An overview of subclinical mastitis in dairy cattle

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Abstract

This manuscript describes economic losses, prevalence, etiology, nutritional approaches, risk factors, diagnostic tests, and control to preclude the causes of subclinical mastitis in dairy cattle. Subclinical mastitis is more disparaging than clinical mastitis. It is almost asymptomatic, with no visible changes observed on the udder; only milk production reduces and milk quality changes. It is a multi-etiological complex that includes nutritional deficiency, number of lactations and unhygienic managemental practices. Diagnosis of subclinical mastitis is quite difficult than clinical mastitis. The detection of subclinical mastitis in suspected quarters can be made using either direct measurement of the somatic cell count (SCC) or indirectly by performing a California Mastitis Test (CMT). Intramammary antibiotics are widely used to treat cows infected with *Streptococcus agalactiae* and have a positive response in production and decrease bulk tank SCC as well. Penethamate is efficacious and sustains a positive effect to reduce SCC in milk against subclinical mastitis.

Key words: Asymptomatic, CMT, Economic loss, Intramammary antibiotics, Subclinical mastitis

Introduction

Livestock productions play a major role in the economy of the country and the life of human beings. Mastitis, in particular, subclinical mastitis is a very common disease that occurs in dairy cows and causes more significant economic loss due to its prolonged effect throughout the lactation period. The term ‘mastitis’ originated from the Greek word *Mastos* or *Mammae* meaning breast and *itis* meaning inflammation. Radostits *et al.* (2007) defined mastitis as an infection or inflammation of the parenchyma of the mammary gland. Drug residues in milk enable easy transmission of the organism to the human population and also create public health issues and economical threats (Kader *et al.*, 2002).

Mastitis is inflammation of the parenchyma of mammary glands characterized by bacteriological changes in milk, milk production and pathological alteration in

glandular tissues. It is one of the leading diseases of dairy cattle that potentially affect farmers’ economy due to reduction in milk yield, milk discard after treatment, cost of treatment, and premature culling. Mastitis is categorized as clinical and subclinical mastitis based on signs and changes in milk quality and quantity of affected cows. The main features of clinical mastitis are edema, udder inflammation with constant and steady pain, and change in colour and consistency of milk, whereas only quality and quantity are reduced with no visible changes in udder observed in subclinical mastitis which make them complex in detection. So, diagnosis of subclinical mastitis (SCM) is difficult than clinical mastitis. When a cow is in heat, or there is a climate change, subclinical mastitis is converted into clinical mastitis (Saroj *et al.*, 2015). On the other hand, many microorganisms are implicated in mastitis infection, few of them are environmental

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pathogens, and some are contagious (Ruegg, 2017).

The milk from affected quarters seems to be normal, even when millions of somatic cells (SC) present in milk, and hence SCM can also be defined as when milk is normal but there may be a decrease in production and may have SCC level of 200,000 cells/mL or greater and can be detected through California Mastitis Test. Therefore, herd screening for SCM infection should be done regularly to prevent milk production loss (Halasa *et al.*, 2009). In the current scenario, MALDI-TOF MS has been used in many countries to detect mastitis because of its speed and reliability (Nonnemann *et al.*, 2019). Therapeutic and prevention strategies have been practiced over the years to improve the health and production of dairy animals which contributes to the economy of the country (Barkema *et al.*, 2006; Pérez *et al.*, 2009; Pereira, 2011; Keefe, 2012).

Economic impacts of subclinical mastitis in dairy cow

Subclinical mastitis has a negative influence on the performance of dairy cows and buffaloes. It has a significant impact on production, nearly 10 - 20% decrease in milk production, as it causes an undesirable effect on the milk constituents, which affects its nutritional value and renders it unfit for processing and consumption (Iraguha *et al.*, 2015). It includes reduced milk production, changes in milk quality and treatment cost. Rathod *et al.* (2017) estimated the economic losses due to subclinical form of mastitis were approximately in the range of INR 21,677 to INR 88,340 per animal for a lactation period. It has been reported that SCM causes threefold more production losses as compared to clinical mastitis leading to substantial economic losses of 60-70% all due to mastitis (De Vliegher *et al.* 2012; Sinha *et al.* 2014).

Susanty *et al.* (2018) observed agro climatic effects on milk production and shows sub-clinical mastitis prevalence in dairy cattle.

Another study revealed that the prevalence rate of mastitis in cows was high as environmental temperature increases significantly (summer) due to the rise in somatic cell score (Bouraoui *et al.*, 2002; Khate and Yadav, 2010; Bertocchi *et al.*, 2014). The temperature and humidity index (THI) can regulate the comfort zone of cows. The incidence of clinical mastitis progresses with an increase in the temperature-humidity index (THI). In addition, increase THI is related to mastitis prevalence and somatic cell score (Hammami *et al.*, 2013). A rise in somatic cell count in dairy cattle linked with mastitis also reported by Barkema *et al.* (2013). Any variation of the body temperature above the normal range suggests the level of heat stress in dairy cattle and also affected by wind speed, dry temperature, humidity and solar radiation (Dikmen and Hansen, 2009). It has been observed that room temperature below 23°C and humidity of 68% is most suitable for the highest production of milk by dairy cattle (Bohmanova *et al.*, 2007). Hence, temperature and humidity play a crucial role in the production of dairy cattle.

Prevalence of subclinical mastitis

Balaji and Senthilkumar (2017) reported a prevalence rate of 67.80% of subclinical mastitis in 80 dairy cows of the Theni district. The prevalence of subclinical mastitis in dairy cows varied from 15 to 75%, whereas the involvement of quarters varied between 5 and 40% (Cynthia, 2005). Due to the importance of unseen nature and massive impact on health and production in lactating cows, gathering information about its prevalence across India provide a guideline for strategic management and control of the disease to reduce economic losses in the dairy sector. The states of Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, and Maharashtra had estimates of 53.52%, 51.18%, 39.58 %, 62.49 % and 35.11% subclinical mastitis respectively (Bangar *et al.*, 2015). According to Busato *et al.* (2000), subclinical mastitis prevalence at the quarter level was 21.2% for a lactation period of 7 to 100 days

and 34.5% for 101 to 305 days postpartum in organic certified dairy farms. According to Varshney and Naresh (2004), the prevalence of subclinical forms of mastitis was more common in India. It was 10-50% in cows when compared to clinical mastitis (1-10%). Ghose and Sharda (2003) had depicted the prevalence rate of subclinical mastitis rate in dairy cows was 52.48%.

Sharma *et al.* (2008) reported a high prevalence rate in dairy cows that was 78%. Abrahmsén *et al.* (2014) observed 86.2% cases of subclinical mastitis through CMT screening of one or more quarters in dairy cows. Mpatswenumugabo *et al.* (2017) conducted a cross-sectional study in dairy farms of Rubavu and Nyabihu districts, Rwanda using the California Mastitis Test and found 50.4% prevalence rate of subclinical mastitis in dairy cows. Sumon *et al.* (2017) reported the subclinical prevalence rate in dairy cows was 25%. Yadav *et al.* (2019) found a higher prevalence rate of subclinical mastitis in cows (38.33%) as compared to buffaloes (16.32%). Senthilkumar *et al.* (2020) depicted the subclinical mastitis prevalence rate based on the stage of lactation: early, mid and late stage of lactation, the prevalence of subclinical mastitis was 28.57%, 6.67% and 16.67% respectively. The quarter wise-right forequarter, right hindquarter, left forequarter and left hindquarter, the prevalence of subclinical mastitis was 16.66%; 54.17%; 8.33% and 20.83% respectively.

On the basis of parity, from first to fourth parity, the prevalence of subclinical mastitis was 9.09%, 16.66%, 25.00% and 25.00% respectively. The prevalence rate of subclinical mastitis based on cultural examination was quarter-wise (64.21 %) and cow wise (39.83%) (Pankaj *et al.*, 2012). Bangar *et al.* (2015) studied the prevalence of subclinical mastitis on cow-basis using 6344 cows from 25 farms and found to be 46.35% (95 % CI 39.38; 53.46). Meta-analysis for the quarter-wise prevalence of subclinical mastitis was carried out using 18,721 udder quarters of

dairy cows from 23 studies, and the pooled estimate of the prevalence of subclinical mastitis on quarter-basis was found to be 23.25% (95% CI 18.15; 29.27). Devi and Dutta (2018) reported the incidence rate of subclinical mastitis cow-wise and quarter-wise on the basis of Somatic Cell Count (SCC) as 93.33% and 90.26% respectively in organized and unorganized farms in and around Khanapara, Guwahati, Assam. Mbindyo *et al.* (2020) reported the overall prevalence of clinical and subclinical mastitis was 51.6% (815/1580), 74.4% (294/395) and 76.6% (118/154) at the quarter, cow and farm level, respectively. The prevalence rate of subclinical mastitis was observed high in European crossbreds than in local breeds (Abdeta and Gemechisa, 2020).

Etiology of subclinical mastitis in dairy cattle

The foremost causes of SCM in dairy animals are due to many factors such as unhygienic management, nutritional deficiency and several pathogens. Multiple causative factors are involved in SCM occurrence and its severity depend on various variables of the host, pathogens, and the environment. Cheng and Han (2020) stated that multiple pathogens that contribute to causing mastitis included contagious organisms, environmental bacteria and opportunistic bacteria. Other organisms such as fungi are less frequently a cause of mastitis in dairy cows. The bacterial etiology are *Streptococcus agalactia* and *Staphylococcus aureus*, *Nocardia* spp., *Mycoplasma* spp. and environmental Streptococci. Another report showed that *Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp., *Trueprella pyogenes*, *Corynebacterium bovis* and *Bacillus* spp. were causing bovine mastitis in 82 lactating cows (Mollalegn *et al.*, 2010). Hande *et al.* (2015) identified the bacterial isolates of *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Enterococcus* spp. and *Streptococcus agalactiae* from the cases of subclinical mastitis in dairy cattle in the Samsun district of Turkey.

Host factors and environmental factors play an essential role in the occurrence of the disease in animals. A wide range of microorganisms can enter the mammary gland through the teat canal and cause disease. Sources of environmental exposure are unhygienic managemental conditions such as contaminated manure, bedding, feeds, dirt, mud and water. Fahim *et al.* (2019) observed that *E. coli* strains are causing intramammary infections including subclinical mastitis. Banerjee *et al.* (2017) observed that *Pseudomonas aeruginosa* is one of the etiological pathogens of subclinical mastitis in dairy cows. Karabasanavar *et al.* (2019) reported that Staphylococci were isolated and confirmed using 16S rRNA gene-based genus-specific PCR in 39.2% of SCM affected quarters and detected virulence-associated *nuc* gene in 75% of *Staphylococcus* isolates suggesting their potential pathogenicity.

The high prevalence of subclinical mastitis was associated with different factors, including herd size, milk marketing, agro-climatic conditions of a region, animal owner education level, variations in sociocultural practices, feeding methods and management (Joshi and Gokhale, 2006). Ndahetuye *et al.* (2019) reported the non-aureus Staphylococci (40.2%) most prevalent microbes of subclinical mastitis, followed by *Staphylococcus aureus* (22%). Another study showed that *Streptococcus* species (22.2%) were the most recorded pathogen for subclinical mastitis, followed by *Staphylococcus aureus* (15.7%), *Pseudomonas aeruginosa* (5.1%) and *Enterobacter* species (0.7%) (Mbindyo *et al.*, 2020). A study identified the causative agents of subclinical mastitis in dairy cows as *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus agalactiae*, *S. dysagalactiae* and *S. pyogenes*, *E. coli*, *Proteus* spp. and *Klebsiella pneumoniae* by using PCR techniques (universal 16S primers) (Sahoo *et al.*, 2020).

Nutritional factors: The number of lactations, metabolic stress and protein-energy ratio may affect the immune system and induce the cause of mastitis in animals. Immunosuppression can

be linked to metabolic disturbance and changes in the modulation of immune system, which may lead to mastitis and reduce milk production (Bronzo *et al.*, 2020). Dey *et al.* (2019) stated that proper balance of nutrients prevents mastitis while nutritional imbalance or sudden change in diet may enhance mastitis incidence in the dairy cow.

Vitamin A and zinc play an essential role in maintaining the physical defense barriers as well as epithelial health of the udder. The functions of phagocytic cells in cattle are influenced by vitamins (A and E), copper, zinc and selenium. Nutritional supplementation of dairy cows with vitamin E enhances the killing ability of immune cells (neutrophils). The nutrient imbalance may be occurring due to either excess or deficiency of essential nutrients in the diet of dairy animals (Haq *et al.*, 2016).

Risk factors: The major risk factors of SCM include host, management practices, diet and pathogens. The host factor consists of age, stage of lactation, milking interval, breed, parity, milk yield capacity, udder and teat conformation, somatic cell count, udder defense, dry period, teat injuries and genetic resistance. Cows of certain breeds are more prone to mastitis. Among Swedish breeds, a high prevalence of mastitis has been recorded in Swedish Holstein cows. Crossbred cows are more susceptible to mastitis than local breeds (Persson *et al.*, 2009). Managemental practices comprise shed and udder hygiene, poor teat condition, poor environmental hygiene, milking technique, sanitation and large herd size, use of handwash cloth, teat dipping and hygiene of milking machine.

Diagnostic test for subclinical mastitis

Microbiological status of the affected quarter and the somatic cell count (SCC) used to detect abnormal changes are mainly due to the inflammatory process (Sudhan and Sharma, 2010). Tresamol *et al.* (2019) found that EC estimation to be easier than CMT as a screening test. Langer *et al.* (2014) stated that many direct and indirect tests help to detect SCM. The direct

test includes Somatic Cell Count, and the indirect test includes Modified California Mastitis Test (MCMT), Modified White Side Test (MWST), Cow Side Test, Screening Test [Electrical conductivity (EC) and pH test] and Laboratory Test [Methylene Blue Reduction Test (MBRT)]. These tests determine the quality of milk. However, in the absence of laboratory facilities, a suitable field-level test may be considered in detecting SCM (Sharma *et al.*, 2008).

Inflammation of the mammary gland is directly accompanied by an increase of SCC in milk (Rodriguez *et al.*, 2000). Therefore, SCC is a standard test for SCM (Durr *et al.*, 2008) and represents a direct test for detecting SCM. Reddy *et al.* (2015) reported that the quarter-wise incidence of SCM as detected by CMT and EC was 27.83 and 19.95% respectively whereas the animal-wise incidence was 52.28 and 38.13% for CMT and EC respectively.

Treatment of subclinical mastitis in dairy cow

Several systematic antibiotics such as procaine penicillin G, amoxicillin, ampicillin and oxytetracycline are effective against mastitis (Preez, 2000). Salat *et al.* (2008) found that systemic treatment of subclinical mastitis with penethamate is effective. The bacteriological cure (BC) of infected quarters has a sustained positive effect on milk SCC during the treatment. Nutrition and feeding management can affect the outcome of mastitis, and some of these nutritional components are less specific to older cows due to differences in their nutrient requirements and metabolism (Saroj *et al.*, 2015).

The therapeutic use of oil extracts of *Ocimum sanctum* (tulsi) with *Azadirachta indica* (neem) and aqueous extract of *Tinospora cordifolia* (giloy) gave good results for reducing bacterial load and enhancement of phagocytic capacity (Dash, 2001). Mastitis remains one of the most economically devastating diseases in dairy cows. Pandey and Kumar (2018) observed that herbal spray was effective against

subclinical mastitis in dairy cows. Administration of tylosin along with benethamine penicillin, penethamate hydriodide and framycetin showed effective results in dairy cattle affected with subclinical mastitis (Ismail *et al.*, 2018).

Prevention and control measures

The outbreak of SCM can be prevented either pre and post milking udder disinfection or by changes in milking technique like using machine milking (Mpatswenumugabo *et al.*, 2017). The contribution of dietary management in the development of mastitis has been controversial and difficult to isolate from other confounding effects. Feeding high-concentrate diets to increase milk yield was a risk factor for mastitis and complexity in udder metabolism (Hamilton *et al.*, 2006) but significant effects of nutrition on udder health which predisposes to mastitis and ketosis in dairy cow (O'Rourke, 2009). Dietary deficiencies of selenium and vitamin E cause of increasing incidence as well as the duration of mastitis. So, a balanced diet should be provided to dairy animals along with hygienic managerial conditions. Kumari *et al.* (2018) observed the efficacy of herbal preparations as an internal and external sealant against subclinical mastitis in dairy cow management. They found that TM or R herbal products along with the company as internal teat sealant helps in treating subclinical mastitis in dairy cows. McParland *et al.* (2019) observed that the use of teat sealant alone could be beneficial in dairy cows with an efficient mastitis control program by decreasing the subclinical mastitis cases.

Dry cow therapy has shown effective results in eliminating the existing intramammary infections and preventing the occurrence of new intramammary infections; hence plays a vital role in the mastitis control programme. This therapy includes administering intra-mammary or systemic antibiotics during the dry period (2 weeks before parturition) (Kashif *et al.*, 2016). Leitner *et al.* (2018) utilized acoustic pulse therapy for treating subclinical mastitis and estimated 70.5% of mastitis cattle return to

normal milk yield than the control group (18.4%).

National Mastitis Council (NMC, 2000) developed ten steps list to follow in a mastitis control program which includes: establish goals for udder health, maintain a clean, dry, comfortable environment, follow proper milking procedures, maintain and use milking equipment properly, keep good records, manage clinical mastitis during lactation appropriately, establish an effective dry cow management program, follow a biosecurity program against contagious pathogens, monitor udder health status regularly and periodically review your mastitis control program.

Conclusion

Subclinical mastitis is a major health concern for dairy cows, as it causes silent economic loss without visible alteration in the milk. SCM causes 3-5 times more financial loss

than clinical mastitis. SCM prevalence is also highly associated with the agroclimatic condition affecting milk production in dairy cattle. The following prevention strategies should be followed to overcome SCM, such as implementing good management in a dairy farm, regular checkup of animals for early diagnosis and proper treatment, which may reduce health-related problems and occurrence of concurrent diseases in dairy cows. The hygienic practice of milking has a more significant impact on lowering SCM incidence, which includes dry cow therapy, post milking teat disinfection, and the use of sterile gloves while milking. So, by following the above steps, we can prevent SCM and minimize economic losses by improving the quality and quantity of milk.

Conflict of interest: Authors have no conflict of interest in this study.

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