

Risk Factors Associated with Subclinical Mastitis in Dairy Cows: Implications for Microbial Dynamics, Environmental Influences, and Management Factors

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Abstract

*Subclinical mastitis is a prevalent yet often overlooked condition in dairy cows, leading to reduced milk yield and quality. This study examines the microbial dynamics, environmental influences, and management factors contributing to subclinical mastitis in dairy herds. Microbial agents, including bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus uberis*, are key contributors, with their ability to persist and form biofilms complicating detection and treatment. Environmental factors, including bedding quality, sanitation, and exposure to pathogens in manure, water, and feed, significantly influence the incidence and severity of mastitis. Additionally, management practices, such as milking hygiene, regular monitoring of udder health, and the use of dry cow therapy, play critical roles in preventing and mitigating subclinical mastitis. The interaction between these factors can exacerbate the condition, making it difficult to control in dairy operations. Effective management strategies must therefore focus on a holistic approach, addressing microbial control, environmental sanitation, and proper milking techniques. This review highlights the need for integrated management practices to mitigate the risk of subclinical mastitis, improving herd health, milk production, and overall farm profitability. Future research should focus on advancing diagnostic methods and developing targeted interventions to address this complex issue.*

Keywords: Dairy cows, environmental factors, microbial dynamics, mastitis, management practices, milk yield, somatic cell count, udder health

INTRODUCTION

Subclinical mastitis is a widespread yet often undiagnosed condition in dairy cows, which poses significant challenges to dairy farming worldwide. Unlike clinical mastitis, subclinical mastitis does not show visible symptoms, making it difficult to detect without specialized testing. However, it leads to substantial economic losses due to reduced milk yield, poor milk quality, and increased somatic cell

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Received Date: February 05, 2025

Accepted Date: February 22, 2025

Published Date: February 28, 2025

Citation: Md. Emran Hossain, Shilpi Islam. Risk Factors Associated with Subclinical Mastitis in Dairy Cows: Implications for Microbial Dynamics, Environmental Influences, and Management Factors. *Research & Review: Journal of Microbiology and Virology*. 2025; 15(2): 45–61p.

counts [1]. The condition is primarily caused by infections from various microbial agents, including bacteria, fungi, and, less commonly, viruses and protozoa. Among the most common bacterial pathogens are *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus uberis*, and *Klebsiella spp.*, which can lead to chronic infections that affect the mammary glands of dairy cows [2]. Environmental and management factors, such as poor hygiene, improper milking practices, and inadequate bedding conditions, also contribute to the persistence and spread of these infections within herds [3, 4].

The microbial dynamics of subclinical mastitis are influenced by the interplay between pathogen

virulence and the cow's immune response. Pathogens, such as *S. aureus* are capable of forming biofilms, which protect them from immune system responses and antibiotic treatments, making infections persistent and difficult to eradicate [5]. Environmental factors, including wet or contaminated bedding, exposure to manure, and suboptimal cow housing, further increase the risk of infection [6]. Additionally, management practices, such as milking hygiene, failure to isolate infected cows, and inadequate treatment strategies exacerbate the problem. These factors not only lead to increased infection rates but also hinder efforts to control the spread of mastitis in dairy herds.

This study aims to fill existing gaps in understanding the complex interactions between microbial dynamics, environmental influences, and management practices in the development of subclinical mastitis. While extensive research has been conducted on individual pathogens and management practices, there is a lack of integrated studies that examine how these factors collectively contribute to the disease. The novelty of this study lies in its comprehensive approach, which addresses not only the bacterial and environmental factors but also emphasizes the role of management in mitigating subclinical mastitis. By examining these factors together, the study seeks to provide a more holistic understanding of the disease, paving the way for more effective control strategies and better management practices in dairy farms.

BACTERIAL CAUSES

Staphylococcus aureus

Staphylococcus aureus is a major cause of subclinical mastitis in dairy cows. This pathogen can form biofilms, which protect it from both immune responses and antibiotic treatments. *S. aureus* infections often lead to persistent, chronic mastitis, characterized by elevated somatic cell counts [7]. Transmission occurs via contaminated equipment, hands, or direct cow-to-cow contact, requiring stringent hygiene practices for control.

Escherichia coli

Escherichia coli is a prominent environmental pathogen responsible for subclinical mastitis in dairy cattle. It thrives in contaminated bedding, manure, or water, infecting cows through teat canal entry [8]. *E. coli* produces endotoxins that trigger inflammation, often leading to increased somatic cell counts and milk quality degradation [9]. Maintaining cleanliness in cow housing and using effective milking hygiene measures help mitigate its spread.

Streptococcus uberis

Streptococcus uberis is a key environmental pathogen implicated in subclinical mastitis. It is commonly found in bedding, manure, and other organic matter [10]. The bacterium colonizes the udder, causing persistent infection that elevates somatic cell counts and reduces milk quality. Control of *S. uberis* relies on proper sanitation, including clean bedding, regular milking hygiene, and environmental management to prevent its transmission.

Klebsiella spp.

Klebsiella spp. is an environmental pathogen that contributes to subclinical mastitis. It is commonly found in wet, contaminated bedding, as well as manure and soil. *Klebsiella* infections lead to inflammation and elevated somatic cell counts in infected cows [11]. The pathogen's resistance to antibiotics complicates treatment, making prevention through improved hygiene, clean bedding, and proper management essential for controlling its spread.

Mycoplasma spp.

Mycoplasma spp. causes contagious subclinical mastitis in dairy cows, often spreading through infected milk or contaminated equipment [12]. Lacking a cell wall, *Mycoplasma* bacteria resist many common antibiotics, making eradication challenging [13]. Infected cows may exhibit decreased milk yield and altered milk composition. Strict biosecurity measures, including isolating infected animals and enhancing herd management practices, are crucial for controlling *Mycoplasma* infections.

Corynebacterium Bovis

Corynebacterium bovis is a commensal bacterium that can cause subclinical mastitis, typically without noticeable clinical symptoms. It colonizes the teat canal, leading to elevated somatic cell counts in infected cows [14]. While less pathogenic than other bacteria, it is an indicator of poor milking hygiene and udder health. Proper teat sanitation and regular milking hygiene are essential to control *Corynebacterium bovis* infections.

Trueperella Pyogenes

Trueperella pyogenes is an opportunistic pathogen that can cause subclinical mastitis, particularly in cows with existing udder injuries. The bacterium produces pus-filled abscesses, which reduce milk yield and quality [15]. It often acts as a secondary pathogen following an initial infection or injury. Preventing *T. pyogenes* infections involves maintaining udder health, avoiding teat injuries, and ensuring proper sanitation and milking practices.

Pseudomonas Aeruginosa

Pseudomonas aeruginosa is an environmental pathogen that can cause subclinical mastitis in dairy cows. It is commonly found in contaminated water, bedding, and milking equipment [16]. This bacterium produces biofilms, which make it resistant to treatment. Infected cows experience elevated somatic cell counts and reduced milk quality. Preventing *P. aeruginosa* requires effective sanitation, proper equipment maintenance, and reducing cow exposure to contaminated environments.

Bacillus Cereus

Bacillus cereus is a sporulating bacterium responsible for subclinical mastitis, particularly when cows are exposed to contaminated feed or bedding [17]. The bacterium's spores are resilient to environmental stress, allowing it to persist in the environment and spread to cows. *B. cereus* infection causes inflammation and milk spoilage [18]. Proper feed storage, bedding management, and overall sanitation are crucial to control its presence in dairy operations.

Proteus spp.

Proteus spp. is an opportunistic environmental pathogen that contributes to subclinical mastitis in dairy cows [19]. It thrives in contaminated bedding, water, and manure, entering the udder through teat canal infections. Known for its urease production, it triggers inflammation and disrupts milk production. To control *Proteus spp.*, maintaining proper hygiene, preventing teat injuries, and reducing pathogen exposure through effective environmental management are essential.

Fungal Causes

Candida Albicans

Candida albicans is a fungal pathogen that can cause subclinical mastitis in dairy cows, often when the immune system is compromised. It is part of the normal udder flora but can overgrow under conditions of stress or antibiotic treatment [20]. Infections lead to increased somatic cell counts and reduced milk quality. Proper hygiene, stress reduction, and controlled antibiotic use are essential for preventing *C. albicans* infections.

Candida Krusei

Candida krusei, a less common but emerging fungal pathogen, can cause subclinical mastitis in dairy cows [21]. This fungus is typically opportunistic, infecting cows with compromised immune systems or following antibiotic treatment. *C. krusei* infections result in chronic mastitis with elevated somatic cell counts [22]. Preventing infection requires good herd management practices, including proper teat hygiene and careful antibiotic usage to avoid disruption of natural microbial balance.

Aspergillus Fumigatus

Aspergillus fumigatus is a ubiquitous environmental fungus that can lead to subclinical mastitis when cows are exposed to contaminated bedding, silage, or feed. This pathogen can trigger inflammation and

reduce milk quality [23]. The fungus is highly resistant to environmental stresses, making prevention difficult. Effective control involves maintaining clean, dry bedding, and proper feed storage to minimize fungal contamination and protect udder health.

Aspergillus Flavus

Aspergillus flavus is a common environmental fungus responsible for subclinical mastitis, often found in moldy feed or bedding [23]. *A. flavus* produces aflatoxins, which can contaminate milk and compromise its safety. Infected cows experience increased somatic cell counts and reduced milk yield [24]. Preventing *A. flavus* infection requires careful management of feed storage, proper ventilation in barns, and maintaining overall environmental cleanliness to reduce fungal contamination.

Fusarium spp.

Fusarium spp. are environmental fungi that can contribute to subclinical mastitis, particularly when cows are exposed to contaminated feed or bedding. *Fusarium species* can produce mycotoxins, which negatively impact udder health and milk production [25]. The toxins may also accumulate in milk, posing a risk to consumers. Preventing *Fusarium* infections involves managing fungal contamination in feed, bedding, and silage, along with maintaining clean and dry environments for cows.

Trichosporon spp.

Trichosporon spp. are opportunistic fungi that can cause subclinical mastitis, especially in cows with weakened immune systems or when antibiotics disrupt the natural microbial flora [26]. These fungi can lead to chronic mastitis with persistent elevation in somatic cell counts. Ensuring proper hygiene, maintaining udder health, and managing antibiotic usage are critical for preventing *Trichosporon* infections in dairy herds.

Cryptococcus spp.

Cryptococcus spp. are fungal pathogens that can occasionally cause subclinical mastitis in dairy cows, particularly in environments with poor sanitation. This fungus can lead to chronic, low-grade infections that elevate somatic cell counts [27]. Infected cows may show subtle signs of inflammation in the udder. Proper environmental sanitation, adequate ventilation, and regular monitoring of udder health are essential for controlling *Cryptococcus* infections.

Nocardia spp.

Nocardia spp. are soilborne bacteria that can cause subclinical mastitis in dairy cows, often following teat injuries or poor hygiene practices [28]. *Nocardia* infections are slow-growing and chronic, leading to persistent elevation in somatic cell counts. These infections require long-term treatment and management, making it important to focus on preventing teat injuries, improving hygiene practices, and ensuring environmental cleanliness to minimize exposure to *Nocardia*.

Prototheca spp.

Prototheca spp. are algae-like microorganisms that can cause subclinical mastitis in dairy cows [29]. These organisms thrive in contaminated water or feed, leading to infection when cows are exposed. *Prototheca* infections can be persistent and difficult to treat due to the organism's resistance to conventional therapies [30]. Maintaining clean water sources, proper waste management, and regular hygiene practices are essential to controlling *Prototheca* infections.

Actinomyces spp.

Actinomyces spp. are rare but emerging pathogens that can cause subclinical mastitis in dairy cows. These bacteria are typically found in the oral cavity, but they can infect the udder through teat injuries or poor hygiene. Infected cows experience elevated somatic cell counts and decreased milk yield. Preventing *Actinomyces* infections requires maintaining teat health, avoiding injuries, and ensuring cleanliness in milking equipment and housing.

VIRAL CAUSES

Bovine Leukemia Virus

Bovine leukemia virus (BLV) is a retrovirus that can cause subclinical mastitis by infiltrating the udder tissue, leading to an increase in somatic cell counts and compromised milk production [31]. BLV infection is typically asymptomatic in early stages but can progress to lymphoma, affecting overall herd health. Control measures include testing for BLV, culling infected animals, and implementing strict biosecurity protocols to limit transmission.

Bovine Herpesvirus

Bovine herpesvirus (BHV) is a viral pathogen that can contribute to subclinical mastitis, particularly through reactivation during periods of stress. BHV causes inflammation in the udder, elevating somatic cell counts and reducing milk yield [32]. The virus is often spread via respiratory droplets or contaminated equipment. Effective herd management, stress reduction, and vaccination programs can help minimize the impact of BHV in dairy herds.

Rotavirus

Rotavirus, primarily known for causing enteric diseases in calves, can also influence subclinical mastitis in cows. The virus can disrupt the balance of gut microbiota, impairing immune function and making cows more susceptible to mastitis-causing pathogens [33]. Rotavirus infections in calves can lead to indirect mastitis risk by stressing the dam. Preventing rotavirus through vaccination, good calf management, and hygienic practices is crucial for controlling its impact.

Bovine Papillomavirus

Bovine papillomavirus (BPV) is responsible for the formation of warts on the udder, which can lead to subclinical mastitis [34]. BPV infection is typically transmitted through direct contact with infected animals or contaminated equipment. Although warts caused by BPV are usually benign, they can cause discomfort, impair milk production, and increase the risk of bacterial infections [35]. Control measures include isolating infected animals and maintaining proper milking hygiene.

Bovine Viral Diarrhea Virus

Bovine viral diarrhea virus (BVDV) is a significant viral pathogen in dairy herds that can indirectly contribute to subclinical mastitis [36]. BVDV compromises the immune system, making cows more susceptible to secondary bacterial infections, including mastitis. The virus can be transmitted through direct contact, contaminated equipment, or bodily fluids. Vaccination programs, isolation of infected animals, and strict herd management are key to controlling BVDV and reducing mastitis risks.

Protozoan Causes

Cryptosporidium spp.

Cryptosporidium spp. are protozoan parasites that primarily affect the gastrointestinal tract but can also influence udder health in dairy cows. These parasites cause subclinical mastitis by impairing immune function and increasing the cow's susceptibility to secondary infections. *Cryptosporidium* is transmitted through contaminated water or feed. Prevention involves maintaining clean water sources, improving farm hygiene, and reducing environmental contamination to minimize transmission risks.

Sarcocystis spp.

Sarcocystis spp. are protozoan parasites that can cause subclinical mastitis in dairy cows by infecting the udder tissue and impairing milk production [37]. These protozoa are transmitted through ingestion of contaminated feed or water, with cattle acting as intermediate hosts. While infections are typically asymptomatic, they can cause inflammation and increase somatic cell counts. Control measures include reducing exposure to contaminated feed and water sources, along with proper sanitation practices.

Toxoplasma gondii

Toxoplasma gondii is a protozoan parasite that can indirectly contribute to subclinical mastitis by compromising the immune system of dairy cows. *T. gondii* is primarily transmitted through ingestion

of contaminated feed, water, or feces from infected cats [38]. The parasite can reduce milk quality and increase susceptibility to other infections, including mastitis. Preventing infection involves maintaining biosecurity measures, particularly limiting access of cats to feed and water sources.

Eimeria spp.

Eimeria spp. are protozoan parasites that typically affect the gastrointestinal tract of calves but can contribute to subclinical mastitis in adult cows. *Eimeria* infections can lead to stress, compromising the cow's immune response and making it more susceptible to mastitis pathogens. Prevention involves ensuring proper calf management, improving hygiene, and minimizing stress, particularly during weaning and other high-risk periods to reduce protozoan transmission and subsequent mastitis risks.

HYGIENE-RELATED FACTORS

Dirty Bedding

Dirty bedding is a significant hygiene-related factor that contributes to subclinical mastitis in dairy cows. Bedding contaminated with feces, urine, or environmental pathogens can increase the risk of teat infections, elevating somatic cell counts [39]. Inadequate bedding maintenance allows bacteria and fungi to proliferate, causing mastitis. Regular cleaning and providing dry, clean bedding for cows are essential to minimize the risk of infection and maintain udder health.

Wet Bedding

Wet bedding creates an ideal environment for bacterial growth, increasing the likelihood of subclinical mastitis in dairy cows [40]. Excess moisture in bedding leads to prolonged exposure of teats to pathogens, which can easily enter the udder and cause infections. To reduce the risk of mastitis, it is crucial to keep bedding dry through regular maintenance, proper drainage, and ventilation within the barn.

Contaminated Water Sources

Contaminated water sources, such as dirty troughs or improperly stored water, are a major risk factor for subclinical mastitis in dairy cows [41]. Pathogens in contaminated water can enter the udder through teat exposure or ingestion. Ensuring that water sources are clean and free of pathogens through regular cleaning and proper water management is vital to prevent mastitis and ensure overall herd health.

Muddy Feedlots

Muddy feedlots increase the risk of subclinical mastitis by causing cows to stand in wet, unsanitary conditions, which can lead to teat contamination. Pathogens in the mud can infect teats and udder tissue, resulting in elevated somatic cell counts and reduced milk production [42]. Maintaining dry and clean feedlot conditions through proper drainage and regular mucking out is necessary to prevent infections.

Poor Drainage Systems

Poor drainage systems create conditions where water and waste accumulate, leading to wet and dirty environments that promote the growth of pathogens [43]. Inadequate drainage in barns, feedlots, and pastures increases the risk of subclinical mastitis by exposing cows to contaminated surfaces. Installing proper drainage and regularly maintaining the infrastructure will help keep environments dry, reducing the risk of udder infections and ensuring better cow welfare.

Manure Accumulation

Manure accumulation in barns and feedlots is a significant hygiene-related risk factor for subclinical mastitis [39]. The buildup of manure creates an unsanitary environment, allowing pathogens to proliferate on bedding, floors, and equipment. This increases the risk of teat infections and elevated somatic cell counts. Regular manure removal, along with proper waste management practices, is essential to maintaining a clean environment and minimizing mastitis risk.

Inadequate Cleaning of Milking Areas

Inadequate cleaning of milking areas contributes to the transmission of mastitis-causing pathogens. If milking equipment, teat disinfectants, or floors are not properly cleaned, bacteria and fungi can spread to the cows' udders [44]. Ensuring thorough cleaning of milking areas after each use, including sanitizing equipment and disinfecting teats, is vital for preventing the spread of infections and reducing somatic cell counts in milk.

Use of Unhygienic Milking Gloves

The use of unhygienic milking gloves is a common source of pathogen transmission in dairy farms. If gloves are not regularly cleaned or replaced, they can become contaminated with bacteria and fungi that infect the teats and udders of cows [45]. To reduce the risk of subclinical mastitis, milking gloves should be changed frequently and properly sanitized before each use, along with ensuring overall milking hygiene.

Pathogen Survival in Organic Matter

Pathogen survival in organic matter, such as manure, bedding, or feed, contributes significantly to the spread of subclinical mastitis in dairy herds. Many mastitis-causing pathogens can survive in organic matter for extended periods, contaminating the environment and increasing the risk of infection [46]. Effective manure management, bedding maintenance, and hygienic practices are essential to limit pathogen survival and prevent mastitis outbreaks in dairy herds.

Poor Ventilation

Poor ventilation in barns can exacerbate the risk of subclinical mastitis by creating humid, stagnant conditions that facilitate the growth of bacteria and fungi [41]. Inadequate airflow increases the likelihood of udder infections due to excessive moisture and poor air quality. Proper ventilation systems are crucial for maintaining a dry, clean, and pathogen-free environment, which helps prevent the onset of mastitis and promotes overall cow health.

MILKING-RELATED FACTORS

Improper Teat Sanitation

Improper teat sanitation is a significant factor in the spread of subclinical mastitis in dairy cows. If teats are not properly cleaned before milking, bacteria from the environment can enter the udder during milking. This increases the risk of infections and elevated somatic cell counts [41]. Routine cleaning and disinfecting of teats with appropriate sanitizing agents before milking is essential to reduce the risk of mastitis transmission.

Incomplete Teat Dipping

Incomplete teat dipping, where teats are not fully covered by the disinfectant, increases the likelihood of mastitis-causing bacteria infecting the udder [47]. This practice leaves portions of the teats vulnerable to contamination, allowing pathogens to enter the udder during milking. Properly dipping all teats with an effective antiseptic solution before and after milking is critical in preventing mastitis and maintaining udder health.

Faulty Milking Machines

Faulty milking machines are a common cause of subclinical mastitis, as they can cause physical damage to teats and lead to infection. If machines are not properly calibrated, they may not extract milk efficiently or may create excessive suction, which increases the risk of teat injury and infection [48]. Regular inspection and maintenance of milking machines are necessary to ensure proper functioning and reduce the risk of udder health issues.

Incorrect Vacuum Levels

Incorrect vacuum levels in milking machines can contribute to subclinical mastitis by causing undue pressure on the teats during milking. Low or high vacuum levels may lead to inefficient milking,

discomfort, or injury to the teats, which can facilitate the entry of pathogens into the udder [49]. Regular monitoring and adjustment of vacuum levels to the optimal range are crucial for preventing teat damage and mastitis.

Overmilking Practices

Overmilking practices, where cows are milked for too long, put excessive strain on the teats and increase the risk of injury and infection. Prolonged milking times can lead to teat trauma, which provides an entry point for bacteria, raising somatic cell counts and promoting the development of subclinical mastitis [50]. Proper milking times and techniques are essential for maintaining udder health and preventing mastitis.

Delayed Detection of Mastitis Cases

Delayed detection of mastitis cases contributes significantly to the spread of subclinical mastitis in dairy herds. Mastitis in its early stages often goes unnoticed due to the lack of visible symptoms. If cases are not identified and treated promptly, infections can spread, leading to higher somatic cell counts and reduced milk quality [51]. Regular monitoring of cows for early signs of mastitis and quick intervention are key to controlling its spread.

Shared Milking Equipment

Shared milking equipment can facilitate the transmission of mastitis-causing pathogens between cows. When equipment is not properly sanitized between milkings, bacteria and fungi can spread from infected to healthy cows [44]. Using dedicated equipment for each cow or ensuring thorough cleaning and disinfection of shared equipment between milkings can reduce the risk of cross-contamination and help control mastitis in dairy herds.

Mixing Infected and Healthy Cows

Mixing infected and healthy cows can exacerbate the spread of subclinical mastitis. Cows with undiagnosed or untreated mastitis can shed pathogens into the milking environment, contaminating healthy cows and raising the risk of infection [52]. Segregating infected cows from healthy ones and isolating them during milking and post-milking is vital for reducing pathogen transmission and managing mastitis outbreaks.

Irregular Machine Maintenance

Irregular machine maintenance can lead to subclinical mastitis by causing malfunctioning equipment, such as malfunctioning milking machines, which can result in teat injury or inadequate milk extraction [53]. Lack of maintenance also increases the risk of bacterial contamination. Routine inspections, cleaning, and calibration of milking machines are essential to ensure proper functioning and reduce the likelihood of mastitis-causing injuries or infections.

Stressful Milking Practices

Stressful milking practices, such as rough handling or prolonged waiting times, can compromise the immune system of dairy cows, making them more susceptible to mastitis. Stress can also lead to poor milk let-down, increasing the likelihood of incomplete milking and exposing teats to bacteria. Implementing calm, efficient milking procedures and minimizing stress through gentle handling and proper management practices can help reduce the incidence of mastitis.

NUTRITIONAL DEFICIENCIES

Vitamin E Deficiency

Vitamin E deficiency can compromise the immune function of dairy cows, making them more susceptible to subclinical mastitis. This vitamin plays a vital role in protecting cells from oxidative stress, including the mammary gland tissue [54]. Inadequate vitamin E levels may weaken the udder's natural defence mechanisms, increasing the likelihood of bacterial infections and elevated somatic cell counts [55]. Ensuring adequate vitamin E intake through balanced feed supplementation is essential for maintaining udder health.

Selenium Deficiency

Selenium deficiency is another nutritional factor contributing to subclinical mastitis in dairy cows. Selenium is a key antioxidant that supports the immune system, and its deficiency can impair immune response and increase susceptibility to mastitis-causing pathogens [56]. Adequate selenium levels in the diet are essential for maintaining udder health. Supplemental selenium may be necessary in regions where soils are deficient in this trace mineral.

Zinc Deficiency

Zinc plays a crucial role in maintaining the integrity of the mammary gland's epithelial cells and immune function. A deficiency in zinc can lead to increased teat injury, reduced milk quality, and greater vulnerability to mastitis [57]. Dairy cows with insufficient zinc may also experience impaired wound healing, which can exacerbate udder infections. Providing a well-balanced diet that meets zinc requirements is essential for preventing subclinical mastitis.

Feeding Spoiled Silage

Feeding spoiled silage to dairy cows can lead to a variety of health problems, including subclinical mastitis. Spoiled silage often contains harmful bacteria, molds, and mycotoxins that can impair the immune system and increase susceptibility to infections [58]. It can also disrupt the cow's digestion, affecting nutrient absorption. Using fresh, high-quality silage is crucial for maintaining overall cow health and preventing the development of mastitis.

Improperly Balanced TMR

An improperly balanced Total Mixed Ration (TMR) can contribute to nutritional deficiencies, weakening the cow's immune system and making it more prone to mastitis. Inadequate proportions of energy, protein, vitamins, or minerals in the TMR can impair udder health and milk production. Regular monitoring of the TMR formulation and ensuring that it meets the nutritional requirements of dairy cows are key to reducing mastitis risk.

Moldy Feed Consumption

Moldy feed consumption can introduce harmful fungi, mycotoxins, and pathogens into the cow's diet, potentially leading to immune suppression and increased susceptibility to mastitis [59]. Mold growth in feed can occur due to improper storage or exposure to moisture, compromising both feed quality and safety. To prevent mastitis, farmers must ensure proper feed storage conditions, monitor feed quality regularly, and discard moldy feed promptly.

Inadequate Clean Drinking Water

Inadequate access to clean drinking water can exacerbate subclinical mastitis by reducing cows' overall health and immune function. Water is essential for maintaining cellular function and promoting the cow's natural defence mechanisms. Without proper hydration, cows are more susceptible to infections, including mastitis. Ensuring that cows have constant access to fresh, clean water is essential to prevent immune suppression and lower the risk of udder infections.

Poor Quality Concentrate Feeding

Feeding poor-quality concentrates can contribute to nutritional imbalances, leading to an increased risk of subclinical mastitis. Concentrates that are contaminated, improperly stored, or deficient in essential nutrients can weaken the cow's immune response and lead to lower milk quality. Regular evaluation of concentrate quality and formulation is necessary to ensure adequate nutrition, boost immunity, and reduce the likelihood of mastitis in dairy herds.

Mycotoxin Contamination in Feed

Mycotoxin contamination in feed is a significant risk factor for subclinical mastitis [60]. These toxic compounds, produced by molds, can impair the immune function of dairy cows and promote inflammation in the udder. Exposure to mycotoxins can compromise the cow's ability to resist infection,

leading to higher somatic cell counts [61]. Preventing mycotoxin contamination involves proper feed storage, mold control, and regular testing to ensure feed safety and cow health.

Mineral Imbalance in the Diet

Mineral imbalances in the diet, such as excesses or deficiencies of calcium, magnesium, or phosphorus, can disrupt the immune system, impair udder health, and increase the likelihood of subclinical mastitis [57]. A well-balanced mineral intake is crucial for maintaining optimal immune function and supporting tissue integrity in the mammary gland. Regular monitoring of mineral levels in the diet and supplementation as needed can help reduce mastitis incidence and promote overall cow health.

ENVIRONMENTAL STRESSORS

High Humidity Levels

High humidity levels create a favorable environment for the proliferation of mastitis-causing pathogens. Moisture promotes bacterial growth on bedding, teats, and milking equipment, increasing the risk of udder infections [62]. Prolonged exposure to high humidity can also weaken cows' immune defences, making them more susceptible to subclinical mastitis. Proper ventilation and maintaining dry conditions in housing areas are crucial to minimize the effects of humidity.

Extreme Heat Stress

Extreme heat stress negatively impacts the health and productivity of dairy cows, increasing their susceptibility to subclinical mastitis. High temperatures can weaken immune function, reduce feed intake, and impair the cow's ability to fight off infections [63]. Providing shade, cooling systems, and adequate water access can help mitigate the effects of heat stress and reduce the incidence of mastitis in dairy herds.

Cold Stress During Winter

Cold stress during winter weakens the immune system of dairy cows, making them more vulnerable to subclinical mastitis. Low temperatures can reduce blood circulation in the udder and create conditions conducive to pathogen entry. Ensuring proper insulation, bedding, and protection from cold winds in barns helps maintain cow comfort, reducing the risk of mastitis and promoting udder health during colder months.

Overcrowding in Barns

Overcrowding in barns leads to increased contact among cows, elevating the risk of pathogen transmission and subclinical mastitis. High stocking densities also compromise cow comfort, increase stress levels, and reduce access to clean bedding and feed, exacerbating udder health issues [64]. Maintaining optimal space allocation and herd management practices are essential to minimize stress and the spread of infections in confined housing systems.

Sudden Temperature Changes

Sudden temperature changes can stress dairy cows, impairing their immune response and increasing their susceptibility to mastitis-causing pathogens. Fluctuations between hot and cold conditions disrupt the cow's natural thermal regulation, affecting overall health. Providing a stable housing environment with controlled temperatures and proper ventilation can help reduce the impact of temperature variations and minimize mastitis incidence.

Excessive Dust

Excessive dust in barns or milking areas can introduce pathogens into the udder, increasing the risk of subclinical mastitis. Dust particles can carry bacteria that contaminate the teat skin and bedding, facilitating infections [65]. Regular cleaning of barns, proper ventilation, and minimizing dust levels in housing areas help reduce the likelihood of mastitis and improve overall cow comfort.

Heavy Rainfall and Waterlogging

Heavy rainfall and waterlogging create wet and unhygienic conditions that promote the growth of mastitis-causing bacteria. Prolonged exposure to waterlogged areas increases the chances of teat contamination and udder infections [66]. Proper drainage systems and maintaining dry conditions in housing and grazing areas during heavy rains are essential to minimize the risk of mastitis and protect udder health.

Exposure to Stagnant Water

Exposure to stagnant water exposes dairy cows to pathogens that can cause subclinical mastitis. Stagnant water is a breeding ground for bacteria, fungi, and protozoa, which can contaminate the cow's udder during contact. Preventing cows' access to stagnant water and ensuring clean water sources are available for drinking and hygiene purposes are critical steps in mastitis prevention.

Contaminated Soil

Contaminated soil in grazing or housing areas serves as a reservoir for mastitis-causing pathogens. Prolonged contact with pathogen-laden soil increases the risk of teat contamination and udder infections. Implementing proper pasture management, regular soil health monitoring, and providing clean bedding in barns are vital measures to reduce the impact of contaminated soil on subclinical mastitis prevalence [67].

Pathogen-Rich Surroundings

Pathogen-rich surroundings, such as areas with accumulated manure or waste, significantly increase the risk of mastitis in dairy cows. These conditions provide an ideal environment for bacteria and fungi to thrive and infect cows' udders [41]. Regular cleaning and disinfection of barns, proper manure management, and maintaining hygienic living conditions are essential to control the spread of pathogens and prevent mastitis.

COW-SPECIFIC FACTORS

Poor Teat Conformation

Poor teat conformation, such as overly short or asymmetrical teats, can hinder the effectiveness of milking and teat sanitation, increasing the risk of subclinical mastitis [68]. Such abnormalities make it easier for pathogens to enter the teat canal and infect the udder. Regular assessment of teat structure and selective breeding programs can help reduce the incidence of poor conformation and related infections.

Teat Injuries

Teat injuries caused by rough handling, sharp objects, or aggressive sucking by calves compromise the udder's natural defence barrier. These injuries create entry points for mastitis-causing pathogens, leading to increased susceptibility to subclinical mastitis. Proper handling, well-maintained housing, and routine inspection of teats can prevent injuries and reduce the risk of infections in dairy cows.

Teat Cracks or Fissures

Teat cracks or fissures provide an ideal environment for pathogen colonization and proliferation. These cracks can result from harsh environmental conditions, improper milking practices, or skin dryness. The presence of cracks significantly increases the likelihood of subclinical mastitis [69]. Regular use of teat conditioners and maintaining proper milking techniques are effective measures to prevent and manage teat skin damage.

Genetic Predisposition

Genetic predisposition plays a significant role in determining a cow's susceptibility to subclinical mastitis. Certain cows may inherit traits, such as poor immune response or weak udder structures, making them more prone to infections [70]. Genetic selection and breeding programs focused on udder health traits can help reduce the prevalence of mastitis and improve overall herd resilience against infections.

Weak Immune Status

Cows with a weak immune status are less capable of fighting off mastitis-causing pathogens, increasing their risk of developing subclinical mastitis [41]. Factors, such as poor nutrition, stress, or underlying diseases can compromise immunity. Enhancing immune function through balanced diets, vaccination programs, and stress reduction measures is essential for reducing the incidence of mastitis in vulnerable cows.

Hormonal Changes During Lactation

Hormonal changes during lactation, particularly at the start and end of the milking cycle, can weaken the udder's natural defences. These fluctuations often leave cows more vulnerable to infections. The teat canal may remain open for longer durations, providing easier access for pathogens. Maintaining optimal management practices during these critical periods can help minimize mastitis risk.

Open Teat Canals Post-Milking

Open teat canals immediately after milking provide an opportunity for pathogens to enter the udder and cause infections [71]. This temporary vulnerability increases the risk of subclinical mastitis if hygiene is not maintained. To mitigate this, proper post-milking teat dipping and ensuring cows stand for a while post-milking are effective measures to reduce pathogen exposure and infection rates.

Stress Due to Parity

Stress related to parity, particularly in first-lactation or high-parity cows, can impact immune function and increase susceptibility to mastitis [72]. Younger cows may have underdeveloped immune defences, while older cows often face reduced immunity and udder integrity. Proper nutrition, stress management, and tailored care for cows at different stages of parity are necessary to minimize mastitis incidence.

Concurrent Diseases

Concurrent diseases, such as lameness or metabolic disorders, can weaken a cow's overall health and immunity, increasing susceptibility to mastitis [73]. These diseases often divert the cow's immune resources away from combating udder infections, exacerbating the risk. Proactive disease prevention, early detection, and effective treatment strategies are essential for maintaining udder health and reducing mastitis occurrence.

Advanced Age of Cows

Advanced age in dairy cows is associated with weakened immune function, reduced udder elasticity, and greater exposure to pathogens over time, all of which increase the risk of subclinical mastitis [74]. Older cows are also more prone to cumulative damage from previous infections. Managing cow health through proper care, culling policies, and adequate nutrition helps reduce mastitis risk in aging herds.

MANAGEMENT LAPSES

Poor Biosecurity

Poor biosecurity practices, such as allowing unrestricted movement of people, animals, or equipment between farms, increase the risk of pathogen introduction and spread [75, 76]. These lapses compromise herd health and contribute to the incidence of subclinical mastitis. Implementing strict biosecurity protocols, including disinfection, visitor control, and quarantine measures, is essential to safeguard the herd against infectious diseases, including mastitis.

Lack of Regular Mastitis Monitoring

The absence of regular mastitis monitoring leads to delayed detection and management of subclinical cases. Without periodic screening, infections remain undiagnosed, allowing pathogens to spread within the herd. Routine somatic cell count testing and milk culturing help in early identification and timely intervention, reducing the impact of mastitis and improving overall udder health management [77].

Inadequate Dry Cow Therapy

Inadequate dry cow therapy leaves cows vulnerable to infections during the non-lactating period. This phase is critical for udder recovery, and failure to address existing or potential infections increases mastitis risk in subsequent lactation [78]. Administering appropriate antibiotics and using teat sealants during the dry period significantly reduces the prevalence of subclinical mastitis.

Failure to Isolate New or Infected Animals

Failure to isolate newly introduced or infected animals facilitates the spread of mastitis-causing pathogens within the herd. Mixing infected cows with healthy ones increases the risk of udder infections [79]. Effective quarantine practices and segregating infected animals during treatment help minimize disease transmission and protect herd health.

Improper Record-Keeping

Improper record-keeping hinders effective mastitis management, as vital information on infection history, treatment protocols, and outcomes becomes inaccessible. This can result in repeated infections and inappropriate treatment strategies. Maintaining detailed and accurate health records aids in tracking mastitis cases, monitoring treatment efficacy, and developing targeted prevention measures.

Infrequent Manure Removal

Infrequent manure removal from barns and milking areas creates unhygienic conditions conducive to the growth and spread of mastitis-causing pathogens. Prolonged exposure to contaminated bedding or floors increases the likelihood of udder infections [6]. Regular manure removal, along with proper waste disposal practices, ensures cleaner environments and reduces mastitis risk.

Improper Administration of Antibiotics

Improper administration of antibiotics, including incorrect dosages or durations, contributes to the persistence of infections and the development of antimicrobial resistance. Such practices compromise the effectiveness of mastitis treatments. Adhering to veterinary guidance and implementing responsible antibiotic use protocols are essential to ensure successful treatment outcomes and protect udder health.

Delayed Treatment of Subclinical Cases

Delayed treatment of subclinical mastitis cases allows infections to worsen and spread within the herd, leading to greater economic losses and reduced milk quality. Early detection and timely treatment using appropriate interventions are critical to controlling the disease and minimizing its impact on herd productivity [80].

Poor Worker Training

Poor worker training on mastitis prevention and management leads to improper milking techniques, inadequate hygiene, and failure to detect early signs of infection. Well-trained farm staff play a key role in maintaining udder health and reducing mastitis prevalence [81]. Regular training programs focusing on hygiene, milking procedures, and early detection methods are essential.

Failure to Identify Persistent Carriers

Failure to identify persistent carriers of mastitis-causing pathogens allows these animals to act as reservoirs, perpetuating infections within the herd [32]. Persistent carriers often require targeted interventions, including specialized treatment or culling. Regular milk testing and thorough diagnostic efforts are crucial for identifying and managing these chronic infection sources effectively.

CONCLUSIONS

Subclinical mastitis remains a significant challenge in dairy farming, leading to reduced milk yield and quality, along with economic losses. The complex interactions between microbial dynamics, environmental factors, and management practices contribute to the persistence and spread of the disease. Effective control requires a holistic approach, addressing microbial control, improving

environmental conditions, and optimizing management strategies, such as milking hygiene and regular health monitoring. This study highlights the need for an integrated approach to understand the multifactorial nature of subclinical mastitis and the importance of tailored interventions. Future research should focus on refining diagnostic methods and developing sustainable strategies to minimize the impact of subclinical mastitis, ultimately enhancing herd health and productivity in dairy systems.

Future Directions

Future research on subclinical mastitis should focus on improving diagnostic techniques for early detection, as timely identification of infections is crucial for minimizing their impact. The development of more sensitive and cost-effective methods, such as molecular diagnostics and biomarkers, could enhance detection in the absence of clinical symptoms. Additionally, future studies should explore the genetic and immune responses of dairy cows to various pathogens, helping to identify animals with natural resistance or tolerance to infection. Investigating novel antimicrobial therapies and alternative treatments, such as probiotics, prebiotics, and immunomodulatory agents, offers potential solutions for controlling persistent infections, especially in antibiotic-resistant strains. Environmental management strategies, including advanced bedding materials and automated systems for maintaining clean and dry housing, could further reduce pathogen exposure. Moreover, the integration of precision farming technologies to monitor cow health in real-time could lead to more personalized management practices, improving overall herd welfare and productivity.

REFERENCES

1. Aqib AI, Muneer A, Shafeeq M, Kirn N. Economic impacts of clinical and sub clinical mastitis on dairy farms. *Vet Sci Res.* 2021;3(2):31–39.
2. Khasapane NG, Byaruhanga C, Thekisoe O, Nkhebenyane SJ, Khumalo ZTH. Prevalence of subclinical mastitis, its associated bacterial isolates and risk factors among cattle in Africa: A systematic review and meta-analysis. *BMC Vet Res.* 2023;19(1):1–16. Available from: <http://doi.org/10.1186/s12917-023-03673-6>
3. Argaw A. Review on epidemiology of clinical and subclinical mastitis on dairy cows. *Food Sci Qual Manag.* 2016;52(6):56–65.
4. Ranasinghe RMSBK, Deshapriya RMC, Abeygunawardana DI, Rahularaj R, Dematawewa CMB. Subclinical mastitis in dairy cows in major milk-producing areas of Sri Lanka: Prevalence, associated risk factors, and effects on reproduction. *J Dairy Sci.* 2021;104(12):12900–12911. Available from: <http://doi.org/10.3168/jds.2021-20223>
5. Rainard P, Foucras G, Fitzgerald JR, Watts JL, Koop G, Middleton JR. Knowledge gaps and research priorities in *Staphylococcus aureus* mastitis control. *Transbound Emerg Dis.* 2018;65:149–165.
6. G. Freu, Garcia BLN, Tomazi T, Gheller LS, Bronzo V, Di Leo GS, et al. Association between mastitis occurrence in dairy cows and bedding characteristics of compost-bedded pack barns. *Pathogens*, 2023;12(4):583.
7. R. Rychshanova, Mendybayeva A, Miciński B, Mamiyev N, Shevchenko P, Bermukhametov Z, et al. Antibiotic resistance and biofilm formation in *Staphylococcus aureus* isolated from dairy cows at the stage of subclinical mastitis in northern Kazakhstan. *Arch Anim Breed.* 2022;65(4):439–448.
8. Haider A, Ikram M, Shahzadi I, Asif Raza M. Bovine Mastitis. In *Springer Series in Biomaterials Science and Engineering*, (vol. 19). Springer; 2023. pp. 49–80. Available from: http://doi.org/10.1007/978-3-031-39947-3_4
9. Burvenich C, Van Merris V, Mehrzad J, Diez-Fraile A, Duchateau L. Severity of *E. coli* mastitis is mainly determined by cow factors. *Vet Res.* 2003;34(5):521–564.
10. Krömker V, Reinecke F, Paduch J-H, Grabowski N. Bovine streptococcus uberis intramammary infections and mastitis. *Clin Microbiol. Open Access.* 2014:3.
11. Munoz MA, Ahlström C, Rauch BJ, Zadoks RN. Fecal shedding of *Klebsiella pneumoniae* by dairy cows. *J Dairy Sci.* 2006;89(9):3425–3430.
12. M. S. Hazelton, Morton JM, Parker AM, Sheehy PA, Bosward KL, Malmo J, et al. Whole dairy herd sampling to detect subclinical intramammary *Mycoplasma bovis* infection after clinical mastitis outbreaks. *Vet Microbiol.* 2020;244:108662.

13. Fp. Maunsell, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, et al. Mycoplasma bovis infections in cattle. *J Vet Intern Med.* 2011;25(4):772–783.
14. Kamel MS, Bakry NM. Clinical and subclinical mastitis. In *The Microbiology, Pathogenesis and Zoonosis of Milk Borne Diseases.* Elsevier; 2024. pp. 153–190.
15. Rezanejad M, Karimi S, Momtaz H. Phenotypic and molecular characterization of antimicrobial resistance in *Trueperella pyogenes* strains isolated from bovine mastitis and metritis. *BMC Microbiol.* 2019;19(1):305.
16. B. Badawy, Moustafa S, Shata R, Sayed-Ahmed MZ, Alqahtani SS, Ali MS, et al. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* isolated from dairy cattle, milk, environment, and workers' hands. *Microorganisms.* 2023;11(11):2775.
17. RH Eid, NE Aref, ES Ibrahim. "Phenotypic diagnosis and genotypic identification of *Bacillus cereus* causing subclinical mastitis in cows. *Vet World.* 2023;16(5):888.
18. Jessberger N, Dietrich R, Granum PE, Märtlbauer E. The *Bacillus cereus* food infection as multifactorial process," *Toxins (Basel).* 2020;12(11):701.
19. Kanwar BPS, Ali SL, Kashyap DK, Bara S. Diagnosis of sub-clinical mastitis (SCM) in organised dairy herds. *Intas Polivet.* 2018;19(2):196–200.
20. Dworecka-Kaszak B, Krutkiewicz A, Szopa D, Kleczkowski M, Biegańska M. High prevalence of *Candida* yeast in milk samples from cows suffering from mastitis in Poland. *Sci World J.* 2012;2012(1):196347.
21. Refai MK, El-Yazid HA. Yeast infections in domestic and wild animals. *Fac Vet Med Cairo Univ Dep Microbiol.* 2017.
22. Elad D, Shpigel NY, Winkler M, Klinger I, Fuchs V, Saran A, et al. Feed contamination with *Candida krusei* as a probable source of mycotic mastitis in dairy cows. *J Am Vet Med Assoc.* 1995; 207(5):620–622.
23. Nazmy S. Isolation and identification of fungi from subclinical mastitic milk. *Benha Vet Med J.* 2022;42(2):138–142.
24. Jiang Y, Ogunade IM, Vyas D, Adesogan AT. Aflatoxin in dairy cows: Toxicity, occurrence in feedstuffs and milk and dietary mitigation strategies. *Toxins (Basel).* 2021;13(4):283.
25. A.-M. Ariton, Poroșnicu I, Neculai-Văleanu A-S, Crivei I-C, Sănduleanu C, Postolache A-N, et al. Strategies for identifying and preventing fungal mastitis in dairy cows. *Sci Pap Anim Sci Biotechnol.* 2022;55(2):104.
26. Spanemberg A, Wunder Jr EA, Pereira DIB, Argenta J, Sanches EMC, Valente P, et al. Diversity of yeasts from bovine mastitis in Southern Brazil. *Rev Iberoam Micol.* 2008;25(3):154.
27. Ahmed Kadhim M. Detection of *Cryptococcus* sp. from clinical and subclinical mastitis in dairy cows and assessment antifungal activity to fluconazole, amphotericin B, and nystatin. 2023.
28. Ribeiro MG, Salerno T, de Mattos-Guaraldi AL, Ferreira Camello TC, Langoni H, Siqueira AK, et al. Nocardiosis: An overview and additional report of 28 cases in cattle and dogs. *Rev Inst Med Trop Sao Paulo.* 2008;50(3):177–185. Available from: <http://doi.org/10.1590/S0036-46652008005000004>
29. Chauhan RS, Malik YS, Saminathan M, Tripathi BN. Immunopathology of mastitis. In *Essentials of Veterinary Immunology and Immunopathology.* Springer; 2024. p. 373–403. Available from: http://doi.org/10.1007/978-981-99-2718-0_11
30. Jabłońska W, Gołębiewski M, Kot M, Mardan H, Pawliński B, Kalińska A, et al. Perspectives and possibilities for new antimicrobial agents in the treatment and control of mastitis induced by algae of the genus *Prototheca* spp.: A review. *Int J Mol Sci.* 2024;25(15). Available from: <http://doi.org/10.3390/ijms25158219>
31. Watanabe A, Murakami H, Kakinuma S, Murao K, Ohmae K, Isobe N, et al. Association between bovine leukemia virus proviral load and severity of clinical mastitis. *J Vet Med Sci.* 2019;81(10):1431–1437.
32. A. de J. Da Silva, de Souza FN, Blagitz MC, Batista CF, Bellinazzi JB, Nobre DSM, et al. Implications of bovine viral diseases for udder health. *Brazilian J Vet Res Anim Sci.* 2018;55(3): e140200–e140200.
33. Vlasova AN, Saif LJ. Bovine immunology: Implications for dairy cattle. *Front Immunol.* 2021; 12:643206.

34. Altun S, Özdemir S, Sağlam YS. The presence and prevalence of bovine parainfluenza 3 (BPiV-3), bovine papillomaviruses (BPV), bovine herpesvirus 1 (BHV-1) in subclinical mastitis in cattle. *Kocatepe Vet J.* 2019;12(2):135–143.
35. Khalafalla AI. Papillomaviruses and polyomaviruses. *Recent Adv Anim Virol.* 2019:21–36.
36. Wellenberg GJ, van der Poel WHM, Van Oirschot JT. Viral infections and bovine mastitis: A review. *Vet Microbiol.* 2002;88(1):27–45.
37. Fayer R, Lynch GP, Leek RG, Gasbarre LC. Effects of sarcocystosis on milk production of dairy cows. *J Dairy Sci.* 1983;66(4):904–908.
38. S. Stelzer, Basso W, Silván JB, Ortega-Mora LM, Maksimov P, Gethmann J, et al. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. *Food Waterborne Parasitol.* 2019;15:e00037. Available from: <http://doi.org/10.1016/j.fawpar.2019.e00037>
39. Nogara KF, Busanello M, Tavares MG, De Assis JA, Freu G, Santos MVD, et al. Factors influencing milk quality and subclinical mastitis in dairy herds housed in compost-bedded pack barn system. *Animals.* 2023;13(23):3638.
40. Fávero S, Portilho FVR, Oliveira ACR, Langoni H, Pantoja JCF. Factors associated with mastitis epidemiologic indexes, animal hygiene, and bulk milk bacterial concentrations in dairy herds housed on compost bedding. *Livest Sci.* 2015;181:220–230.
41. Cheng WN, Han SG. Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments—A review. *Asian-Australasian J Anim Sci.* 1699;33(11):1699.
42. Khan MZ, Khan A. Basic facts of mastitis in dairy animals: A review. *Pak Vet J.* 2006;26(4): 204.
43. Bicudo JR, Goyal SM, Pathogens and manure management systems: A review. *Environ. Technol. (United Kingdom).* 2003;24(1):115–130. Available from: <http://doi.org/10.1080/09593330309385542>
44. Bekuma A, Galmessa U. Review on hygienic milk products practice and occurrence of mastitis in cow's milk. *Agric Res Technol Open Access J.* 2018;18(2):1–11.
45. Iraguha B. Bovine mastitis control strategies with emphasis on developing countries. 2023.
46. Kibebew K. Bovine mastitis: A review of causes and epidemiological point of view. *J Biol Agric Healthc.* 2017;7(2):1–14.
47. Gleeson D, Flynn J, Brien BO. Effect of pre-milking teat disinfection on new mastitis infection rates of dairy cows. *Ir Vet J.* 2018;71:1–8.
48. Odorčić M, Rasmussen MD, Paulrud CO, Bruckmaier RM. Milking machine settings, teat condition and milking efficiency in dairy cows. *Animal.* 2019;13(S1):s94–s99.
49. Vermaak P, Petzer I-M, Karzis J. Effects of milking machine settings and teat liners on bovine udder health. *S Afr J Anim Sci.* 2022;52(4):421–432.
50. Natzke RP, Everett RW, Bray DR. Effect of overmilking on udder health. *J Dairy Sci.* 1982;65(1):117–125.
51. Cobirka M, Tancin V, Slama P. Epidemiology and classification of mastitis. *Animals.* 2020;10(12):1–17. Available from: <http://doi.org/10.3390/ani10122212>
52. Zigo F, Vasil M, Ondrašovičová S, Výrostková J, Bujok J, Pecka-Kielb E. Maintaining optimal mammary gland health and prevention of mastitis. *Front Vet Sci.* 2021;8:607311.
53. Romero G, Peris C, Fthenakis GC, Diaz JR. Effects of machine milking on udder health in dairy ewes. *Small Rumin Res.* 2020;188:106096.
54. Yang FL, Li XS, Role of antioxidant vitamins and trace elements in mastitis in dairy cows. *J Adv Vet Anim Res.* 2015;2(1):1–9.
55. Sharma N, Upadhyay SR, Hussain K, Soodan JS, Gupta SK. Role of antioxidants in udder health: A review. *Intas Polivet.* 2007;8(2):284–295.
56. M. Z. Khan, et al. Role of selenium and vitamins E and B9 in the alleviation of bovine mastitis during the periparturient period. *Antioxidants.* 2022;11(4):657.
57. K. Libera, Konieczny K, Witkowska K, Żurek K, Szumacher-Strabel M, Cieslak A, et al. The association between selected dietary minerals and mastitis in dairy cows—A review. *Animals.* 2021;11(8):2330.

58. Driehuis F, Wilkinson JM, Jiang Y, Ogunade I, desogan ATA.. Silage review: Animal and human health risks from silage. *J Dairy Sci.* 2018;101(5):4093–4110.
59. Fink-Gremmels J. The role of mycotoxins in the health and performance of dairy cows. *Vet J.* 2008;176(1):84–92.
60. R. Falkauskas, Bakutis B, Jovaisiene J, Žilaitis V, Pridotkas G, Stankevicius R, et al.. Mycotoxin risk management for dairy cows by monitoring blood parameters, reproduction status and SCC in milk. *Arq Bras Med Veterinária e Zootec.* 2022;74:281–290.
61. P. Brodzki, Marczuk J, Lisiecka U, Krakowski L, Szczubiał M, Dąbrowski R, et al.. Assessment of selected immunological parameters in dairy cows with naturally occurring mycotoxicosis before and after the application of a mycotoxin deactivator. *J Vet Res.* 2023;67(1):105–113.
62. Vitali A, Bernabucci U, Nardone A, Lacetera N. Effect of season, month and temperature humidity index on the occurrence of clinical mastitis in dairy heifers. *Adv Anim Biosci.* 2016;7(3):250–252.
63. Vitali A, Felici A, Lees AM, Giacinti G, Maresca C, Bernabucci U, et al. Heat load increases the risk of clinical mastitis in dairy cattle. *J. Dairy Sci.* 2020;103(9):8378–8387.
64. Krawczel P, Grant R. Effects of cow comfort on milk quality, productivity and behavior. in *NMC Annual Meeting Proceedings.* 2009.
65. Abebe R, Markos A, Abera M, Mekbib B. Incidence rate, risk factors, and bacterial causes of clinical mastitis on dairy farms in Hawassa City, southern Ethiopia. *Sci Rep.* 2023;13(1):10945.
66. Jessé González Arenas D, Vergara-Galván M, Jiménez-Escobar C, Zambrano-Varón J. Risk factors associated with the incidence of clinical mastitis in pasture-based dairy herds in Colombia. 2022:1–16.
67. Hogan J, Smith KL. Managing environmental mastitis. *Vet Clin Food Anim. Pract.* 2012;28(2):217–224.
68. Singh AK. A comprehensive review on subclinical mastitis in dairy animals: Pathogenesis, factors associated, prevalence, economic losses and management strategies. *CABI Rev.* 2022;(2022).
69. Blowey R, Edmondson P. *Mastitis control in dairy herds.* (2nd Edition). 2010;53(9).
70. Miles AM, Huson HJ. Graduate student literature review: Understanding the genetic mechanisms underlying mastitis. *J Dairy Sci.* 2021;104(1):1183–1191.
71. M. Hovinen, S. Pyörälä. “Invited review: Udder health of dairy cows in automatic milking,” *J. Dairy Sci.* 2011;94(2):547–562.
72. Corset A, Remot A, Graulet B, Poton P, Philau S, Ricoulet JF, et al. Effects of parity and week after calving on the metabolic, redox and immune status of dairy cows. *J Dairy Sci.* 2024;107(10):8592–8608. Available from: <http://doi.org/10.3168/jds.2024-24706>
73. Stanek P, Żółkiewski P, Januś E. A Review on mastitis in dairy cows research: Current status and future perspectives. *Agriculture* 2024;14(8):1292.
74. Abdel-Rady A, Sayed M. Some epidemiological studies on subclinical mastitis in dairy cows in assiut governorate. *J Mastitis/Udder Heal Milk Qual.* 2008;25(551):53.
75. Cardona CJ. Farm and regional biosecurity practices. *Avian Infl.* 2008:353–367.
76. Brennan ML, Christley RM. Biosecurity on cattle farms: A study in north-west England. *PLoS One.* 2012;7(1):e28139.
77. Ruegg PL, Pantoja JCF. Understanding and using somatic cell counts to improve milk quality. *Irish J Agric Food Res.* 2013:101–117.
78. L. Filippone Pavesi, Pollera C, Sala G, Cremonesi P, Monistero V, Biscarini F, et al. Effect of the selective dry cow therapy on udder health and milk microbiota. *Antibiotics.* 2023;8:1259.
79. Elbayoumy MK, Allam AM, Ghazy AA, Nasr SM. Advances in controlling bacterial mastitis in dairy cows. *Egypt J Vet Sci.* 2024;55:1–21.
80. Nielsen C, *Economic Impact of Mastitis in Dairy Cows*, no. November. Department of Animal Breeding and Genetics, Swedish University of ..., 2009.
81. Rodriguez Z, Lopez-Benavides M, Gentilini MB, Ruegg PL. Impact of training dairy farm personnel on milking routine compliance, udder health, and milk quality. *J Dairy Sci.* 2024. Available from: <http://doi.org/10.3168/jds.2024-25609>