may be due to the abundance of vectors or mixing with other animals.

Lumpy Skin Disease was identified as the primary cattle health issue, was found to cause significant economic loss because it interfered cattle from gaining weight, permanently damaged their hides, reduced their ability to produce milk, had a protracted and debilitating clinical course, caused bulls to become infertile or even sterile, and caused pregnant cows to be aborted. Farmers and dairy farm owners in the study area were fully aware of LSD's somewhat increased morbidity and mortality, which might result in both direct and indirect economic losses. LSD also produced a moderate rise in morbidity and death. The findings will be useful in developing effective ways for diagnosing and controlling LSD in Bangladesh, as well as preventing the economic losses associated with dairy farming.

ETHICAL APPROVAL

The study was performed according to ethical guidelines of the ethical committee of the Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University (approval No. #AUP2020020).

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AUTHOR CONTRIBUTIONS

Saad Muhammad Rafe-Ush-Shan: Sample collection and processing, methods, formal analysis, writing of the article.
Ruhena Begum: Sample collection and processing, methods, formal analysis, writing of the article.
Milton Roy: Sample collection and processing, methods, formal analysis, writing of the article.
Muhammad Mujahidul Islam: Sample collection and processing, methods, formal analysis, writing of the article.
Hemayet Hossain: Formal analysis, software, writing of the article.
Md. Shahidur Rahman Chowdhury: Methods, formal analysis, writing of the article.
Md Bashir Uddin: Conceptualization, methods, project administration, writing - review and editing.
Md. Mahfujur Rahman: Methods, supervision, formal analysis, writing - review and editing.
Md. Mukter Hossain: Conceptualization, methods, supervision, project administration, fund acquisition, formal analysis, writing - review and editing.

CONFLICT 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 and/or publication of this article.

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