

ASSOCIATION OF COW-LEVEL FACTORS WITH MASTITIS OCCURRENCE IN DAIRY CATTLE IN SAMDRUPJONGKHAR DISTRICT

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ABSTRACT: *Mastitis continues to be a major concern impacting dairy production and herd productivity in Bhutan, and evidence on cow-level factors associated with mastitis is limited. Routine surveillance data offer an opportunity to explore host-related associations under field conditions, to assess the association between selected cow-level factors (age, parity, and breed) and mastitis occurrence among dairy cattle. A cross-sectional study was conducted using routine mastitis surveillance data from 355 lactating cows screened across six sub-districts under Samdrupjongkhar District. Mastitis status was determined using the California Mastitis Test (CMT), with cows classified as positive if at least one udder quarter tested positive. Age and parity were categorized into biologically relevant groups, and breed was classified as Pure Jersey or Jersey cross on phenotypic characteristics. Firth penalized logistic regression was applied to address sparse outcome events and unequal subgroup sizes. Odds ratios (OR) with 95% confidence intervals (CI) were estimated, and model fit was assessed using the likelihood ratio test. The overall mastitis prevalence was 12.1% (43/355). Unadjusted prevalence was higher among Pure Jersey cows (42.1%) compared with Jersey cross cows (10.4%). In the multivariable Firth regression model, breed was the only factor significantly associated with mastitis occurrence. Jersey cross cows had markedly lower odds of mastitis compared to Pure Jersey cows (OR = 0.15, 95% CI: 0.06-0.42, $p < 0.001$). Age and parity were not significantly associated with mastitis. The overall model was statistically significant (LR $\chi^2 = 19.55$, $df = 6$, $p = 0.0033$). Mastitis occurrence in the study population was relatively low, and breed was the only cow-level factor significantly associated with mastitis. Due to the use of routine surveillance data and a limited number of positive cases, the findings should be interpreted cautiously. Further studies incorporating larger sample sizes and management-level risk factors are recommended to better characterize mastitis epidemiology in Bhutan.*

Keywords: Age; Breed; Dairy cattle; Firth logistic regression; Mastitis; Parity; Samdrupjongkhar; Surveillance.

1. INTRODUCTION

Bovine mastitis is the inflammation of the mammary glands and udder tissues due to microbial infections or physical injury (Dhital et al. 2026). Globally, mastitis remains a common cattle disease and an economically significant disease in dairy cattle, causing economic losses due to decreased milk production and reduced milk quality (Dhital et al., 2026). The economic

losses due to mastitis are estimated at around USD 147 per cow per year and USD 146 billion worldwide (Cheng and Han 2020; Dhital et al. 2026)

Depending on the visibility of the effects of inflammation of the mammary gland, mastitis can be classified as clinical and subclinical. In clinical mastitis (CM),

symptoms like swelling, heat, hardness, redness, and pain on the udder, as well as a change in the colour and consistency of milk are observed. However, subclinical mastitis (SCM) does not produce any visible effects on the udder or milk quality (Abdulkhader et al. 2022). Among its forms, subclinical mastitis is the most widespread, causing decreased milk yield and increased somatic cell levels, and an increased risk of developing clinical mastitis during lactation, even in the absence of obvious symptoms (Kumar et al. 2025).

Bacterial intra-mammary infection (IMI) is considered to be the main cause of bovine mastitis; however, the host factors, including breed, age, and parity, affect susceptibility and resistance to mastitis (Cheng and Han 2020).

In Bhutan, 78.0% of the Bhutanese population practices dairy farming (Tamang and Bhujel 2025). The high prevalence of subclinical and clinical mastitis was reported at 67.0% and 20.7%, respectively by Rai et al. (2018). The common prevalence of the condition in dairy cattle underscores the need to identify host factors that may predispose cows to mastitis. Assessing the association between host factors, including age, breed, and parity, and mastitis occurrence may improve understanding of their contributory role in disease susceptibility.

Thus, the study was conducted to assess the association between selected cow factors and the occurrence of mastitis in dairy cattle in Samdrupjongkhar District.

2. MATERIALS AND METHODS

2.1 Study area and population

The study was conducted in six Sub-districts under Samdrupjongkhar District (Figure 1). The district lies in the subtropical climate

zone, at elevations ranging from 200 to 3,600 meters above sea level (National Center for Hydrology and Meteorology [NCHM] 2025). During the peak summer months, temperatures in the district range from a minimum of 14°C to a maximum of 36°C, with a mean annual temperature of 23.8°C (NCHM2025). The region receives substantial rainfall, with an average annual precipitation of 5,309.4 mm recorded at the Arong station, contributing to its lush environment (NCHM2025).

The district has a total cattle population of 10,670, of which 3,024 are milking cows, producing 2,310.43 metric tons (MT) of milk in 2024 (Integrated Agriculture and Livestock Census of Bhutan 2025). A total of 42 dairy groups are present in the district (Commercial Agriculture Resilient Livelihood Enhancement Programme, Project Endline Survey Report 2025).

Lactating dairy cows belonging to different dairy farmer groups in the study Sub-districts were screened for mastitis as part of routine surveillance activities.

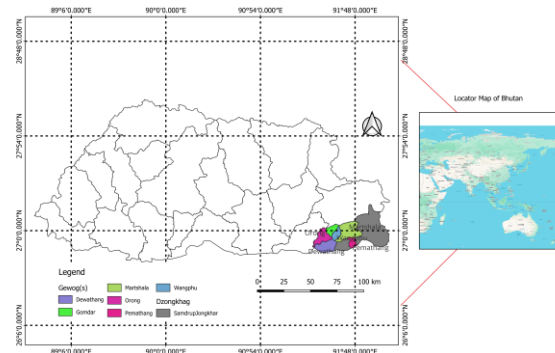


Figure 1: Map showing the study areas.

A total of 358 lactating cows of varying breeds were screened during the study period. Due to the very small number of Holstein Friesian and local breed cows present in the area at the time of screening,

these breeds were excluded from the analysis. Consequently, 355 lactating Jersey cows classified as Pure Jersey and Jersey cross cows were included in the final study population (Table 1).

Table 1: Number of dairy cow samples screened for mastitis in Samdrupjongkhar District.

Sub-district	Total Tests
Orong	146
Dewathang	73
Wangphu	55
Gomdar	36
Pemathang	35
Martshala	13

2.2 Study design

This was a cross-sectional study based on secondary analysis of data collected during routine mastitis screening activities conducted by the Regional Veterinary Hospital and Epidemiology Center (RVH&EC), Dewathang, as part of its surveillance program. The screening was not specifically designed to investigate associations with cow-level factors; however, the existing dataset was utilized to explore potential relationships between age, parity, breed, and mastitis occurrence.

All lactating cows presented during the screening period were eligible for inclusion. Lactating cows with apparently healthy udders, cows suspected of subclinical mastitis (e.g., reduced milk yield), or cows showing clinical signs of mastitis were included for screening. Inclusion of cows across the full disease spectrum enabled assessment of mastitis prevalence and its association with host-related factors under field conditions.

2.3 Data collection

Data were collected during the routine subclinical and clinical mastitis screening

program conducted between October 2023 and October 2024 by the RVH&EC, Dewathang. The surveillance was conducted to determine the prevalence of mastitis cases to enable early treatment intervention and to guide affected farmers on appropriate management practices. Data were collected using a structured, closed-ended questionnaire developed in the Epicollect5 mobile app. Detailed information on owner-level and animal, including age, parity, and breed were recorded. Each udder quarter-Left Fore (LF), Right Fore (RF), Left Hind (LH), and Right Hind (RH)-were assessed, and the findings were classified and recorded as 'Negative' and 'Positive' against each quarter. The breed, particularly Jersey cows, was classified phenotypically as Pure Jersey (purebred) and Jersey cross (crossbred) based on observable morphological traits.

2.4 Mastitis diagnosis

Mastitis screening was performed on all lactating cows presented during the screening program using the California Mastitis Test (CMT). A detailed anamnesis was recorded. The udder quarters were physically examined individually to identify clinical signs of mastitis, including swelling, heat, pain, and induration of the udder.

Quarters showing no apparent clinical signs were classified as apparently healthy and were screened for subclinical mastitis using the CMT. Thereafter, quarters exhibiting clinical signs of mastitis were examined. Testing of apparently healthy quarters was conducted prior to affected quarters to minimize the risk of cross-contamination.

Milk from each quarter was tested for subclinical mastitis using the CMT

following the standard procedure described by National Mastitis Council (1999).

The absence of reaction in the milk sample on a cup of CMT paddle was classified as negative. Similarly, the appearance of slight thickening (weak positive) to strong gel formation (strong positive) was classified as a positive sample. This binary classification is commonly used in epidemiological studies to estimate prevalence and analyze risk factors, reducing subjectivity in scoring (Sharma et al. 2018).

2.5 Data analysis

Age was categorized into three groups: 1-3 years (>1 to ≤ 3 years), 3-5 years (>3 to ≤ 5 years), and >5 years. Parity was categorized as parity 1 (first parity), parity 2, parity 3-4, and ≥ 5 parity. Overall mastitis status for each cow was determined binarily: 'Positive' if any quarter tested positive, and 'Negative' if all quarters were negative. The data cleaning was performed using the standard *R* function.

Breed was categorized as Pure Jersey and Jersey cross. Although other breeds, including local and Holstein Friesian cattle, were recorded, they were excluded from the inferential analysis due to their small sample size.

Descriptive statistics were used to summarize cow-level characteristics, including mean age, parity distribution, and breed. Mastitis prevalence was calculated as the proportion of positive cases among the examined lactating cows and was estimated overall, as well as stratified by breed, age group, and parity group. Differences in mastitis prevalence between breeds were assessed using Fisher's exact test due to small expected cell counts.

Inferential analysis was performed using Firth logistic regression to assess

associations between mastitis status (binary outcome) and explanatory variables (age group, parity group, and breed).

Odds ratios (OR) with 95% confidence intervals (CI) were estimated using 1-3 years for age, parity 1, and Jersey breed as reference categories. Model significance was evaluated using likelihood ratio tests. All statistical analyses were conducted in *R* software (version 4.3.2) using the *logistf*, *dplyr*, *knitr*, *janitor*, *tibble*, and *flextable* packages. Results were considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Mean age and parity distribution

The mean age of cows was similar between breeds, with Pure Jersey averaging 7.22 ± 2.84 years and Jersey cross 7.19 ± 3.97 years (Table 2). Median parity was 3 for both breeds, although Jersey cross exhibited a wider parity range (1-15) compared to Pure Jersey (1-11), indicating greater variability in lactation experience within the Jersey cross group.

Table 2: Age and parity distribution of dairy cows by breed in the study population.

Breed	Age (years, Mean \pm SD)	Median Parity (Range)
Pure Jersey	7.22 ± 2.84	3 (1–11)
Jersey cross	7.19 ± 3.97	3 (1–15)

3.2 Prevalence of mastitis

3.2.1 Overall prevalence of mastitis

Out of 355 lactating cows sampled across six sub-districts, 43 cows were positive for mastitis, resulting in an overall prevalence of 12.1% in the studied population (Table 3).

The majority of cows (312/355; 87.9%) were mastitis-negative. The highest mastitis prevalence of 38.9% (14/36) was observed in Gomdhar sub-district (Figure 2).

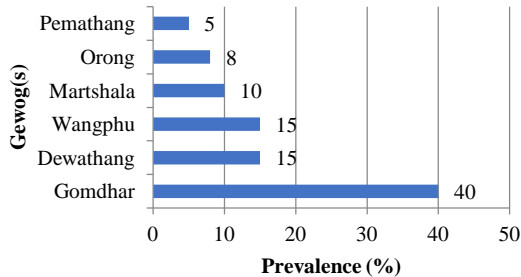


Figure 2: Prevalence of mastitis by sub-district (in %) during the screening period.

Table 3: Overall mastitis prevalence among lactating dairy cows in the study population under Samdrupjongkhar District.

Total lactating cows studied	Positive	Negative	Prevalence (%)
355	43	312	12.1

3.3 Mastitis prevalence (Breed-wise)

Mastitis prevalence differed notably between breeds. Overall, 42.1% of Pure Jersey cows and 10.4% of Jersey cross cows were positive for mastitis (Table 4). This unadjusted difference was statistically significant (Fisher’s exact test, $p < 0.001$), with Jersey cross cows having markedly lower odds of mastitis compared to Jersey cows (OR = 0.16, 95% CI: 0.05–0.49).

Table 4: Distribution of mastitis status among dairy cows by breed.

Breed	Yes (mastitis+)	No (mastitis-)
Pure Jersey	42.1%	57.9%
Jersey	10.4%	89.6%

cross		
Total	12.1% (43)	87.9% (312)

3.4 Mastitis prevalence by breed and age group, and parity group

Mastitis prevalence varied across breed and age groups. Among Pure Jersey cows, mastitis prevalence was highest in cows older than 5 years (46.2%), followed by 33.3% in both the 1–3 years and 3–5 years age groups. Among Jersey cross cows, prevalence was generally lower, ranging from 6.9% in 3-5 years to 12.8% in cows >5 years, with 7.6% prevalence observed in 1-3-year-old cows. These results indicate that mastitis prevalence tends to be higher in older cows (>5 years) in both breeds (Figure 3).

Mastitis prevalence was higher in Pure Jersey (33.0-50.0%) compared to Jersey cross (7.0-18.0%), suggesting breed susceptibility. Prevalence tended to increase with parity in both breeds, particularly in cows with ≥ 5 calvings (Figure 4).

3.5 Firth penalized logistic regression result

The overall Firth logistic regression model was statistically significant based on the Likelihood Ratio test (LR $\chi^2 = 19.55$, $df = 6$, $p = 0.0033$), and indicated that the predictors collectively improved model fit compared with a null model.

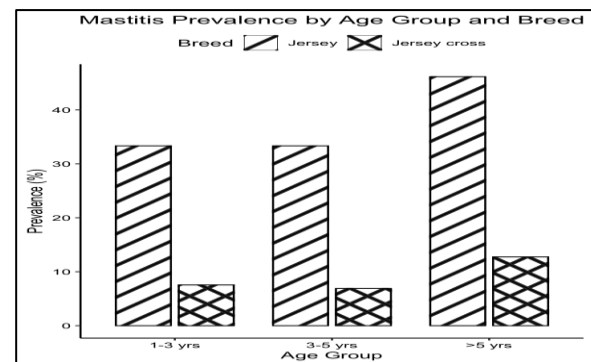


Figure 3: Prevalence (%) of positives in cows by Breed across Age Groups (1-3 yrs, 3-5 yrs, >5 yrs).

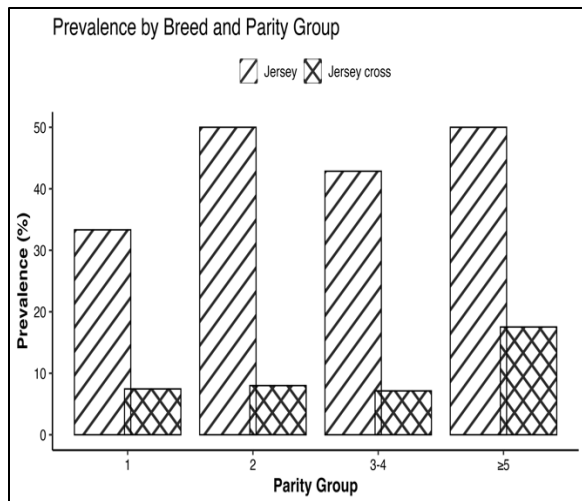


Figure 4: Prevalence (%) of positives in cows by breed across parity Groups (1, 2, 3-4, ≥5).

When individual predictors were assessed, age and parity were not significantly associated with mastitis occurrence. Compared to cows aged 1-3 years, those aged 3-5 years (OR = 1.05, 95% CI: 0.17-5.27, $p = 0.950$) and >5 years (OR = 1.84, 95% CI: 0.21-15.03, $p = 0.575$) had similar odds of mastitis. Similarly, parity was not a significant predictor; cows with parity 2 (OR = 0.92, 95% CI: 0.19-5.58, $p = 0.916$), 3-4 (OR = 0.56, 95% CI: 0.08-4.83, $p = 0.584$), or ≥5 (OR = 1.29, 95% CI: 0.17-11.36, $p = 0.806$) showed no significant differences compared with first-parity cows (Table 5).

After adjusting for other factors in the Firth logistic regression model, Jersey cross cows had markedly lower odds of mastitis compared to Pure Jersey cows (OR = 0.15, 95% CI: 0.06-0.42, $p < 0.001$). Pure Jersey was used as the reference category.

3.6 Discussion

In this study, mastitis prevalence was evaluated in dairy cows, and associations with age, parity, and breed were assessed using Firth logistic regression.

Table 5: Multivariable Firth logistic regression analysis of host-related factors associated with mastitis in lactating dairy cows.

Variables	OR	95% CI	P-value
Age group			
1-3 yrs (Ref)	-	-	-
3-5 yrs	1.05	0.17-5.27	0.950
>5 yrs	1.84	0.21-15.03	0.575
Parity group			
1 (Ref)	-	-	-
2	0.92	0.19-5.58	0.916
3-4	0.56	0.08-4.83	0.584
≥5	1.29	0.17-11.36	0.806
Breed			
Pure Jersey (Ref)	-	-	-
Jersey cross	0.15	0.06-0.42	<0.001

Overall, mastitis was present at low levels in the population, and breed was identified as the only predictor associated with mastitis in the multivariable model in this study, although the small number of Pure Jersey cows and positive events may affect the precision of this association.

The observed association between breed and mastitis occurrence, with Jersey cross cows showing lower odds compared to Pure Jersey cows, aligns with evidence that genetic and breed differences influence susceptibility to mastitis in dairy cattle. Genetic studies have identified loci associated with mastitis resistance across multiple breeds, highlighting the role of genetic background in disease susceptibility (Cai et al. 2024). Furthermore, breed-dependent differences in immune responses

and mastitis prevalence have been reported, supporting the biological plausibility of breed effects on mastitis risk (Bannerman et al. 2008).

Although age and parity were not significantly associated with mastitis in this study, this does not contradict existing evidence. The lower aged-group's prevalence among Jersey cross cows and the increase in mastitis prevalence (12.76%, 25/196) with higher parity (>5 yrs) are consistent with previous epidemiological studies and meta-analyses reporting age and parity-related increases in mastitis risk (Chen et al. 2023).

The mastitis prevalence trends among Jersey cross cows and older age groups align with expectations reported in the literature (Abebe and Bakala 2019; Khasapane et al. 2023). However, in this study, mastitis prevalence among Pure Jersey cows aged 1–3 years was similar to that of cows aged 3–5 years, which contrasts with the commonly reported increase in mastitis risk with advancing age. This finding is influenced by the small number of Pure Jersey cows in this age group and a positive event recorded during the screening period in this group, which may have inflated prevalence estimates. Therefore, the age-specific findings in young Pure Jersey cows should be interpreted with caution.

In this study, breed was associated with mastitis, with Jersey cross cows showing lower odds compared to Pure Jersey cows (OR = 0.15, 95% CI: 0.06-0.42, $p < 0.001$). The overall mastitis prevalence was higher in pure Jersey cows (33.0-50.0%), which is consistent with the finding reported by Fesseha et al. (2021). Age and parity were not significantly associated with mastitis. Overall, the age and parity distributions were comparable between breeds in this

study, suggesting that differences in mastitis prevalence are unlikely to be confounded by age or parity.

The overall model was statistically significant based on the Likelihood Ratio (LR) test (LR $\chi^2 = 19.55$, $df = 6$, $p = 0.0033$), indicating that the set of predictors collectively improved model fit compared with the null model (Hosmer et al. 2013). Such discrepancies are observed to be common in multivariable analyses with limited event counts or correlated covariates, where individual predictors may not reach statistical significance despite contributing to overall model performance (Dohoo et al. 2012).

The lack of significance in this model reflects limited statistical power due to a relatively low number of positive cases in each category, as well as the use of routine surveillance data rather than purpose-designed epidemiological sampling.

In the Firth logistic regression analysis, cows were categorized into age groups of 1-3, 3-5, and >5 years, and parity groups of 1, 2, 3-4, and ≥ 5 , with the youngest age group (1-3 years) and first-lactation cows (parity 1) set as reference categories. This categorization is biologically meaningful, as 1-3 years-old cows and first-lactation cows generally have the lowest cumulative udder exposure and are expected to have lower mastitis risk compared with older or higher-parity cows. Using these reference categories allows the resulting odds ratios to be interpretable in terms of increasing age and parity, and aligns with common practice in mastitis epidemiological studies that stratify cows by age and lactation history to assess risk patterns (Khasapane et al. 2023; Sarba and Tola 2017).

The use of Firth logistic regression was appropriate given the small number of mastitis-positive cases and the very low count of Pure Jersey cows, which could have caused separation or instability in standard logistic regression. By applying penalized likelihood estimation, Firth regression provided more reliable odds ratio estimates, confidence intervals, and *p*-values, reducing bias associated with sparse events and unequal distribution of cases across breed categories (Heinze and Schemper 2002; Wang 2014). This methodological choice strengthens confidence in the observed significant association between breed and mastitis despite the rarity of the outcome and the large difference in sample sizes between the breed categories.

4. CONCLUSION

This study assessed the association between selected cow-level factors and mastitis occurrence among lactating dairy cows in Samdrupjongkhar district using routine surveillance data. The overall prevalence of mastitis was relatively low in the study population. Among the variables examined, breed was the only factor significantly associated with mastitis occurrence, with Jersey cross cows showing lower odds of mastitis compared with Pure Jersey cows. Age and parity were not significantly associated with mastitis in the multivariable analysis.

These findings should be interpreted cautiously due to the limited number of mastitis-positive cases and the small number of Pure Jersey cows included in the analysis. Nevertheless, the results suggest that breed-related differences may influence mastitis susceptibility under field conditions in the study area. The use of Firth penalized logistic regression allowed for more reliable estimation of associations in the presence of sparse data and unequal subgroup sizes.

Further studies incorporating larger sample sizes and a broader range of management- and farm-level risk factors are warranted to better elucidate the epidemiology of mastitis in dairy cattle populations in Bhutan and to inform evidence-based mastitis control strategies.

5. Limitations

This study has some limitations that should be considered when interpreting the findings. The analysis was based on routine mastitis surveillance data rather than a study specifically designed to investigate risk factors, which may limit causal inference and introduce selection bias related to farmer participation and cow availability during screening.

Furthermore, the relatively small number of mastitis-positive cases, particularly among Pure Jersey cows, may have limited statistical power and resulted in imprecise estimates for some predictors. Although Firth penalized logistic regression was applied to reduce small-sample bias and address data sparsity, the observed associations-especially those related to breed-should be interpreted with caution. Additionally, breed classification was based on phenotypic characteristics rather than pedigree or genetic information, which may have led to some degree of misclassification.

Despite these limitations, the study provides useful baseline information on mastitis prevalence and cow-level associations under field conditions and highlights areas for future, more comprehensive epidemiological investigations.

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