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Subclinical Mastitis and Antibiotic Resistance in Dairy Cows, Bharatpur, Nepal

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ABSTRACT

Subclinical mastitis is a significant health concern in dairy cows, leading to decreased milk production and quality. This study aimed to determine the prevalence of subclinical mastitis in lactating cows from Bharatpur Municipality, Chitwan, Nepal, and assess the antibiotic resistance patterns of pathogens isolated. A total of 200 milk samples from 50 cows (Jersey, Holstein, and crossbreeds) were tested using the California Mastitis Test (CMT), with 30 samples (15%) testing positive. The prevalence of subclinical mastitis was higher in older cows and those with higher parity, with Jersey cows showing an increasing trend from 34.62% (3-6 years) to 100% (9 years or above). Staphylococcus aureus was the most frequently isolated pathogen (53.85%), followed by Coagulase-negative Staphylococci (CONS) and Bacillus spp. The antibiotic susceptibility test revealed that Staphylococcus aureus isolates were most sensitive to gentamicin (100%), followed by doxycycline and cotrimoxazole. These findings highlight the need for regular monitoring of mastitis and prudent use of antibiotics to prevent resistance.

Keywords: Suclinical Mastitis, Antibiotic Resistance, Staphylococcus aureus, Cow.

Introduction

Subclinical mastitis, an inflammation of the mammary gland in dairy cows, is a major issue affecting dairy production globally. Unlike clinical mastitis, subclinical mastitis is asymptomatic but leads to significant economic losses due to reduced milk yield, altered milk composition, and increased veterinary costs (Smith & Hogan, 1995). The condition is commonly caused by bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus* species (Radostits et al., 2007).

Subclinical mastitis is a prevalent issue in dairy cows, leading to significant economic losses due to decreased milk production, lowered milk quality, and increased treatment costs (Bradley, 2002; Ruegg, 2017). The silent nature of subclinical mastitis means it often goes undetected, allowing the infection to persist and spread within herds (Smith & Hogan, 1995).

The emergence of antibiotic-resistant strains among these pathogens has become a major concern for veterinary medicine and public health. Widespread and often inappropriate use of antibiotics in animal husbandry has accelerated the development of resistance, making treatment difficult and less effective (Bradley, 2002). Monitoring antibiotic resistance patterns in pathogens from subclinical mastitis cases is crucial to guide effective treatment and reduce the reliance on antibiotics, which contributes to antimicrobial resistance (Oliver & Murinda, 2012; WHO, 2021).

The widespread use of antibiotics to treat this condition has led to the emergence of antibiotic-resistant pathogens, making it more difficult to control and treat mastitis (Oliver & Murinda, 2012; WHO, 2021). Despite the significant impact of antibiotic resistance in veterinary medicine, there is limited data on the specific antibiotic resistance patterns of pathogens isolated from subclinical mastitis cases in cows in many regions, including Nepal. A clear understanding of the resistance profiles of these pathogens is essential to guide treatment decisions and develop strategies to mitigate the spread of antimicrobial resistance.

Thus, this study aims to analyze the antibiotic resistance patterns of pathogens isolated from subclinical mastitis cases in cows, providing insights into the prevalence of resistant strains and the effectiveness of current treatment protocols.

Methodology

Study Area:

This study was conducted on lactating cows from Bharatpur Municipality, Chitwan district, Nepal, be-

tween April 22 and June 25, 2016. Milk samples were collected from 50 dairy cows (34 Jersey, 12 Holstein, and 4 Jersey-Holstein crossbreeds), with a total of 200 quarters sampled.

Sampling and Sample Site:

A pre-tested questionnaire was filled out by farmers whose cows were suspected of having subclinical mastitis. Out of 50 cows tested, 30 milk samples tested positive for subclinical mastitis and were sent to the Microbiology Laboratory at Balkumari College for pathogen isolation and identification.

Sample Collection:

Milk samples were collected under sterile conditions:

- Hands and cow udders were cleaned and sterilized.
- 15 ml of milk was collected from each teat into sterile beakers after discarding foremilk.

Processing and Testing of Samples:

Samples were transported to the laboratory in a cool box for immediate testing. The California Mastitis Test (CMT) was used to assess somatic cell counts in the milk, following the manufacturer's instructions. The results were categorized into negative, trace, weak positive, distinct positive, and strong positive based on the reaction.

Culture Examination:

Primary Isolation of Bacteria:

Milk samples were streaked on Nutrient Agar, Mannitol Salt Agar, and MacConkey Agar plates and incubated at 37°C for 48 hours. Colony characteristics and smears stained by Gram's method were used to identify bacterial growth.

Identification and Purification of Bacteria:

Bacterial isolates were purified by subculturing, and identification was confirmed using biochemical tests.

Antibiotic Susceptibility Test:

Antibiotic susceptibility of the isolates was tested using the modified Kirby-Bauer disk diffusion method as recommended by CLSI standards. Antibiotics tested included Gentamicin, Ceftazidime, Ciprofloxacin, Cotrimoxazole, and Doxycycline, which are commonly used for treating subclinical mastitis.

Result

Prevalence of Subclinical mastitis

The California Mastitis Test (CMT) results reveal that out of 200 quarters of milk samples taken from 50 dairy cows, 30 quarters (or 15%) tested positive for subclinical mastitis. This indicates that 15% of the sampled quarters were infected with bacteria, despite the cows showing no visible symptoms of mastitis. This level of prevalence highlights the need for regular screening and careful management to prevent the spread of infections and ensure timely treatment.

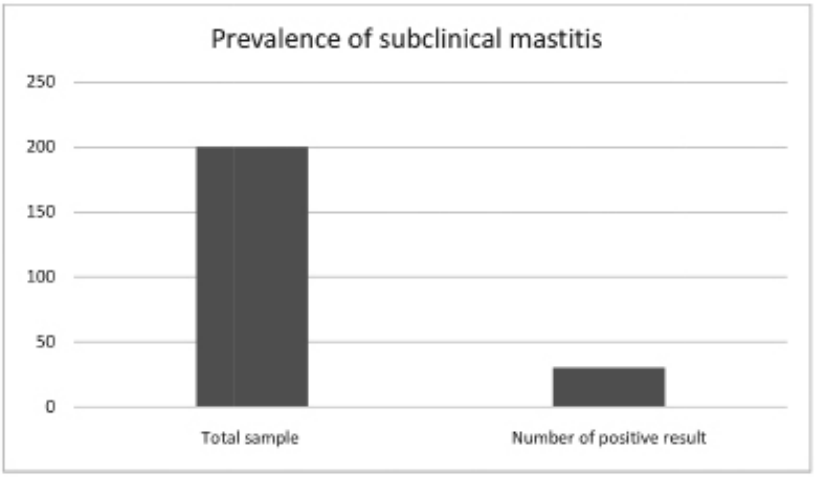

Figure1: Prevalence of Subclinical mastitis

Photograph 1: California Mastitis Test (Sample C11)

Occurrence of Mastitis Based on Breed

Out of the 50 cows sampled, among Jersey breed: 34 cows were tested, and 15 (44.12%) were found to have subclinical mastitis based on the CMT test**.** Holstein breed: 12 cows were tested, and 7 (58.33%) tested positive for subclinical mastitis and Crossbreed (Jersey-Holstein)**:** 4 cows were tested, and none showed positive results for subclinical mastitis. This data suggests that Holstein cows had a higher percentage of subclinical mastitis cases (58.33%) compared to Jersey cows (44.12%), while no cases were detected in the crossbreeds.

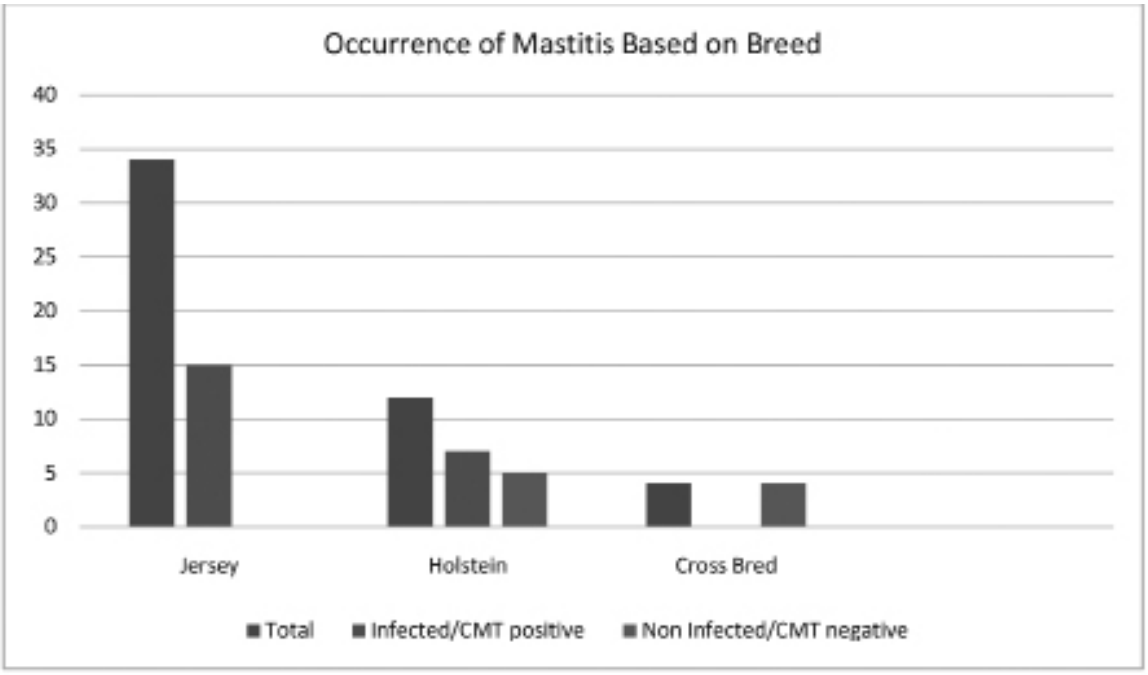

Figure2: Occurrence of Mastitis Based on Breed

Bacteriological Isolates from Milk Samples

Out of 30 CMT-positive samples, 26 bacteria were isolated, Staphylococcus aureus **i**s the most frequently isolated pathogen, present in 14 samples (53.85%). This indicates that S. aureus is the dominant pathogen causing subclinical mastitis in this study. Coagulase-Negative Staphylococci (CONS) is identified in 6 samples (23.07%). Bacillus spp. Is found in 5 samples (19.23%). Citrobacter freundii is detected in 1 sample (3.85%), making it the least frequently isolated pathogen.

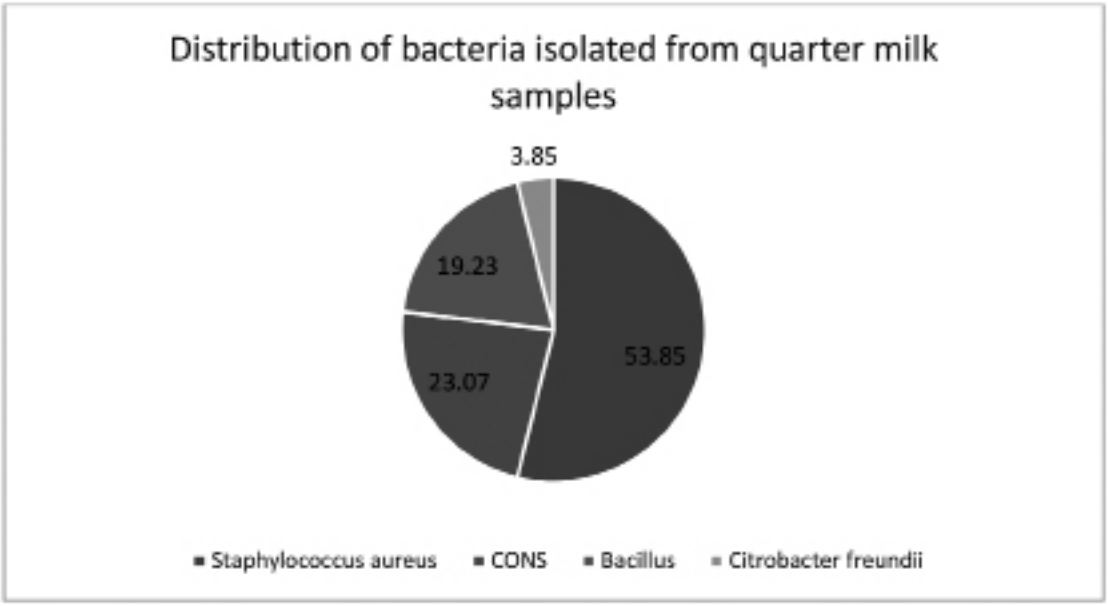

Figure 3: Distribution of bacteria isolated from quarter milk samples.

Prevalence of subclinical mastitis based on age and parity in different cow breeds

The study recorded the prevalence of subclinical mastitis based on age and parity in different cow breeds. In Jersey cows, the prevalence increased with age: 34.62% (3-6 years), 66.67% (6-9 years), and 100% (9 years or above). For Holstein cows, prevalence was 60% (3-6 years) and 100% (6-9 years). No cases were found in crossbreeds. Regarding parity, Jersey cows showed increasing prevalence with higher parity: 55.55% (parity 2), 46.15% (parity 3), 33.33% (parities 4 and 5), and 100% for parities 6 and above. For Holstein cows, subclinical mastitis was recorded as 50% for parities 1 and 3, and 100% for parities 4 and 5. Crossbreeds had no positive cases across any parity.

Table 1

Prevalence of subclinical mastitis based on age and parity in different cow breeds

	Jersey		Holstein		Cross Breds	
	Sample tested	Positive(%)	Sample tested	Positive(%)	Sample tested	Positive(%)
0-3	-	-	1	0	-	-
3-6	26	9 (34.62)	10	6 (60)	4	0
6-9	6	4 (66.67)	1	1 (100)	-	-
9 or above	2	2 (100)	-	-	-	-

Antibiotic susceptibility test on *Staphylococcus aureus*

The antibiotic susceptibility test conducted on *Staphylococcus aureus* isolates revealed that all were sensitive to gentamicin (100%), followed by doxycycline (85.72%), cotrimoxazole (85.72%), ciprofloxacin (71.42%), and ceftazidime (57.14%). No resistance was observed for gentamicin, but some resistance was noted for ciprofloxacin and ceftazidime.

Table 2

Antibiotic susceptibility test on *Staphylococcus aureus.*

Antibiotic	Sensitive	Intermediate	Resistance
Doxycycline	12 (85.72%)	2 (14.28%)	0
Ciprofloxacin	10 (71.42%)	1 (7.15%)	3 (21.43%)
Gentamicin	14 (100%)	0	0
Ceftazidime	8 (57.14%)	3 (21.43%)	3 (21.43%)
Cotrimoxazole	12 (85.72%)	1 (7.14%)	1 (7.14%)

Discussions

Subclinical mastitis remains a silent but critical problem in dairy cows, as it does not present visible symptoms but significantly impacts milk production and quality. In this study, the overall prevalence of subclinical mastitis was 15%, consistent with other studies reporting subclinical mastitis rates ranging from 12% to 18% in dairy herds in similar settings (Zeryehun et al., 2013; Abebe & Daniel, 2010). The California Mastitis Test (CMT) was used to detect the presence of subclinical mastitis, as it is a simple and effective diagnostic tool for somatic cell count (SCC) estimation, widely recommended for field conditions (Quinn et al., 2011).

The breed-wise prevalence showed a higher rate in Jersey cows compared to Holstein and crossbreeds, with Jersey cows showing a prevalence of up to 100% in older age groups. This finding aligns with studies suggesting that Jersey cows are more prone to mastitis due to their genetic predisposition and udder conformation (Seegers et al., 2003). Age-related susceptibility is also well documented, with older cows being more prone to infections due to prolonged exposure to pathogens and weakened immune responses. Furthermore, increased parity was associated with higher mastitis prevalence, consistent with the findings of previous studies that show a direct relationship between parity and mastitis incidence (Barkema et al., 2006).

The bacteriological analysis revealed *Staphylococcus aureus* as the predominant pathogen (53.85%), which is in agreement with other studies identifying *S. aureus* as a leading cause of bovine mastitis worldwide (Oliver & Murinda, 2012). Its ability to form biofilms and evade the host immune system makes it a persistent and difficult pathogen to treat (Cramton et al., 1999). Coagulase-negative staphylococci (CONS) and *Bacillus* spp. were also isolated, which are commonly associated with environmental contamination in dairy farms (Hogan & Smith, 2003). The isolation of *Citrobacter freundii* in one case, although rare, has been reported in other studies and indicates environmental contamination as a possible source (Moser et al., 2013).

The antibiotic susceptibility test results showed 100% sensitivity to gentamicin, which is commonly used for treating mastitis. The high sensitivity to doxycycline and cotrimoxazole (85.72%) suggests these antibiotics are still effective options for treatment. However, the resistance to ciprofloxacin (21.43%) and ceftazidime (21.43%) highlights the growing concern of antibiotic resistance, which is a significant issue in veterinary medicine (Erskine et al., 2002). This finding underscores the need for prudent use of antibiotics and adherence to antimicrobial stewardship programs to mitigate the risk of resistance development.

Conclusion

This study highlights the significant prevalence of subclinical mastitis in dairy cows in Bharatpur Municipality, with an overall infection rate of 15%. Notably, older and multiparous cows, particularly Jersey breeds, exhibited a higher susceptibility to this condition. The primary pathogen identified was *Staphylococcus aureus*, which underscores the need for targeted interventions to manage this prevalent issue. The antibiotic susceptibility testing revealed that while gentamicin demonstrated 100% effectiveness, resistance to ciprofloxacin and ceftazidime raises concerns regarding the sustainability of treatment options. These findings emphasize the critical need for regular monitoring of mastitis and prudent use of antibiotics to mitigate the risk of developing resistance. Continuous efforts in education, monitoring, and responsible antibiotic usage are essential to improve dairy health and productivity in the region.

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