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Full Length Research

Impact of lumpy skin disease on the first-service artificial insemination conception rate of dairy cattle in Northern Bangladesh

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ABSTRACT: Lumpy skin disease (LSD) is an emerging transboundary viral disease of cattle causing substantial economic and reproductive losses in Bangladesh. This study aimed to assess the epidemiology of LSD, identify modifiable farm-level risk factors, evaluate therapeutic management, examine farmers' knowledge, attitudes, and practices (KAP), and determine the effect of LSD on the conception rate (CR) of first artificial insemination (FAI) in northern Bangladesh. A 'prospective observational cohort' in Methods was conducted from January 18 to October 30, 2025, across 942 dairy farms in 10 northern districts of Bangladesh. Trained veterinarians or interns clinically examined cattle for LSD, and structured interviews were used to collect socioeconomic data, management practices, and KAP indicators. Logistic regression analysis was performed to identify significant risk factors associated with LSD occurrence and reproductive outcomes. The animal-level prevalence of LSD was 21.3%, while herd-level prevalence was 55.1%. Mortality and case-fatality rates were 4.3% and 22.2%, respectively, with calves under six months accounting for 42.9% of deaths. Vector abundance, particularly biting midges, stable flies, and tabanids, significantly increased disease risk. LSD exposure adversely affected reproductive performance; however, cows with higher Red Chittagong (RC) inheritance showed superior FAI conception rates. Only 35.3% of farmers practised adequate preventive measures despite moderate to good knowledge and attitudes. In conclusion, LSD significantly impacts cattle health and reproductive efficiency in northern Bangladesh. Strengthening vaccination coverage, vector control, farmer education, and biosecurity practices is essential to improve disease control and enhance conception rates following the first artificial insemination.

Keywords: Bangladesh, KAP, LSD, Risk Factors, Therapeutic management.

Abbreviations: FAI, first artificial insemination; **LSD,** Lumpy Skin Disease; **KAP,** knowledge, attitudes, and practices.

INTRODUCTION

Lumpy skin disease (LSD) is an emerging transboundary viral disease of cattle caused by lumpy skin disease virus (LSDV), a member of the genus Capripoxvirus. Since its first official detection in Bangladesh in 2019, LSD has

rapidly spread across many districts and has become endemic in several regions. Clinically, the disease is characterised by fever, multiple cutaneous nodules, lymphadenopathy, and secondary bacterial infections. Affected animals frequently exhibit reduced feed intake, weight loss, decreased milk yield, hide damage, and a range of reproductive disorders, including abortion, infertility, and repeat breeding. These production and reproductive impairments result in substantial economic losses for both smallholder and commercial dairy farms.

The biological and clinical features of LSD have been extensively described in global and regional literature. Comprehensive reviews have detailed the molecular pathogenesis of LSDV, host immune responses, clinical manifestations, and diagnostic options, while emphasising the significant impacts of the disease on animal welfare and productivity (Haider *et al*., 2024a, b; Ahmed, 2024). Systemic illness associated with LSD, such as fever, anorexia, and immune activation, together with local lesions involving the mammary gland and genital tract, can disrupt endocrine function and reproductive physiology. Elevated inflammatory mediators and metabolic stress may impair oestrous cyclicity, oocyte quality, timing of ovulation, and early embryo survival, thereby reducing reproductive efficiency and conception rates following artificial insemination (AI).

Following the emergence of LSD in Bangladesh, multiple epidemiological studies have documented widespread exposure and variable clinical prevalence across the country. A nationwide prospective observational cohort serological survey conducted between October 2021 and March 2022 demonstrated substantial natural exposure to LSDV in unvaccinated cattle populations, highlighting ongoing virus circulation and the importance of sero-monitoring for epidemiological assessment (Parvin *et al*., 2025). District-level investigations, including studies conducted in Khulna, have further reported high morbidity and significant clinical impacts, as well as the frequent occurrence of secondary bacterial complications requiring therapeutic intervention (Begum *et al*., 2024; Kumar *et al*., 2025). Additional regional studies from northern Bangladesh (Dinajpur, Thakurgaon, Nilphamari, and Rangpur) have characterised the clinical, pathological, biochemical, and molecular features of LSD, providing valuable context for assessing its broader production and reproductive consequences (Roy *et al*., 2025a).

Evidence increasingly indicates that LSD has a pronounced negative effect on reproductive performance. Field observations and clinical studies have reported increased rates of abortion, calf losses, repeat breeding, and infertility in LSD-affected herds. A recent study from India reported a repeat-breeding rate of approximately 45.2% and an abortion rate of 13.1% in cattle affected by LSD, underscoring the disease's potential to compromise reproductive efficiency (Roy *et al*., 2025b). Despite these findings, quantitative evaluations of conception outcomes at first AI in LSD-affected versus non-affected cows remain limited, particularly under field conditions in Bangladesh, where AI is widely used in dairy development programs.

In the present study, LSD exposure was defined as the presence of clinical signs and/or serological evidence of lumpy skin disease virus infection occurring within the periconception period, specifically from 14 days before to 28 days after artificial insemination (AI).

Accurate early pregnancy diagnosis is essential for assessing reproductive outcomes and optimising herd management. Pregnancy-associated glycoprotein (PAG) enzyme-linked immunosorbent assays (ELISAs) have been validated as reliable tools for early pregnancy detection in dairy cows, with reported accuracies exceeding 90% around 27–32 days after timed AI (Silva *et al*., 2007). Comparative evaluations of commercial PAG tests, including whole-blood point-of-care assays and laboratory-based milk ELISAs, have demonstrated their practical utility for both research and on-farm applications (Safak *et al*., 2025). In settings where access to skilled ultrasonography is limited, PAG ELISA offers a robust and feasible approach for determining conception status at first AI, with ultrasound confirmation used where feasible to enhance diagnostic confidence.

Given the substantial exposure to LSDV in Bangladesh, the widespread use of AI, and the recognised links between systemic disease and impaired fertility, a focused evaluation of the impact of LSD on conception at first AI is warranted. The study hypothesised that cows experiencing clinical LSD or demonstrating evidence of LSDV exposure during the peri-conception period would have a significantly lower probability of conception at first AI compared with LSD-free cows, after adjusting for parity, breed, body condition score, timing of insemination, and herd-level factors. Demonstrating such an association would quantify an important and actionable component of LSD's economic burden and provide evidence to support targeted reproductive management, disease control, and vaccination strategies in Bangladesh. Direct studies evaluating the relationship between lumpy skin disease (LSD) and first-service artificial insemination (AI) conception rates in Bangladesh are still very limited. Therefore, to address this important research gap, the present study was conducted to investigate the association between LSD and first-service AI outcomes in cattle under field conditions in northern Bangladesh.

MATERIALS AND METHODS

Ethical approval

This research was ethically approved by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC), Institute of Biological Sciences, University of Rajshahi, with ethical approval number 110/320(48)/IAMEBBC/IBSc.

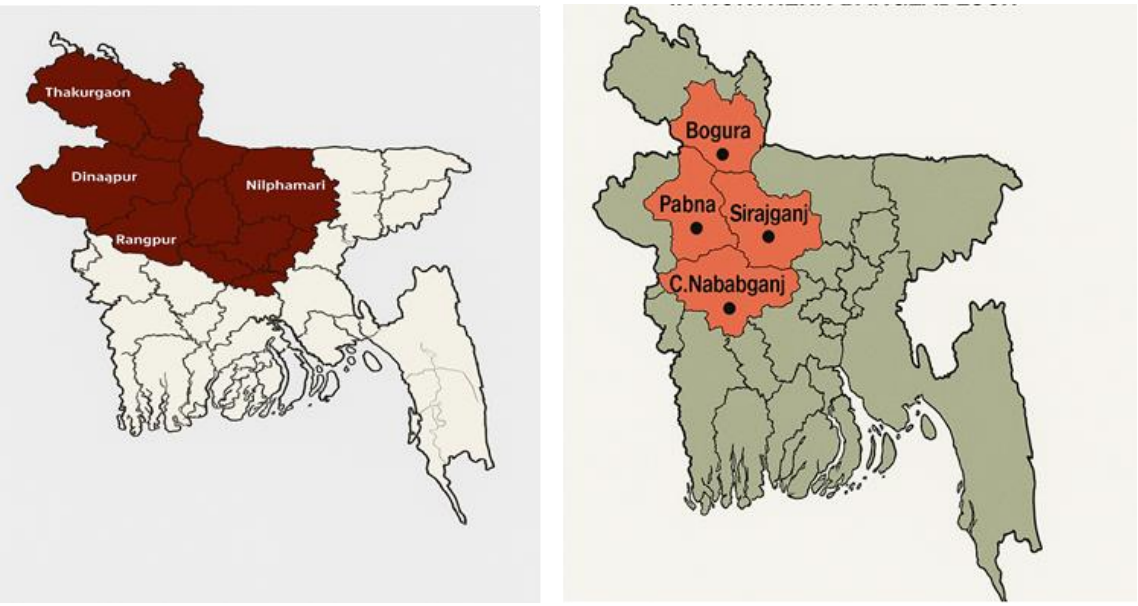

Figure 1. Geographic location with sampling.

Study design and study area

A prospective observational cohort study was conducted to evaluate the effect of lumpy skin disease (LSD) exposure during the periconception period on reproductive outcomes in dairy cattle. The study was carried out across different commercial dairy farms located in LSD-endemic regions of Bangladesh. Farms were selected based on herd size, routine use of artificial insemination (AI), and availability of reproductive and health records. The geographical regions were mentioned in Figure 1.

The study design calculation considered the following assumptions:

- Confidence level (α) = 95%
- Statistical power (1−β) = 80%
- Expected conception rate among non-exposed cows = assumed from previous field records/literature
- Expected reduction in conception rate among LSD-exposed cows = biologically meaningful detectable difference
- Two-sided hypothesis testing

The formula used was:

$$n = \frac{(Z_{\alpha/2}+Z_{\beta})^2[p_1(1-p_1)+p_2(1-p_2)]}{(p_1-p_2)^2}$$

Where: n= required sample size per group, p_1= expected conception rate in unexposed cows, p_2= expected conception rate in LSD-exposed cows, $Z_{\alpha/2}$= standard normal deviate at 95% confidence level (1.96), Z_{β}= standard normal deviate corresponding to 80% power (0.84).

Study population

A total of 942 dairy cattle presented for artificial insemination from 10 northern districts of Bangladesh were enrolled in the study. Eligible animals included clinically healthy cows and heifers with no history of reproductive disorders unrelated to LSD at the time of AI. Animals were followed from insemination until pregnancy diagnosis.

Case definition and exposure assessment (LSD)

LSD exposure was defined as clinical and/or serological evidence of LSD virus infection occurring during the periconception window, defined as 14 days before to 28 days after artificial insemination. Clinical diagnosis was based on the presence of characteristic generalised skin nodules accompanied by fever and/or lymphadenopathy, assessed through standardised field clinical scoring performed by trained veterinarians. Serological diagnosis was conducted using a commercially available LSD virus ELISA, following the manufacturer's (The company name is IDEXX Laboratories, Inc. (often referred to simply as IDEXX), which produces the Alertys Pregnancy Tests range for cattle. These tests, including the cowside Alertys OnFarm ruminant pregnancy test and Alertys Ruminant Pregnancy Test, detect pregnancy-associated glycoproteins (PAGs) in blood or milk, EPD ELISA: IDEXX,

Headquarters USA, Supplier Netherlands) instructions.

To maximise diagnostic sensitivity, combined clinical scoring and serological testing were used to classify animals as LSD-exposed or non-exposed during the defined risk window.

Sample collection and laboratory confirmation

Among clinically suspected animals, 120 skin biopsy samples were aseptically collected from active nodular lesions (PCR assay detected LSDV positivity in 28% of the tested samples). These samples were subjected to: Molecular detection of LSD virus using polymerase chain reaction (PCR), and histopathological examination to identify characteristic capripoxvirus-associated lesions.

A concise methodological PCR : Molecular detection of LSD virus (LSDV) was performed using conventional PCR targeting the capripoxvirus P32 gene. DNA was extracted from skin biopsy tissues using a commercial genomic DNA extraction kit according to the manufacturer's instructions. The primer sequences used were: Forward primer 5′-TTTCCTGATTTTTCTTACTAT-3′ and Reverse primer 5′-AAATTATATACGTAAATAAC-3′, amplifying a specific fragment of the P32 gene.

PCR amplification was carried out in a 25 µL reaction mixture containing template DNA, PCR master mix, forward and reverse primers, and nuclease-free water. Thermocycling conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50–55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes.

PCR products were analysed by 1.5% agarose gel electrophoresis, stained with ethidium bromide or an equivalent nucleic acid stain. Samples showing the expected amplicon size under UV illumination were considered positive for LSDV DNA, while samples without visible amplification were considered negative.

Selected PCR-positive samples underwent partial genome sequencing, and phylogenetic analyses were performed to confirm viral identity and genetic relatedness with circulating LSDV strains, following established protocols (Siddiqui *et al*., 2013). A phylogenetic tree was constructed based on the obtained PCR sequences to evaluate the genetic relationship of the detected LSDV strains with reference capripoxvirus isolates reported worldwide.

Reproductive management and artificial insemination

Artificial insemination was conducted according to standard practices used in Bangladesh. Briefly, healthy, cycling cows were restrained properly, and heat detection was confirmed prior to insemination. Frozen semen straws were thawed in a water bath at 35–37°C for 30–45 seconds, loaded into a pre-warmed AI gun, and deposited into the uterine body via the recto-vaginal technique under hygienic conditions. Post-insemination, animals were observed for any immediate complications and recorded for pregnancy diagnosis at the appropriate interval. Estrus detection, timing of insemination, semen handling, and inseminator identity were recorded. These procedures followed nationally accepted protocols, allowing adjustment for AI timing, technician effects, parity, breed, and farm-level management variables during statistical analysis (Siddiqui *et al*., 2013).

Semen quality parameters, including progressive motility and sperm morphology, were assessed prior to use as they are key determinants of fertility and potential confounders of conception rate. Semen samples were obtained from approved artificial insemination (AI) centres in Bangladesh, and details regarding bull breed, semen batch, and production AI station were recorded to ensure traceability and control for variability in semen source.

Clinical scoring of Lumpy Skin Disease (LSD) cases was performed using a standardised case definition and scoring guideline. To reduce observer bias, assessors were trained prior to field work and used a uniform scoring checklist during clinical examination. Where possible, clinical assessors were blinded to laboratory PCR results to minimise diagnostic expectation bias. In addition, periodic supervisory field visits and cross-checking of recorded scores were conducted as part of quality control to ensure consistency and reliability of clinical data collection.

Pregnancy diagnosis and outcome measures

Pregnancy status was determined using pregnancy-associated glycoprotein (PAG) ELISA, performed on blood samples collected 28 days post AI. The assay was conducted and interpreted according to validated sensitivity and specificity thresholds, with clear decision rules for positive, negative, and ambiguous results (Silva *et al*., 2007).

The primary reproductive outcome was first-service conception rate, defined as confirmed pregnancy following the first AI. Secondary outcomes included early embryonic loss, where follow-up data were available.

Follow-up and Data Collection: Animals were monitored from the time of insemination through pregnancy diagnosis. Structured data collection forms were used to record:

- Animal-level factors (age, parity, body condition score),
- Disease status and timing of LSD infection,
- AI-related variables.

Farm-level management practices

The selection of follow-up intervals and relevant covariates was guided by previous studies evaluating the direct effects of LSD on reproductive performance (Parvin *et al*., 2025).

Body condition score (BCS) was assessed to evaluate the nutritional status of animals using a standard 1–5 scale (1 = emaciated, 5 = obese), with increments of 0.25 where applicable. Scoring was performed by trained assessors following a standardised visual and palpation-based method focusing on key anatomical regions such as ribs, spine, and tail head. Prior to field data collection, assessors underwent training to ensure a uniform understanding of the scoring criteria. To minimise observer bias, inter-rater reliability was evaluated through joint scoring sessions, and consistency among assessors was maintained throughout the study period.

Statistical analysis

All data were analysed using SPSS software (Version 26.0). Descriptive statistics were generated for all variables. Associations between LSD exposure and conception outcomes were evaluated using chi-square or Fisher's exact tests, as appropriate. Multivariable logistic regression models were constructed to estimate the effect of LSD exposure on first-service conception while controlling for potential confounders, including parity, AI technician, farm, and vaccination status (Siddiqui *et al*., 2013). Results were expressed as odds ratios with 95% confidence intervals, and statistical significance was set at $p<0.05$.

Data were analysed using Fisher's Exact Test to compare categorical variables among study groups. For multiple pairwise comparisons, a post-hoc Bonferroni correction was applied to adjust the significance threshold and minimise the risk of Type I error. Statistical significance was considered at adjusted p-values, with *** indicating $p<0.001$. The Regular Vaccination group was used as the reference category for comparison. Effect size was estimated using the Effect Size Ratio, calculated as: (Healthy Group CR) / (Intervention Group CR). The parameter CR1% was defined as the first-service conception rate. All statistical analyses were performed using appropriate statistical software.

RESULTS AND DISCUSSION

LSD remains a major emerging threat in Northern Bangladesh, with high animal-level (21.3%) and herd-level (55.1%) prevalence (Figure 2). Mortality (4.3%) and case-fatality (22.2%) rates indicate substantial disease impact, especially among calves and certain crossbred types. Vector abundance, particularly biting midges, stable/horse flies, and tabanids, significantly increases LSD risk across seasons (Figure 3). Farm practices such as introducing new animals (AOR = 1.36) and cattle movement (AOR = 1.32) further elevate outbreak probability (Figure 4). Breed composition influences reproductive outcomes; HF-87.5% cows showed the highest exotic-type conception rate (82.7%), while Sahiwal crossbreds (67.5% exotic blood) achieved the highest FAI success (84.2%). Therapeutic management and timely supportive care improve recovery but vary by clinical form. Farmers demonstrated a level of good knowledge (52.8%), and positive attitudes (68.0%) among farmers were generally comparable with previous regional studies, whereas the lower level of adequate preventive practices (35.3%) remained a common challenge reported in similar settings (Figure 5). The study provides the first integrated evaluation of epidemiology, reproduction, and farmer behaviour for LSD in Bangladesh. Strengthened farm-level biosecurity, targeted farmer training, and improved awareness are essential to reduce the LSD burden.

The analysis reveals that vaccination status has the most significant impact on bovine reproductive efficiency, with vaccinated cattle showing 4.6× higher conception rates than their non-vaccinated counterparts ($p<0.001$). Disease status also substantially affects outcomes, with disease-affected cattle showing 1.5× lower conception rates than healthy cattle ($p<0.001$). Deworming shows a positive trend but lacks statistical significance, likely due to the small sample size in the non-dewormed group (n=4). These findings emphasise the critical importance of vaccination protocols and disease prevention in maximising reproductive efficiency in dairy operations (Tables 1 and 2).

Both Chi-square and Fisher's exact tests demonstrated a highly significant association between LSD status and conception rate ($\chi^2 = 86.28$, df = 1, $p < 0.001$; Fisher's exact $p < 0.001$). There is a highly significant association between LSD status and conception rate ($p < 0.001$). The odds of conception in non-affected cows are about 4.9 times higher than in LSD-affected cows, and this difference is statistically significant ($p < 0.001$) (Table 3). Reporting 95% confidence intervals helps assess the precision and statistical significance of the estimates. Cows that recovered from LSD, which implies difficulty in conceiving on the first AI and lower first service conception rates after infection. One investigation (in Bangladesh) reported repeat breeding rates of ~45.2% in LSD-affected cattle, suggesting lower conception success at first AI (Muktafi *et al*., 2024). The study revealed a strong and statistically significant correlation between First Artificial Insemination (FAI) outcome and the occurrence of Lumpy Skin Disease (LSD) in dairy cows of Northern Bangladesh. Our findings demonstrate that LSD has a marked negative effect on the conception rate of first artificial insemination, highlighting the disease as a critical reproductive

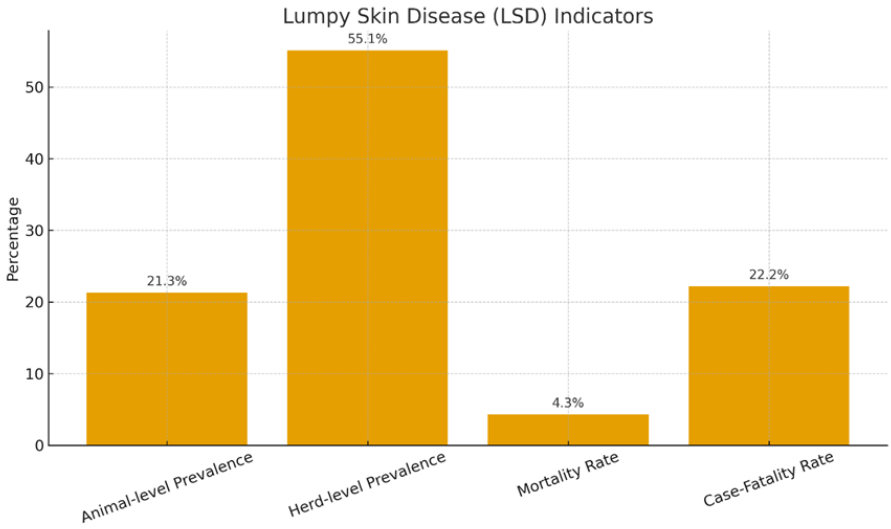

Figure 2. Animal-level prevalence of LSD.

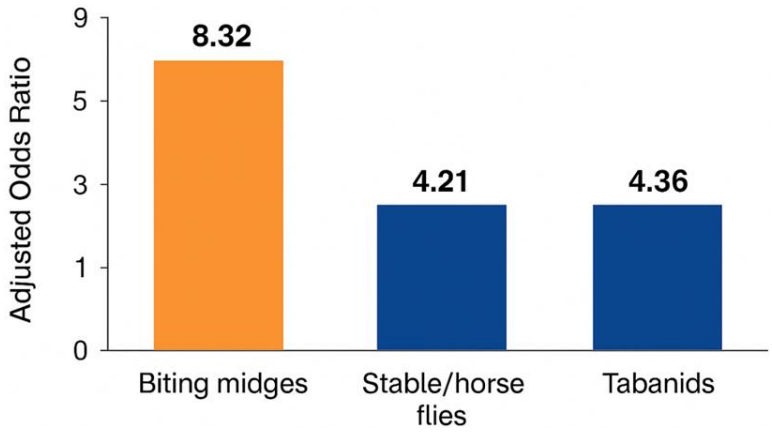

Figure 3. Seasonal vector abundance significantly increased risk.

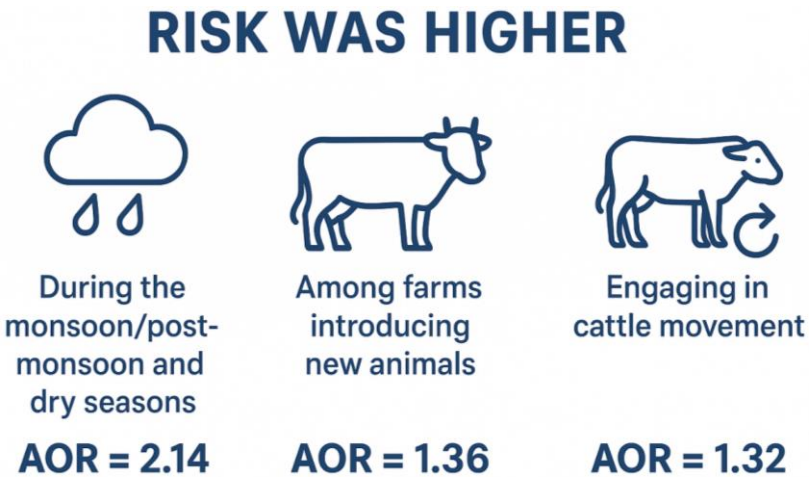

Figure 4. Risk assessment.

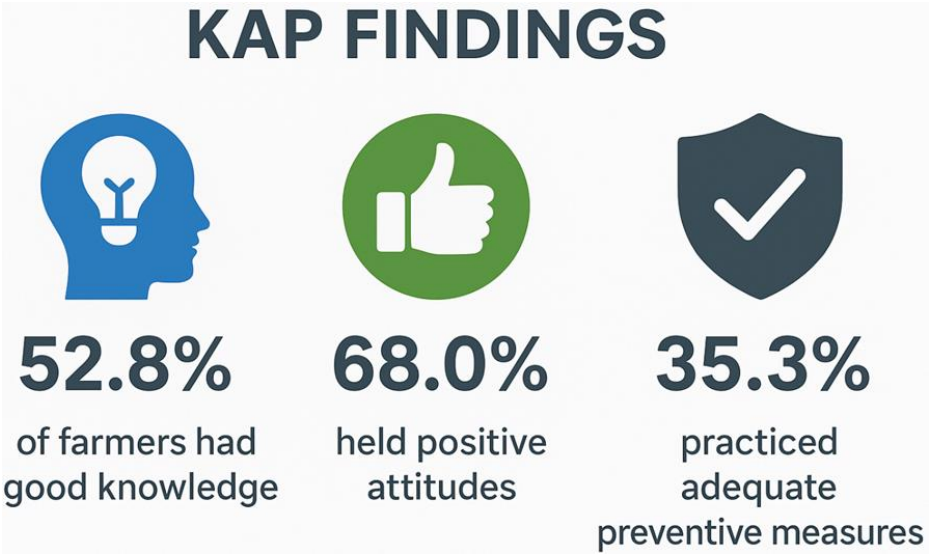

Figure 5. Knowledge, Attitude & Practice (KAP) Level I measure of dairy farmers.

Table 1. Advanced analysis of bovine reproductive efficiency by health status.

Health intervention status	Reproductive performance metrics			Comparative analysis	
	AI attempts (n)	Successful conceptions (n)	Conception rate (CR1%)	Effect size ratio	Statistical significance
Regular Vaccination	163	149	**91.4**	Reference	
Non-Vaccination	05	01	**20**	4.6	p < 0.001***
Cow-non-affected Diseases	78	57	73.1	Reference	
Cow-affected Diseases	140	68	**48.6**	1.5	p < 0.001***

Statistical analysis: Fisher's Exact Test with Bonferroni correction; *** p<0.001; Effect Size Ratio = (Healthy Group CR)/(Intervention Group CR); Reference Group: Regular Vaccination; Key: CR1% = First-service conception rate.

Table 2. Conception rate based on breed.

Breed	AI attempts	Successes	Failures	Success rate (%)	% of Total AI	% of Total Successes	p-value
HFC	667	417	250	62.4	71.2	68.7	0.0488
SLC	259	180	79	69.5	27.1	29.2	
RC	16	13	3	81.2	1.7	2.1	

Statistical Summary: Chi-square test: $X^2 = 6.04$, df = 2, p = 0.048816.

constraint in this region. Analysis of reproductive records showed that cows affected by LSD around the time of breeding exhibited a substantially lower conception rate following FAI compared to non-infected cows. The strength of the correlation indicates that LSD is not merely an incidental health condition but a major determinant of reproductive failure at first service. This strong association suggests that the physiological and systemic impacts of LSD directly interfere with the cow's ability to conceive following insemination.

LSD is commonly associated with high fever, anorexia, and systemic inflammatory responses. Elevated body temperature during the peri-insemination period can impair oocyte quality, disrupt fertilisation, and negatively affect early embryonic development. LSD-infected cows often experience reduced feed intake and body condition loss. Negative energy balance is well known to delay ovarian activity and lower conception rates, particularly at first insemination. The stress response induced by LSD may alter the secretion of key reproductive hormones such as

Table 3. Association between lumpy skin disease (LSD) status and conception rate in cows.

LSD status	Conceived	Not conceived	Total	p-value
Cow-non affected	488	191	679	p < 0.001
Cow-affected	108	155	263	

Table 4. Conception outcomes in cattle according to lumpy skin disease vaccination status.

Vaccination	Conceived	Not conceived	Total AI	CR1 (%)	p-value
LSD Vaccinated	288	107	395	72.9%	p < 0.001
Non-vaccinated	257	290	547	47.0%	

Conception rate was significantly higher in LSD-vaccinated cattle (72.9%) compared with non-vaccinated cattle (47.0%) (χ^2 = 62.18, df = 1, p < 0.001).

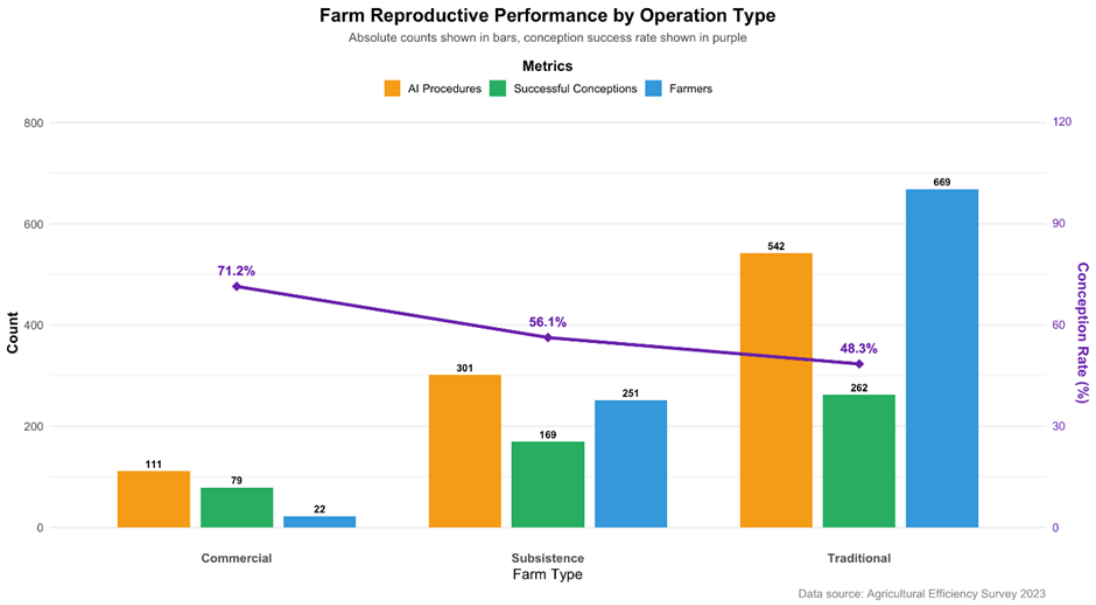

Figure 6. Conception rate based on farm type or management.

GnRH, LH, and progesterone. Hormonal disruption around estrus and early luteal phases can compromise ovulation, fertilisation, and embryo implantation. The difference in conception rate (CR1%) between LSD-vaccinated and non-vaccinated cattle is highly statistically significant (p < 0.001). LSD vaccinated cattle show a substantially higher conception rate (72.9%) than non-vaccinated cattle (47%) (Table 4). Previously published studies, Muktafi *et al.* (2024), reported that the vaccinated cow conception rate was 32.3%. Our study strongly reported that there was a strong association between LSD and reproduction and milk production of cows, where we found that LSD-infected cow milk production was reduced, and conception rate was also lower due to delayed estrus, reduced fertility, and infertility in cows and bulls. Indigenous cattle achieved the highest conception rate (81.2%), while purebred Holstein Friesian (HF) and Sahiwal Cross (SLC) cows performed less well, likely due to reduced adaptability to Bangladesh's climate and nutritional conditions. This aligns with Bahar *et al*. (2024), who reported a 63.8% conception rate in indigenous breeds. In Bangladesh in 2024, one study was conducted where they found the highest success rate of SLXF 97.1% and SL 95%, which supports our findings (Siddiqui *et al*., 2013; Bahar *et al*., 2024). The significantly higher conception rate observed in commercial farms (71.2%) compared to subsistence (56.1%) and traditional farms (48.3%) indicates that farm management practices play an important role in reproductive performance (Figure 6). In previously published studies in Bangladesh, there were several

studies on FAI and LSD, but no studies found the association between FAI and conception rate with LSD. Our novel findings stated that a strong association exists between FAI and LSD, which significantly affects the conception rate of cows in northern Bangladesh.

Conclusion

This study demonstrates that Lumpy Skin Disease remains highly prevalent in Northern Bangladesh and is associated with substantial impacts on dairy cattle health and reproductive performance. Vector abundance, introduction of new animals, and uncontrolled cattle movement were identified as important epidemiological risk factors for disease occurrence. Although farmers showed moderate awareness regarding LSD, implementation of preventive biosecurity and vector-control practices was limited. A key finding of this study was the significant association between LSD and failure of the first artificial insemination (FAI). Cows affected by LSD had approximately 4.9 times higher odds of FAI failure compared with non-affected cows, indicating a considerable negative effect of the disease on reproductive efficiency. This finding has important clinical and economic implications because the first-service conception rate is a major determinant of dairy herd productivity and profitability in Bangladesh. Since artificial insemination is widely used to improve genetic merit and production performance, the persistence of LSD may substantially reduce the effectiveness of breeding programs.

Overall, the findings highlight the need for strengthened LSD prevention and control measures, including improved farm biosecurity, vector management, farmer training, and disease surveillance, to protect both animal health and reproductive productivity in dairy farms of Northern Bangladesh.

CONFLICT OF INTERESTS

The authors have no conflicts of interest.

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