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# Subclinical Mastitis in Dairy Cattle: Diagnosis, Impact, and Control in Nepal

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## Abstract

Subclinical mastitis (SCM) is a significant but mostly ignored threat to Nepal's dairy industry. Early identification is challenging because, unlike clinical mastitis, SCM does not exhibit any obvious symptoms of udder or milk abnormalities. This paper summarizes the prevalence, causal agents, diagnostic techniques, effects, and control approaches of SCM in dairy cattle from Nepal. With prevalence rates ranging from 13.6% to 60%, Streptococcus species, Escherichia coli, and Staphylococcus aureus are the most prevalent pathogens. The development of antibiotic resistance, especially to commonly used drugs, complicates treatment. Diagnostic techniques, including PCR, bacteriological culture, somatic cell count, and the California Mastitis Test, are crucial to identification. SCM reduces milk yield and quality, promotes infertility, and results in significant financial losses. Regular screening, proper milking practices, dry cow therapy, and farmer education are all essential for effective management. In order to protect animal health, milk safety, and the long-term viability of Nepal's dairy industry, these actions must be strengthened.

## Keywords

Antimicrobial resistance; Diagnostics; Hygiene management; Milk quality; Udder health

## 1. Introduction

### 1.1 Background

Mastitis is the most economically significant disease affecting dairy herds due to its high prevalence, high treatment costs, increased labor, loss of milk quality and output, and early animal culling [1]. Mastitis is an inflammatory disorder of the mammary gland that occurs when microorganisms invade the udder tissue [2]. It falls into one of two categories: clinical and subclinical. Acute onset, altered milk composition and appearance, reduced milk production, and inflammation a key indicators of an infected udder and are the hallmarks of clinical mastitis [3]. In contrast, subclinical mastitis (SCM) is the most harmful kind of mastitis, with no noticeable changes in the appearance of milk and udder [1].

### 1.2 Statement of the problem

Due to inadequate management techniques, a lack of early detection facilities, and a lack of understanding among farmers, sub-clinical mastitis in bovines is prevalent in Nepal [4]. Research indicates that subclinical mastitis usually affects more than 50% of animals in Africa and Asia, endangering the health of animals, farmers, dairy processors, and consumers [5]. The frequency of SCM in dairy animals (cattle and buffalo) from smallholder farms to institutional farms in different districts of Nepal ranges from 13.6 to 60% [5]. Housing, diet, and milking practices are

important management aspects that affect the type of mastitis pathogen that can infect the mammary gland to differing degrees [5].

### 1.3 Rationale of the study

There isn't much consolidated data specifically about subclinical mastitis in Nepal, despite its enormous impact. A comprehensive review is essential to summarize current knowledge, identify gaps, and guide better management practices for farmers, veterinarians, and policymakers.

### 1.4 Objectives

Therefore, the goal of this review is to provide a comprehensive analysis of subclinical mastitis in Nepali dairy cattle, with a focus on the condition's prevalence, causes, diagnostic methods, impacts on productivity and reproduction, and preventative measures. Finding research gaps, compiling available data to provide better options for managing mastitis, and promoting informed decision-making for farmers, veterinarians, and lawmakers are the objectives.

## 2. Methods

The basis for this review was a thorough search of peer-reviewed publications using keywords associated with subclinical mastitis, pathogens, diagnosis, and control in Nepal across databases such as PubMed, Google Scholar, and ScienceDirect. To guarantee relevance and current developments, articles published between 2000 and 2024 were given priority. Studies that addressed the etiology, diagnosis, management, effects, or epidemiology of subclinical mastitis in dairy cattle were included. To find trends, knowledge gaps, and useful suggestions, data were retrieved, critically examined, and qualitatively synthesized. Zotero was used to manage referencing, and Google Docs was used to prepare the manuscript in accordance with IJFSA journal guidelines. Studies with well-defined methodologies and those carried out in environments similar to Nepalese dairy systems were preferred.

## 3. Etiology and Pathogenesis

Without producing obvious clinical symptoms, several pathogenic microorganisms, primarily bacteria, infiltrate the mammary gland and cause subclinical mastitis [6]. The most common causes of disease are environmental pathogens like *Escherichia coli*, *Klebsiella spp*, *Streptococcus uberis*, and infectious pathogens like *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma spp* [7]. In most regions of Nepal, Streptococcus species are the primary cause, but S.aureus is more prevalent in certain areas [8]. Sick udders, milking equipment, or the hands of the people who milk can spread infectious microorganisms. When the teat canal is left open after milking, microorganisms from the environment can get into the teat through bedding, manure, water, or mud [9]. When the teat sphincter relaxes during or after milking, pathogens enter through the teat canal and proceed into the alveoli and teat cistern [10]. *S. uberis* adheres to and gets inside epithelial cells using special adhesion molecules (SUAM) and even pedestals to avoid being detected by the immune system [11]. Additionally, coagulase-negative staphylococci, *Enterococcus species*, and *S. aureus* produce biofilms, which increase their resistance to drugs and host defenses [12]. Numerous pathogens generate virulence factors, including hemolysins, leukotoxins, capsules, and biofilms, which allow neutrophils and macrophages to evade the body and result in chronic, long-lasting infections [13]. Despite the lack of obvious clinical symptoms, the presence of pathogens causes leukocyte infiltration, which raises somatic cell counts (typically >200,000 cells/mL), a characteristic of SCM [13]. The composition of milk is changed by SCM, which results in decreased lactose, casein, calcium, and phosphorus and increased salt, chloride, immunoglobulins, enzymes (such as NAGase), and whey proteins, all of which indicate tissue changes from inflammation [14]. If left undetected, many SCM cases become chronic and persist throughout lactation. Persistent inflammation results in fibrosis, impaired milk production, epithelial damage, and increased vulnerability to reinfection [14].

## 4. Diagnostic Approaches

Given that SCM doesn’t result in any noticeable changes in the udder or milk, it is difficult to diagnose. Correct diagnosis, however, is crucial to minimizing financial losses for farmers as well as minimizing the suffering and stress. Some of the diagnostic approaches are as follows.

### 4.1 California Mastitis Test (CMT)

The California Mastitis Test is a semi-quantitative, cow-side assay that uses lysed leukocytes and a surfactant reagent to visually evaluate gel formation [15]. The amount of cellular nuclear protein in the milk sample is the basis for the CMT principle, which is correlated with SCC [16].

According to the results of the California Mastitis Test (CMT) in Bharatpur, 30 quarters (15%) of the 200 quarters of milk samples obtained from 50 dairy cows tested positive for subclinical mastitis [17].

### 4.2 Somatic Cell Count (SCC)

Somatic Cell Count (SCC) is a quantitative laboratory method that measures the number of leukocytes in milk. Counts of ⩾200,000 cells/mL are routinely used as regulatory standards globally and indicate subclinical mastitis [18].

According to the study, normal milk had a mean SCC of 176 (30 to 800 × $10^3$ cells/mL), whereas subclinical mastitic milk had a mean SCC of 958 (300 to 6,500 × $10^3$ cells/mL). 200,000 cells/mL was established as the practical diagnostic upper limit for normal milk [19]. With a reported sensitivity of 30—89% and specificity of 60—90% at quarter-level sampling, SCC provides greater diagnostic precision when measured with automated cell counters [20].

### 4.3 Bacteriological Culture

The "gold standard" for diagnosing mastitis is bacteriological culture, which involves plating milk samples on selective media, incubating them for 24 to 48 hours, and identifying bacterial colonies using morphological and biochemical techniques [21], streptococci (11.1%), coliforms (9.5%), and staphylococci (7.9%) were the most common pathogens in Lamjung, where culture confirmed SCM in 28.6% of animals and 24.2% of quarters [22].

### 4.4 Polymerase Chain Reaction (PCR)

Both cultivable and fastidious mastitis pathogens can be detected using PCR, a quick and highly specific technique. Its multiplex and real-time variants can also detect genes that provide resistance to antibiotics [23]. A PCR-based analysis of CMT-positive samples in Chitwan revealed that 15.2% of the samples had Staph. aureus, and 6.9% of those samples had the mecA gene, making 1.05 percent of the samples MRSA-positive. This demonstrates the effectiveness of PCR in detecting resistance genes and molecular pathogens [8].

## 5. Impact

In eight major dairy regions of Nepal, 384 households were surveyed. Annual losses from SCM alone were Rs 8.32 billion (approximately US$64 million), while clinical mastitis contributed Rs4.43 billion (approximately US$34 million). The incidence of infertility in affected cows was 11% higher, indicating its substantial impact on reproductive performance [24]. According to the study conducted in Lamjung, high-yielding cows (>3 L) were significantly more likely to have SCM, suggesting a direct relationship between yield and infection risks [22]. CMT-positive samples in multiple studies revealed decreased milk production and an elevated prevalence of S. aureus [25]. Escherichia coli was isolated from 16.5% of subclinical mastitis cases in a 2021 study carried out in Chitwan. The effectiveness of commonly used antibiotics in treating SCM in Nepal is seriously questioned, as evidenced by the alarmingly high resistance rates found in antimicrobial susceptibility testing (38.8% to ceftriaxone and 37.7% to ciprofloxacin) [26].

## 6. Control and Prevention Strategies

Maintaining dairy cows' cleanliness, particularly around the udder, can guarantee good UH and milk quality while also greatly lowering the risk of SCM [27]. Antiseptic remedies (such as iodine-based teat dips) used to disinfect teats before and after milking effectively lower the incidence of SCM and bacterial contamination [28]. Avoid overmilking and ma-chine-milking faults, which can cause teat injury and increase infection risk [29]. Instruction in Good Husbandry Practices enhanced smallholders' adoption of sanitary measures in Nepal and decreased the prevalence of SCM in dairy cows from 55.0 to 35.0% [30]. Frequent screening lowers the risk of transmission and financial losses by enabling the early detection and treatment of SCM [31]. During the dry period, giving intramammary antibiotics aids in the removal of chronic intramammary infections and guards against the development of new ones [32].

## 7. Research Gaps and Future Directions

Subclinical mastitis in Nepal has been extensively studied; however, there are still a number of unanswered questions. The following is required:

- Mapping the prevalence of SCM across ecological zones using nationwide epidemiology surveys.
- Longitudinal research on SCM's effects on the economy at the national and farm levels.
- Research on probiotics and herbal remedies, as well as alternative treatments for antibiotic resistance.
- Creation of quick and inexpensive cow-side diagnostic instruments suited to smallholder circumstances.
- Evaluations of the effects of farmer education initiatives on the prevalence of SCM and milk quality.

## 8. Conclusion

Subclinical mastitis remains a silent but significant issue for Nepal's dairy industry, compromising animal health, reducing milk production, and resulting in financial losses. The prevalent illness, which is made more challenging by the emergence of antibiotic resistance, is primarily caused by Streptococcus species, E. coli, and S. aureus. In addition to control measures like dry cow therapy, improved milking techniques, antiseptic teat dips, and farmer education, reducing the burden of SCM necessitates early and accurate detection using tests like PCR, SCC, and CMT. Strengthening these community-based efforts and filling research gaps are essential to safeguarding Nepal's milk supply and improving the lives of dairy farmers.

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