

Clinico-epidemiological investigation of Lumpy skin disease in Maheshkhali, Cox's Bazar



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A Clinical Report Submitted as per approved styles and Contents

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List of Abbreviation

List of Abbreviation	Elaboration
UUVH	Upazila Livestock Office and Veterinary Hospital
LSD	Lumpy Skin Disease Capri pox viruses
LSDV	Lumpy Skin Disease Virus
GDP	Gross Domestic Product
CI	Confidence Interval
OR	Odds Ratio
SD	Standard deviation
DLS	Department of Livestock Services
LRT	Likelihood ratio test
P	Probability
N	Number
et. al	and others
TP	Total protein
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
CaPVs	Capri pox viruses
OIE	Office International des Epizooties
e.g.	For example
DVM	Doctor of Veterinary Medicine

Abstract

Lumpy skin disease (LSD) is known as an economically significant viral disease of cattle, causing nodular eruptions in the skin, including the respiratory and gastrointestinal tract. This study was based on the current scenario of LSD for determining the alterations in haematological and serum biochemical values in Lumpy Skin Disease Virus (LSDV) infected cattle, as well as their clinical features, prevalence, and associated risk factors. Clinico-epidemiological data of total 231 cattle (where 64 cases were LSDV infected) were collected from Upazila Veterinary Hospital, Maheshkhali, Cox's Bazar. Blood samples were randomly collected from lumpy skin disease virus (LSDV) infected cattle and examine for haematological and biochemical parameters. The overall clinical prevalence of LSD in the study population was 26.84% (95% confidence interval [CI]: 0.21-0.33). Calves aged <1 year (OR = 10.42; P = 0.0069), cross breeds (in Sahiwal cross, OR = 3.5; P = 0.002; Holstein Friesian cross, OR = 2.8; P = 0.01), and male (OR = 3.28; P = 0.0001) animals were significantly at higher risk than adults, indigenous breed, and female individuals, respectively. Fever, skin nodules, swollen lymph nodes, edema causing limb swelling, nasal discharge, lacrimation, and respiratory distress in severe cases were the most prevalent clinical signs observed in LSD-infected cattle. Haematological examination revealed LSD-infected patients were anemic, and red blood cells (RBC), haemoglobin (HGB) and packed cell volume (PCV) parameters were below their corresponding reference ranges in infected cattle. Biochemical analyses showed that total protein (TP), creatinine, aspartate aminotransferase (AST) with alkaline phosphatase (ALP) were decreased. These findings may be helpful for diagnosing LSDV infection, developing effective treatment strategies, preventing further relapses or outbreaks of this disease, and also serving as a baseline for LSD research in respective fields.

Keywords: LSD, prevalence, risk factor, clinical signs, haematology, biochemistry, Maheshkhali.

Chapter 1: Introduction

Lumpy skin disease (LSD) is an emerging and infectious disease of cattle caused by double-stranded DNA virus called lumpy skin disease virus (LSDV). The Lumpy skin disease virus (LSDV) belonging to the genus Capripoxvirus (CaPVs) of Chordopoxvirinae subfamily and the family Poxviridae. It is also known as the Neethling virus. Lumpy skin disease (LSD) is thought to be highly economically significant and have a significant impact on livelihoods as well as food security, especially for smallholders in Bangladesh. About 24.86 million cattle are raised in Bangladesh, and they account for 1.85% of the country's gross domestic product (GDP) (Livestock economy, 2022-23; DLS). On the other hand, the infection of LSDV in cattle had established as a major health issue in this country. Cattle with LSD may have an acute, sub-acute manifestation as fever, lacrimation, nasal discharge, enlargement of superficial lymphnode, anorexia, emaciation and circumscribe skin nodules that eventually necrotize which can result in chronic debility in affected animals. The climate, management challenges, vector prevalence, animal mobility, and preventive and control measures are all conducive to the LSD outbreak in Bangladesh. According to the DLS, Situation Report, LSD 2019, there was 10-20% morbidity and 1-5% mortality in Bangladesh. In a recent scenario, the situation has worsened. Consequently, in the near future, LSD might pose a serious threat to Bangladesh cattle health. Similarly, the disease causes a sharp decline in milk yield due to a high fever brought on by the viral infection itself and secondary bacterial mastitis that is predisposed by the development of lesions on the teats, resulting in a significant loss of milk production as LSD is more severe in cows during the peak period of lactation (Radostits et al., 2006). Along with this, the total cost of veterinary treatment and assistance is added to the direct losses. Besides, haematological, biochemical, and immunological changes can be observed as a result of these infections (Neamat-Allah 2015). The following conditions result in significant economic losses: myiasis, low weight gain, abortion, decrease milk production, emaciation, and permanent damage to hide that lowers their market value

(Abutarbush et al., 2015; Abera et al., 2015;). Due to the fact that none of the countries impacted by LSDV will be interested in purchasing meat or hide from another. Thus the entire economy of the impacted nation will be seriously distorted (Babiuk et al., 2008). Because of this, trade losses related to LSD may be significantly larger than direct losses if partners in trade take action by prohibiting the importation of cattle products from countries where the disease is present. This could lead to a decrease in investments in the cattle industry. According to a study conducted in Ethiopia, the yearly financial cost was estimated to be USD 6.43 per head for indigenous zebu and USD 58 per head for crossbred cattle (Gari et al., 2011). Given that China, Bangladesh, and India have some of the largest populations of cows worldwide, the recent LSD arrivals in Asia are cause for concern. Up to USD 1.45 billion was estimated to have been lost economically in direct livestock and production losses as a result of LSD in the South, East, and Southeast Asia. In the Middle East, reported cases of LSD outbreaks impacted 10.5% of cattle on average. According to Sudhakar et al. (2020), in India backyard small holdings had a lower morbidity rate than the country's overall 7.1% rate. According to Food and Agriculture Organization (FAO) Nepal, preliminary data for Nepal show that overall morbidity is 4.85%, with higher rates in cattle 7.23% (2020) private correspondence. The COVID-19 lockdown, which is being enforced in many countries, makes it more difficult for veterinary services and research facilities to investigate outbreaks promptly and diagnose diseases. This could cause delays in the detection of diseases, the reporting of cases, and the execution of control measures. In that situation LSD in cattle had reached to an alarming issue in livestock industry in Bangladesh and around the globe as well. The World Animal Health Organisation (OIE) has listed LSD because of the serious economic effects it has on affected herds and the possibility of rapid viral spread in vulnerable cattle populations (Bowden et al., 2007). The Maheshkhali island, located on Bangladesh's eastern coastline shore, has a distinctive geological, tectonic, and geomorphologic structure with a hilly topography encircled by a coastal plain. About 27840 cattle population are raised in Maheshkhali. These animals are vital for this region's socioeconomic framework. But it is shocking to the farmers that the recent LSD outbreak in this cattle population caused both direct and indirect losses to the local economy. The majority of cattle

owners are small-scale farmers and their livelihoods are being significantly impacted by the LSD outbreak in that region. For many of these low-income families, the expense of providing supportive treatment for two to three months during the recovery period is unaffordable.

The first outbreak in Bangladesh is known to have occurred on July, 2019, in three upazilas (Anowara, Karofuli, and Patia) of Chattogram Division. Later, it was confirmed as LSD using real-time PCR on August 27, 2019 by the Department of Livestock Services (DLS), Bangladesh (DLS, 2019). The disease rapidly spread to every region of the country. As per the situation report released by DLS, out of the 25 million cattle in the population, there have been a total of 553,528 cases and 97 recorded deaths since December 3, 2019 (DLS, 2019). However, the incidence was highest in Chattogram as 8.26% and Khulna as 6.52% and lowest in Sylhet as 0.01%. Once more, multiple regions of the country including Barishal, Dinajpur, Sylhet, Sirajgonj-Pabna, Naogon reported LSD outbreaks in 2020 and 2021.

In Bangladesh, there are some research gap or very few reports available on LSD like spatial epidemiology of LSD, risk assessments or risk models addressing introduction or spread of LSD, molecular characterization and virus isolation, rapid diagnostic kit development for LSD, haematological and serum biochemical analysis, vaccine efficacy determination, vector identification and seasonal variation of LSDV infection etc. In order to prevent further outbreaks, it is important that policies and research that can be implemented enable the most effective control strategy. Considering this context, the following objectives were included in the research plan:

1. To assess the prevalence of LSD in study area.
2. To explore the potential risks factors associated with LSD.
3. To analyse the haematological and biochemical features of blood in LSDV infected cattle.

Chapter 2: Materials and Methods

2.1. Study area & Study period

The study area was Maheshkhali Upazila under the district of Cox's Bazar. The study was carried out in Upazila Livestock Office and Veterinary Hospital (UUVH), Maheshkhali, Cox's Bazar, Bangladesh. This study was undertaken during clinical rotation at UUVH as internship programme of DVM from 16th April to 8th June 2023. The animals, which came from various parts of Maheshkhali island, were chosen from UUVH. Using a pre-made questionnaire survey, the total number of clinically sick cattle was first recorded, the number of information for clinically suspected lumpy skin disease (LSD) were recorded.

2.2. Study design and cases

During the placement, around 231 cattle were treated at Upazila Veterinary Hospital for various diseases and disease conditions. These cattle were all part of the study population and were assigned to various case groups (e.g., lumpy skin disease, acidosis, aspiration pneumonia, dermatophytosis, etc). This study focused on 62 cases of lumpy skin disease out of 231 cattle, taking follow-up cases into account. The cases of LSD were diagnosed by registered veterinarian (Upazila livestock officer and veterinary surgeon) based on the clinico-epidemiological history, physical examination and clinical signs.

2.3. Clinical and epidemiological data collection

A standardised questionnaire was used to obtain the necessary data related to the lumpy skin disease from the owner. The following information are included in the questionnaire: Demographic information of patient (age, breed, sex, body weight), socio-economic condition of the farm owner (Name, address, sex, age, occupation, education, job), patient information (duration of illness, history of deworming, vaccination, previous illness, number of infected animal), management system (housing system, floor type, type of feed, grazing system, water source, use of fly repellent, biosecurity). Clinical examination include rectal temperature (°F), heart rate, respiration rate, dehydration test, mucous membrane examination, lymphnode palpation, observation on presence or absence of nasal discharge and lacrimation, inspection of skin nodule and other necessary complaint. Data on diagnoses,

medication prescriptions, recovery time and complications of LSD were also documented.

2.4. Sample collection

Along with collection of data through questionnaires, ten samples of whole blood (with and without EDTA) were aseptically drawn from the jugular vein of clinically affected LSD cattle in a vacutainer tube. After separation of serum from whole blood, serum sample was kept in -20°C for biochemical analysis.

2.5. Haematological and Biochemical analysis

One automated cell counter (Cell Tech Alpha, Japan) was used to determine the haematological profile (RBCs, Hb, PCV, etc.) of LSDV infected cattle. Biochemical analyses were performed using an analyzer (Humalyzer, 3000) to evaluate aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP) and creatinine from serum sample.

2.6. Management of data and statistical analysis

All data were entered into Microsoft Excel 2010 (Microsoft corporation, USA). Data were then coded and re-coded in MS excel 2010. Finally exported into STATA 13 (Stata Crop, 4905, Lakeway Drive, College Station, Texas 77845, USA) for descriptive, univariable and multivariable statistical analysis.

The prevalence of various diseases and disease conditions was determined with dividing the number of cases in each category by the total number of cases among all disease categories. Frequency distribution of cases(LSD) were presented according to categories of each selected clinical sign (temperature, skin nodule, lymphnode, edema in leg, nasal discharge, lacrimation, respiratory distress) and managerial factors (grazing, water source, floor type, use of fly repellent, biosecurity).

Fisher's exact test was utilised to evaluate the association between the categorised variable of LSD (e.g., LSD, yes or no) and chosen independent factors (age, breed, gender). The binomial approximation was utilised to calculate the 95% confidence intervals (CI). A multivariate logistic regression analysis was considered for potential inclusion of significant factors (at $p \leq 0.2$). The ultimate multivariable model was constructed through a backward stepwise method and likelihood ratio tests (LRT) to assess models with and without each variable. Variables were kept if the

LRT's P-value was ≤ 0.05 . While assessing the significant difference, a cutoff point probability value of $p \leq 0.05$ was taken into consideration.

In case of different haematological and biochemical parameter analysis, difference between the obtained value of LSD infected cattle and normal reference value in healthy cattle were calculated by applying the two-sample *t*-test. A value of $P \leq 0.05$ was taken to be statistically significant. The analysis of spatial data was performed using ArcGIS software version 10.8 for the spatial distribution of LSD infection.

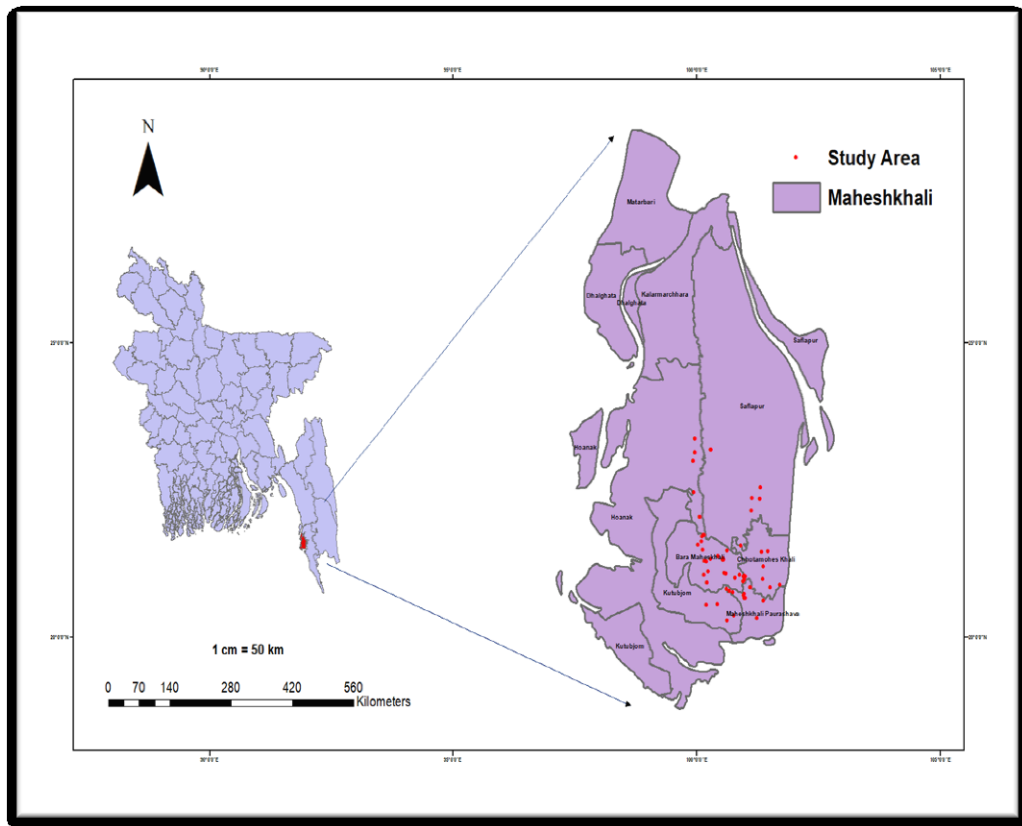


Figure 1: Spatial distribution of study area from which cases of LSD were presented to UUVH.

Chapter 3: Results

3.1. Prevalence of LSD in cattle

During this study period, LSD affected cattle (26.84%; 95% CI 0.21-0.33) had the highest prevalence among different clinical cases of cattle that were presented to UUVH (Table 1).

Table 1: Frequency Distribution of different disease and diseases condition of cattle presented at Upazila Veterinary Hospital, Maheshkhali, Cox's Bazar (231 cases).

Diseases	Frequency	Percentage	95% CI
LSD	62	26.84	0.21-0.33
Acidosis	18	7.79	0.05-0.12
Aspiration pneumonia	22	9.52	0.06-0.14
Dermatophilosis	1	0.43	0.0006-0.03
Dermatophytosis	6	2.60	0.01-0.05
Parasitic infestation	11	4.76	0.03-0.08
Papillomatosis	7	3.03	0.01-0.06
Mastitis	9	3.90	0.02-0.07
Myiasis	6	2.60	0.01-0.05
Abscess	6	2.60	0.01-0.05
Foot and Mouth Disease	8	3.46	0.01-0.07
Calf diarrhoea	13	5.63	0.03-0.09
Naval ill	11	4.76	0.02-0.08
Hypocalcaemia	12	5.19	0.02-0.08
Stunted growth	1	0.43	0.0006-0.03
Rabies	1	0.43	0.0006-0.03
Dystokia	1	0.43	0.006-0.03
Retained placenta	2	0.87	0.002-0.03
Pregnant	4	1.73	0.006-0.04
Fracture	3	1.30	0.004-0.04
Synovitis	3	1.30	0.004-0.03
Wound	11	4.76	0.26-0.08
Unidentified	13	5.63	0.03-0.09

Again, LSD patient less than 1-year old (40.20%), Sahiwal (SW) cross breed (40.54%) and male (38.39%) cattle had higher prevalence when compared with the patients 1 to 3 years (21.98%) followed by more than 3 years (2.63%), breed Holstein Friesian (HF) with (35.29%) followed by local breed (16.26%) and female patients (15.97%), respectively ($p \leq 0.2$) (Table 2). Moreover, cattle rearing without using fly repellent (62.90%), grazing in field (61.29%), feeding water from ponds (70.97%), a floor type made of mud (58.06%) with poor biosecurity (50%) had higher prevalence of LSD than cattle rearing with applying fly repellent (37.10%), confined (not grazing) animal (61.29%), feeding ground water (29.03%), with bricked floor type (27.42%) followed by cemented floor (14.52%) and with average biosecurity (33.87%) followed by good biosecurity (16.13%) condition, respectively ($p \leq 0.2$) (Table 2).

Table 2: Univariate association between LSD and selected factors through Fisher's exact test (231 cases total, 62 cases LSD infected)

Factors	Categories	Lumpy skin disease		P-value
		Yes N (%)	No N (%)	
Age (Year)	<1	41 (40.20)	61	0.000
	1-3	20 (21.98)	71	
	>3	1 (2.63)	37	
Breed	SW cross	30 (40.54)	44	0.000
	HF cross	12 (35.29)	22	
	Local	20 (16.26)	103	
Gender	Female	19 (15.97)	100	0.000
	Male	43 (38.39)	69	
Fly repellent	Used	23 (37.10)	106	0.001
	Not used	39 (62.90)	63	
Grazing	Field	38 (61.29)	74	0.025
	Confined	24 (38.71)	95	
Water	Pond	44 (70.97)	59	0.000
	Ground water	18 (29.03)	110	
Floor type	Mud	36 (58.06)	39	0.000
	Brick	17 (27.42)	56	
	Cemented	9 (14.52)	74	
Biosecurity	Poor	31 (50)	50	0.003
	Average	21 (33.87)	58	
	Good	10 (16.13)	61	

3.2. Risk factors for LSD

The risk of LSD in cattle had 10.42 (OR) times and 24.86(OR) times significantly ($p \leq 0.05$) higher where cattle aged less than 1 year and between 1 to 3 years compared to cattle aged more than 3 years. Multivariable logistic regression also revealed that Sahiwal cross (OR=3.5) breed had significantly ($p \leq 0.05$) highest risk followed by Holstein Frisien (OR=2.8) than local breed. Again, male individuals were in significantly ($p \leq 0.05$) higher risk (OR=3.28) than female individuals. In addition, there was strong statistical evidence that without controlling fly, grazing freely in field (than confined), water supply from ponds (rather ground water) , a floor type of mud (than brick and cement) and poor biosecurity condition (than average and good) of the farm serve as possible risk factors for the occurrence of LSD in cattle (Table 3).

Table 3: Multivariable association between potential factors with the prevalence of LSD in cattle (Logistic regression model output) (62 cases)

Factors	Categories	OR	95% CI	P value
Age(year)	<1	10.42	1.25-86.53	0.0069
	1-3	24.86	2.8-220.61	0.0000
	>3	Reference	-	-
Breed	SW cross	3.5	1.75-7.03	0.002
	HF cross	2.8	1.17-6.70	0.01
	Local	Reference	-	-
Gender	Female	Reference	-	-
	Male	3.28	1.72-6.24	0.0001
Fly control	Yes	Reference	-	-
	No	2.85	1.53-5.30	0.0005
Grazing	Field	2.03	1.11-3.71	0.0186
	Confined	Reference	-	-
Water	Pond	4.55	2.33-8.90	0.000
	Ground water	Reference	-	-
Floor type	Mud	7.58	3.05-18.85	0.000
	Brick	2.49	1.02-6.10	0.000
	Cemented	Reference	-	-
Biosecurity	Poor	3.78	1.63-8.75	0.0008
	Average	2.20	0.94-5.15	0.0600
	Good	Reference	-	-

3.3. Clinical findings

A range (mild, moderate, severe) of obvious skin nodule with high fever, swollen lymphnodes (pre-scapular and pre-femoral), edematous swelling in leg, lacrimation, nasal discharge with severe respiratory distress leading to pneumonia are most prevalent clinical findings in LSDV infected cattle. Most of the cattle had moderate (40.32%) to severe (32.26%) skin nodule, high rectal temperature (75.81%), Swollen lymphnode, swollen leg, lacrimation, nasal discharge, respiratory distress were observed in 64.52%, 40.32%, 51.61%, 35.48% and 35.81% cases, respectively (Table 4) at the time of clinical examination. And, the duration illness varies from 10days to 30days or more.



Figure 4: Skin nodule covering entire body of LSDV infected cattle



Figure 3: Enlargement of Lymphnode



Figure 2: Lacrimation in calf



Figure 5: Edematous swelling of leg



Figure 6: Sloughing off nodule results lameness



Figure 7: Open grazing in field showing fly infestation

Table 4: Frequency distribution of observable clinical signs of LSD infected cattle (62 cases).

Variable	Co variable	Frequency	Percentage (%)
Temperature (°F)	103-105	12	24.19
	>105	47	75.81
Skin nodule	Mild	17	27.42
	Moderate	25	40.32
	Severe	20	32.26
Lymphnode	Swollen	40	64.52
	Not swollen	22	35.48
Leg swollen/edema	Yes	25	40.32
	No	37	59.68
Nasal discharge	Yes	22	35.48
	No	40	64.52
Lacrimation	Yes	32	51.61
	No	30	48.39
Respiratory distress	Present	16	35.81
	Absent	46	74.19
Duration of illness (Days)	<20	24	38.70
	20-30	15	24.19
	>30	23	37.09

3.4. Haematological and Biochemical analysis

Ten blood samples from randomly selected cattle that had been infected with LSD were separately used for haematological and biochemical analysis. The biochemical parameters obtained from LSD infected cattle were presented in Table 5. The findings reveal that cattle infected with LSD had considerably lower levels of red blood cells (RBC), haemoglobin (HGB) and Packed Cell Volume (PCV) than reference value observed in healthy cattle. Additional blood parameters as White Blood Cell (WBC), Differential Leukocyte Count (DLC) and Erythrocyte Sedimentation Rate (ESR) stay within the range of their reference values. Besides, there was a significant increase in the concentrations of total protein and creatinine, as well as in the serum Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) levels ($p < 0.05$).

Table 5: Significant difference of Hematological and biochemical parameters in LSD infected and their reference range, mean \pm SD.

Analysis type	Parameters	Mean \pm SD	Normal value	P-value
Haematological analysis	WBC (10^3 / μ L)	4.25 \pm 1.20	4-12	0.058
	RBC (10^6 / μ L)	4.1 \pm 0.34	5-10	0.051
	HGB (g/dl)	7.3 \pm 0.141	8-15	0.059
	PCV (%)	22.6 \pm 0.5	24-46	0.230
	ESR (mm in 1 st hour)	0.12 \pm 0.23	0-1	0.034
	Lymphocyte (%)	57.5 \pm 3.53	45-75	0.043
	Neutrophil (%)	37.5 \pm 3.53	15-75	0.326
	Eosinophil (%)	4.5 \pm 2.12	0-20	0.124
	Monocyte (%)	3 \pm 1.41	0-8	0.065
	Basophil (%)	0.21 \pm 0.62	0-2	0.051
Biochemical analysis	Total protein (g/L)	89.75 \pm 1.90	67.4-74.6	0.033
	Creatinine (mg/dl)	2.7 \pm 0.14	1-2	0.008
	AST(U/L)	155.8 \pm 4.70	78-132	0.02374
	ALP(U/L)	407.75 \pm 55.64	25-127	0.061

(Source of Reference ranges : Latimer KS, *Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology*, 5th ed., Wiley-Blackwell, 2011)

Chapter 4: Discussion

In this study, the overall LSD prevalence was found to be 26.84% in Moheshkhali Upazila, Bangladesh. These results are quite similar to those of Badhy et al., (2021) in Chittagong, Prank et al., (2020) in Sirajgonj-Pabna, Molla et al., (2018) in Ethiopia, Body et al., (2012) in Oman reported that the prevalence of LSD were 23%, 26.5%, 27.9%, respectively. But the present study doesn't support the results of Hasib et al., (2021) 10% in Chittagong, Khalil et al., (2021) 21% in Barishal, Pory et al., (2021) 13.65%) in Sylhet, Sarkar et al., (2020) 41.06% in Dinajpur, Haque et al., (2020) 49% in Naogon, Bangladesh. There are numerous factors that could have contributed to the prevalence of LSD including geography, climate, animal health and nutrition , management practises and biosecurity, immunity, seasons, populations and distribution of potential insect vectors in different habitats; virulence of the virus etc.

Risk factor analysis of LSD in cattle revealed that calves (< 1 year) were at higher risk than adults. Calves may have a higher incidence of the virus because of their lower immunity, malnutrition and early-life susceptibility to it. The results of Ahmed and Zaher (2008), Vorster and Mapham (2008) were consistent with this finding. As per Bangladesh Cattle Breeding Policy, she has a large population of cattle, mostly crossbred Holstein Friesian and Sahiwal cattle and native zebu cattle. However, this study revealed that indigenous breeds were significantly at lower risk than cross breeds which was in agreement with other authors (Kiplagat et al. 2020). Because of their strong immune responses, genetic differences and excellent environmental adaptation, local breeds were less vulnerable than cross-breeds, according to Salib et al., (2011), Hasib et al., (2021) and Abera et al., (2015). According to Tageldin et al. (2014), crossbred cattle may be more susceptible to disease because they are less able to fend off infections than cattle from native breeds. Besides, in this study, male cattle are more susceptible to LSD that finding was in consistent with Abd Elmohsen et al., (2019); Badhy et al., (2021); Kalil et al., (2021); and Pory et al., (2021). However, other researchers observed that females were more likely to be infected with LSD than male (Magori-Cohen et al., 2012; Ayelet et al., 2014;). Abera et al.,

(2015) claimed as male individual were at high risk of disease than female because they were exposed to stressors like exhaustion from hard work.

On the other hand, this study also found some other factors related to farm management systems that significantly contributed to the occurrence of LSD in cattle. According to research by Tuppurainen and Oura (2012), the prevalence of LSD was significantly related to the existence of insect vectors, grazing, water source, husbandry systems, and other factors. This study was in line with the findings of Alemayehu et al., (2015) who found applying fly repellent had positive effect on lowering the risk of LSD, as controlling vectors is another most efficient techniques for limiting LSD spread. Additionally, cattle grazing in field act as potential risk factor for LSD. Because biting flies may have made it easier for the virus to spread among cattle raised for open grazing in fields or hilly terrain, but farms with zero-grazing (confined) management kept their cattle protected from arthropod vectors Hasib et al., (2021) and Ochwo et al., (2019). Furthermore, supply of water from ponds and muddy floor types of house also has significant role to give favourable condition for flies than their counterparts. Similar observations from previous research corroborated these results as stated by Babiuk et al., (2008); Tuppurainen and Oura (2012). Even though infectious transmission is thought to be an ineffective means of transmission, contamination of the water and pasture could be regarded as a possible risk when it comes to communal grazing and watering point usage (Waret-Szkuta et al., 2011). The findings of current study indicated that a higher percentage of cattle with LSD infection were found in households or farms with poor biosecurity practises than in those with good or medium biosecurity practises. Alemayehu et al., (2015) reported that poor biosecurity and farm waste management were also responsible for the transmission of LSD virus.

The most prevalent clinical featured noticed in current study were high fever, characteristics skin nodule, enlargement of lymphnode, edema in joint results in limb swelling , lachrimation, nasal discharge results in respiratory distress and pneumonia in severe cases. These observation had similarities with the findings of other authors (Abdulqa et al., 2016; OIE, 2017). According to El-mandrawy and Alam (2018), clinical signs of LSD that were taken into consideration for a diagnosis included

nasal discharge, ocular discharge, anorexia, emaciation, lymph node swelling, and lesions in the oral mucosa and skin. Animal that showed clinical sign were also examined for their blood parameters as changes may occur in viral infection.

Haematological analysis of blood sample from cattle infected with LSD showed reduced levels of red blood cells (RBC), haemoglobin (HGB) and packed cell volume (PCV) in current study. These findings are corroborated by a prior study of Jalali et al., (2017) and Ghosh et al., (2023). Anorexia, decreased serum iron levels, lower responsiveness of bone marrow to erythropoietin, and anaemia may also be contributing factor for this pan-reduction of vital erythrocyte parameters (Morceau et al., 2009 and Jalali et al., 2017). But this result contradict with Morris (2002) who stated that absolute erythrocytosis has been linked to long-term illnesses in large animals. Furthermore, the level of White Blood Cell (WBC), Differential Leukocyte Count (DLC) and Erythrocyte Sedimentation Rate (ESR) stay within the range of their reference values. This result showed inconsistency with other prior studies as Shefaa et al., (2018) reported, due to viral infection LSDV infected cattle displayed leukopenia. Additionally, lymphopenia is brought on by a high dose of corticosteroid hormone release (Ismail and Yousseff, 2006).

Along with the above mentioned changes in blood parameters, the present study showed that cattle with LSDV infection had higher concentrations of total serum protein. This is to be expected as it shows that the immune system has become activated after an infection. Changes in albumin and total protein concentrations have been linked to the humoral immune response to infectious pathogens, according to Dudek et al., (2010) and Matei et al., (2010). According to certain reports, a decrease in the glomerular filtration rate is reflected in an increase in creatinine concentration (Gowda et al., 2010; Samra and Abcar, 2012). This study revealed cattle infected with LSDV had a significantly higher serum creatinine concentration. Conversely, cattle that were naturally infected with LSDV had low creatinine concentrations, according to Abutarbush, (2015). Besides, significantly higher AST concentrations were found in the current study's LSDV-infected cattle. According to reports, increased serum AST concentration in cattle with LSDV infection may be associated with hepatic damage caused by viremia (Sevik et al., 2016). Muscular injuries may

also be associated with elevated AST concentrations in infected cattle (Stockham and Scott, 2008). Moreover, the concentration of ALP level in LSD infected cattle was found to have significantly increased in this study, which appears to be at odds with a prior study (Abutarbush, 2015) that found no change in ALP concentrations in LSDV-infected cattle . Consequently, the hepatic damage caused by the presence of LSDV in cattle may be associated to the elevation in AST and ALP levels in these animals.

Conclusion

The purpose of this study was to evaluate the prevalence of Lumpy Skin Disease (LSD) in study area, exposing the associated risk factors along with their clinical features. To sum up, the findings of this investigation demonstrated that LSD outbreak had a noteworthy correlation between the age, breed, and gender of cattle. Furthermore, there are fewer trends of LSD occurrences due to the management status, particularly fly control, individually grazed cattle, the water source, the types of floors, and the good biosecurity conditions. The current investigation unequivocally demonstrated that haematological and biochemical stress markers were altered in LSDV-infected cattle. These findings contribute to a deeper comprehension of pathogenesis and may provide more insight for improving treatment plans. This study can support the work of researchers, practitioners, decision-makers, and planners. Furthermore, it could support their efforts in disease surveillance and control to reduce risks and enhance animal health.

Limitation

PCR is the quickest and most effective method for identifying and locating the agent responsible for the viral outbreak. In skin nodule samples, PCR demonstrated high sensitivity for detecting LSD virus DNA (Sharawi and Abd El-Rahim, 2011; Tuppurainen et al., 2005). However, it is very unfortunate that, owing to limited resources, we were unable to use the PCR method for virus diagnosis. Only reported cases with cardinal clinical signs were used to diagnose the data in this study. Sometimes it was challenging to assess the case history properly because the owners were mostly illiterate, followed by a few with only a primary level of education.

References

- Abd Elmohsen, M., Selim, A., and Abd Elmoneim, A. E. (2019). Prevalence and molecular characterization of Lumpy Skin Disease in cattle during period 2016-2017. *Benha Veterinary Medical Journal*, 37(1):173–176.
- Abdulqa, H. Y., Rahman, H. S., Dyary, H. O. and Othman, H. H. (2016). Lumpy Skin Disease. *Reproductive Immunology*, 1(25): 233-240.
- Abutarbush, S. M. (2015). Hematological and serum biochemical findings in clinical cases of cattle naturally infected with lumpy skin disease. *Journal of Infection in Developing Countries*, 9(3):283–288.
- Abera, Z., Degefu, H., Gari, G., and Kidane, M. (2015). Sero-prevalence of lumpy skin disease in selected districts of West Wollega zone, Ethiopia. *BMC Veterinary Research*, 11(1), 1–9.
- Ahmed, W.M. and Zaher, K.S. (2008). Observations on lumpy skin disease in local Egyptian cows with emphasis on its impact on ovarian function. *African Journal of Microbiology Research*, 2(10): 252-257.
- Alemayehu, G., Leta, S., Eshetu, E and Mandefro, A. (2015). Incidence of lumpy skin disease and associated risk factors among export-oriented cattle feedlots at Adama District, Central Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 7: 128–134.
- Ayelet, G., Haftu, R., Jemberie, S., Belay, A., Gelaye, E., Sibhat, B., Skjerve, E. and Asmare, K. (2014). Lumpy skin disease in cattle in central Ethiopia: outbreak investigation 30 and isolation and molecular detection of the virus. *Reveiw of Science and Technology*, 33: 877- 887.
- Babiuk, S., Bowden, T. R., Boyle, D. B., Wallace, D. B., and Kitching, R. P. (2008). Capripoxviruses: An emerging worldwide threat to sheep,goats and cattle. *Transboundary and Emerging Diseases*, 55(7), 263–272.
- Badhy, S. C., Chowdhury, M. G. A., Settypalli, T. B. K., Cattoli, G., Lamien, C. E., Fakir, M. A. U., Akter, S., Osmani, M. G., Talukdar, F., Begum, N., Khan, I. A., Rashid, M. B., and Sadekuzzaman, M. (2021). Molecular characterization of lumpy skin disease virus (LSDV) emerged in Bangladesh

- reveals unique genetic features compared to contemporary field strains. *BMC Veterinary Research*, 17(1), 1–11.
- Body, M., Singh, P. K., Hussain, H. M., Al-rawahi, A., Al-maawali, M., Al-lamki, K., and Al-habsy, S. (2012). Clinico-histopathological findings and PCR based diagnosis of lumpy skin disease in the Sultanate of Oman. *Pakistan Veterinary Journal*, 32(2), 206–210.
- Bowden, T. R., Babiuk, S. L., Parkyn, G. R., Copps, J. S., and Boyle, D. B. (2007). Capripoxvirus tissue tropism and shedding: a quantitative study in experimentally infected sheep and goats. *Virology*, 371:380–93.
- Department of Livestock Services (DLS), (2019). Situation Report: Lumpy skin disease in Bangladesh.
- Department of Livestock Services (DLS), (2023). Livestock Economy: Source- BBS, 2022-23; Prepared by Dr. Hossan Md. Salim.
- Dudek, K., Bednarek, D. and Szymańska-Czerwińska, M. (2010). Acute phase response in calves as a result of experimental challenge with. *Bulletin of the Veterinary Institute in Pulawy*, 54(4): 517–520.
- El-mandrawy, S. A. M., and Alam, R. T. M. (2018). Hematological , biochemical and oxidative stress studies of lumpy skin disease virus infection in cattle. *Journal of Applied Animal Research*, 46: 1073–1077.
- FAO. (2020). EMPRES-i Global Animal Disease Information System. In: FAO Animal Production and Health Division, Rome.
- Gari, G., Bonnet, P., Roger, F. and Waret-Szkuta A. S. (2011). Epidemiological aspects and financial impact of lumpy skin disease in Ethiopia. *Preventive veterinary medicine*, 102(4): 274-283.
- Ghosh, K., Logno, T. A. M., Imtiaz, A., Das, T. and Biswas, P.K. (2023). Clinico-epidemiological features of lumpy skin disease affecting cattle in Bangladesh. *Asian Journal of Animal and Veterinary Advances.*, 18: 66-73.
- Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V., Math, A. A. K. and Vernekar, S. N. (2010). Markers of renal function tests. *North American Journal of Medical Sciences*, 2(4): 170–173.
- Hasib, F. M. Y., Islam, M. S., Das, T., Rana, E. A., Uddin, M. H., Bayzid, M., Nath, C., Hossain, M. A., Masuduzzaman, M., Das, S., and Alim, M. A. (2021).

- Lumpy skin disease outbreak in cattle population of Chattogram, Bangladesh. *Veterinary Medicine and Science*, 7:1616–1624.
- Haque, M. H., Roy, R. K., Yeasmin, F., Fakhruzzaman, M., Yeasmin, T., Sazib, M. R. I., Uddin, M. N. and Sarker, S. (2021). Prevalence and Management Practices of Lumpy Skin Disease (LSD) in Cattle at Natore District of Bangladesh. *European Journal of Agriculture and Food Sciences*, 3(6): 76–81.
- Ismail, S. M., and Yousseff, F. M. (2006). Clinical, hematological, biochemical and immunological studies on lumpy skin disease in ismailia governorate. *Suez Canal Veterinary Medical Journal*. 1:393–400.
- Jalali, S. M., Rasooli, A., Seifi, A. S. M., and Daneshi, M. (2017). Clinical, hematologic, and biochemical findings in cattle infected with lumpy skin disease during an outbreak in southwest Iran. *Archives of Razi Institute Journal*, 72(4):255-265.
- Khalil, M. I., Sarker, M. F. R., Hasib, F. M. Y., and Chowdhury, S. (2021). Outbreak investigation of lumpy skin disease in dairy farms at Barishal, Bangladesh. *Turkish Journal of Agriculture - Food Science and Technology*, 9(1), 205–209.
- Kiplagat, S. K., Kitala, P. M., Onono, J. O., Beard, P. M., and Lyons, N. A. (2020). Risk factors for outbreaks of lumpy skin disease and the economic impact in cattle farms of Nakuru County, Kenya. *Frontiers in Veterinary Science*, 7: 259.
- Magori-Cohen, R., Louzoun, Y., Herziger, Y., Oron, E., Arazi, A., Tuppurainen, E., Shpigel, N. Y. and Klement, E. (2012). Mathematical modelling and evaluation of the different routes of transmission of lumpy skin disease virus. *Veterinary research*, 43(1): 1.
- Matei, S. T., Groza, I., Andrei, S., Bogdan, L., Ciupe, S. and Petrean, A. (2010). Serum metabolic parameters in healthy and subclinical mastitis cows. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine*, 67(1): 110–114.

- Molla, W., Frankena, K., Gari, G., Kidane, M., Shegu, D., and de Jong, M. C. M. (2018). Seroprevalence and risk factors of lumpy skin disease in Ethiopia. *Preventive Veterinary Medicine*, 160: 99–104.
- Morceau, F., Dicato, M., and Diederich, M. (2019). Pro-inflammatory cytokine-mediated anemia: regarding molecular mechanisms of erythropoiesis. *Mediators Inflammation*, 12(2): 221-230.
- Morris, D. D. (2002). Alterations in the erythron. In: Smith BP, editor. Large animal internal medicine. 2nd edition, New York: Mosby; p. 415–419.
- Neamat-Allah, A. N. F. (2015). Immunological, hematological, biochemical, and histopathological studies on cows naturally infected with lumpy skin disease. *Veterinary World*, 8(9):1131–1136.
- Ochwo, S., VanderWaal, K., Munsey, A., Nkamwesiga, J., Ndekezi, C., Auma, E., and Mwiine, F. N. (2019). Seroprevalence and risk factors for lumpy skin disease virus seropositivity in cattle in Uganda. *BMC Veterinary Research*, 15(1), 1–9.
- OIE (World Organisation for Animal Health), (2017). Lumpy Skin Disease: Aetiology, Epidemiology, Diagnosis, Prevention and Control. International des Epizootics, OIE Terristerial Manual.
- Prank, M. R., Singha, S., Das, P., and Paul, P. (2020). An outbreak of Lumpy skin disease in Dairy herds of Pabna and Sirajganj districts, Bangladesh. *Bangladesh Journal of Veterinary and Animal Sciences*, 8(2): 106-112.
- Pory, F. S., Lasker, R. M., Islam, N. and Siddiqui, S. I. (2021). Prevalence of Lumpy Skin Disease at District Veterinary Hospital in Sylhet District of Bangladesh. *International Journal of Research and Innovation in Applied Science*, 6(10): 111-115.
- Radostits, O. M., Gay, C. C., Hinchcliff, K. W. and Constable, P.D. (2006). *Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Edition 10th. Sounders Elsevier, Spain. P. 1424-1426.
- Salib, F. A., and Osman, A. H. (2011). Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate, Egypt. *Veterinary World*, 4(4): 162–167.

- Samra, M. and Abcar, A. C. (2012). False estimates of elevated creatinine. *The Permanente Journal*, 16(2):51–52.
- Sarkar, S., Meher, M. M., Parvez, M. M. M., and Akther, M. (2020). Occurrences of Lumpy Skin Disease (LSD) in Cattle in Dinajpur Sadar of Bangladesh. *Research in Agriculture Livestock and Fisheries*, 7(3), 445–455.
- Sevik, M., Avci, O., Dogan, M., and Ince, O. B. (2016). Serum biochemistry of lumpy skin disease virus-infected cattle. *BioMed Research International*, 2:1–6.
- Sharawi S, and Abd El-Rahim I. (2011). The utility of polymerase chain reaction for diagnosis of lumpy skin disease in cattle and water buffaloes in Egypt. *Revue Scientifique et technique*, 30(3):821–830.
- Shefaa, A. M., El-Mandrawy and Rasha T. M. (2018). Hematological, biochemical and oxidative stress studies of lumpy skin disease virus infection in cattle, *Journal of Applied Animal Research*, 46(1):1073-1077.
- Stockham, S. L., and Scott, M. A. (2008). Fundamentals of veterinary clinical pathology, 2nd ed. Ames (Iowa): Blackwell. Szasz G, Waldenstrom J, Gruber W. 1979. Creatine kinase in serum: 6. Inhibition by endogenous polyvalent cations, and effect of chelators on the activity and stability of some assay components. *Clinical Chemistry*, 25:446–452.
- Sudhakar, S.B., Mishra, N., Kalaiyarasu, S., Jhade, S.K., Hemadri, D., Sood, R., Bal, G.C., Nayak, M.K., Pradhan, S.K. and Singh, V.P. (2020). Lumpy skin disease (LSD) outbreaks in cattle in Odisha state, India in August 2019: Epidemiological features and molecular studies. *Transboundary and Emerging Diseases*, 1:1–15.
- Tageldin, M. H., Wallace, D. B., Gerdes, G. H., Putterill, J. F., Greyling, R. R., Phosiwa, M. N., Al Busaidy, R. M., & Al Ismaaily, S. I. (2014). Lumpy skin disease of cattle: An emerging problem in the Sultanate of Oman. *Tropical Animal Health and Production*, 46(1): 241– 246.
- Tuppurainen, E. R., Afonse, C. L., Zsak, L.Z., Kutish, G. F and Rock, D. L. (2005). The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. *Onderstepoort Journal of Veterinaey Research*, 72: 153-164.

- Tuppurainen, E. S. M., and Oura, C. A. L. (2012). Review: Lumpy skin disease: An emerging threat to Europe, the Middle East and Asia. *Transboundary and Emerging Diseases*, 59(1), 40–48.
- Waret-Szkuta, A., Ortiz-Pelaez, A., Pfeiffer, D. U., Roger, F. and Guitian, F. J. (2011). Herd contact structure based on shared use of water points and grazing points in the Highlands of Ethiopia. *Epidemiology and Infection*, 139: 875–885.
- Vorster, H. and Mapham, H. (2008). Pathology of lumpy skin disease. *Livestock Health Production Review*, 1:16-21.

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