



A REVIEW ON *STREPTOCOCCUS AGALACTIAE*: THE CAUSE OF BOVINE MASTITIS

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Abstract: Around 150 species of microorganisms, typically bacteria, are capable to cause mastitis in dairy cows. Based on the source of infection, these microorganisms are categorized into contagious and environmental pathogens. The main contagious mastitis pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* species. *Streptococcus agalactiae* is a gram-positive coccus with a tendency to form chains and it is beta-hemolytic, fastidious, catalase-negative, and facultative anaerobes. The objectives of this review are to give a brief overview of *Streptococcus agalactiae* and to assess diagnostic methods of the bacteria. GBS is surrounded by a capsule composed of polysaccharides (exopolysaccharide). The species is subclassified into ten serotypes (Ia, Ib, II- IX) depending on the immunologic reactivity of their polysaccharide capsule. *Streptococcus agalactiae* is known for its high infectivity, rapid spread, and silent nature. The bacteria show the existence of two transmission cycles: a contagious transmission cycle via milking machine and a fecal-oral cycle, via drinking water. California mastitis test (CMT) is very simple and can be performed on farm for somatic cell count (SCC) estimation. The CMT reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time. *S. agalactiae* are CAMP test positive which gives a sharp arrow-head enhancement of haemolysis caused by the beta-haemolysin of *Staphylococcus aureus*. First choice antimicrobials for treating mastitis caused by streptococci and penicillin susceptible staphylococci are β -lactam antimicrobials, particularly penicillin G. Measures aiming at preventing new cases of mastitis include fly control, optimal nutrition, improvement of milking hygiene, avoidance of inter-sucking among young ones, implementation of post-milking teat disinfection, regular control of the milking equipments and implementation of milking order.

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1. INTRODUCTION

In Ethiopia livestock plays a major national resource; the majority of agricultural output is generated from crop and livestock integrated production systems. The country has the largest livestock population of any African country with estimated 52.13 million and dairy cows are representing around 7.2 million (CSA, 2012). Milk produced from these animals provides an important dietary source for the majority of rural as well as considerable number of the urban and peri-urban population. Milk is considered as a nearly complete food since it is a good source of protein, fat and major minerals. Several studies have reported the distribution and occurrence of the essential components in various animal milks (Gasmalla *et al.*, 2017). However, milk production often does not satisfy the country's requirement, due to a multitude of factors, out of which disease of the mammary glands known as mastitis is among the various factors contributing to reduced milk production (Fayera *et al.*, 2019).

Mastitis, inflammation of the parenchyma of mammary gland, is a multi-etiological and complex disease of

animals and it is characterized by physical, chemical, and usually, bacteriological changes in the milk, and pathological changes in the glandular tissues. The occurrence of the disease is an outcome of interplay between three major factors: infectious agents, host resistance like breed, the shape of the udder and teats, age as well as stage of lactation, and environmental factors (FAO, 2014). Around 150 species of microorganisms, typically bacteria, are capable to cause mastitis in dairy cows. Based on the source of infection, these microorganisms are categorized into contagious and environmental pathogens. The main contagious mastitis pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* species. They primarily infect the cow's udder and they can spread from cow to cow during the time of milking (AbdEl-razik *et al.*, 2021) and the disease is often classified as subclinical or clinical depending on the pathological changes in the milk, teats and udder (Andrews *et al.*, 2003).

Streptococcus agalactiae is the major etiologic agent of invasive neonatal infections in humans, causing sepsis,

pneumonia, meningitis, osteomyelitis, and soft tissue infections (Baker, 2000). Unlike *Staphylococcus aureus* which can multiply in different tissues, *Streptococcus agalactiae* is one of the mastitis bacteria that can usually grow and multiply in the udder (Andersen *et al.*, 2003). However, these bacteria can survive for short time periods on hands, milking machine parts, and teat skin, leading to its spread from cow to cow during milking. *S. agalactiae* is most commonly introduced into a clean herd when an infected cow is purchased. Because of the silent nature of infections and highly contagious nature, infections can spread quickly (Sandy, 2011).

Streptococcus species are non-motile, fastidious bacteria which require the addition of special nutrients like blood or serum to their culture media. These bacteria can be differentiated by Lancefield group antigens, type of haemolysis and biochemical fermentation patterns. Streptococci can exhibit three types of haemolysis (alpha, beta and gamma). Alpha (α) haemolysis: green or partial haemolysis, Beta (β) haemolysis: clear zone of haemolysis, and Gamma (γ) haemolysis: no haemolysis. The type of haemolysis depends on factors like the species of Streptococcus, the type of blood used in the culture medium, and environmental conditions. The optimal temperature for Streptococcus species is around 37°C, although most can grow in the range of 20-42°C (Kerorsa, 2020).

Group B Streptococcus (*Streptococcus agalactiae*) is a gram-positive coccus with a tendency to form chains (as reflected by the genus name Streptococcus). It is beta-hemolytic, catalase-negative, and facultative anaerobes (Ryan *et al.*, 2004). The bacterium, *Streptococcus agalactiae*, was a major cause of mastitis in the pre-antibiotic era. It remains a significant cause of chronic mastitis in many herds, even though it can be readily eliminated. The bacteria have the ability to adhere to the mammary tissue of cows and the specific microenvironment of the bovine udder is necessary for the growth of the bacterium. The virulence of various strains is related to differences in their ability to adhere to the mammary epithelium of the animals. Procedures for the diagnosis and treatment of intramammary infections due to the bacteria are well established (Keefe, 1997). If detected early, antibiotic therapy is very effective in curing and controlling the spread of contagious pathogens. However, antibiotic therapy is not effective against environmental pathogens, especially coliform bacteria. Culling is another method of control especially when dealing with chronically infected animals. This eliminates the potential source of infection at the expense of purchasing a replacement animal (Tomita and Hart, 2001). Therefore, the objectives of this review are to give a brief overview of *Streptococcus agalactiae* and to assess identification methods of the bacteria.

2. STREPTOCOCCUS AGALACTIAE: THE CAUSE OF BOVINE MASTITIS

2.1. General Characteristics of Streptococci

Streptococci are Gram positive cocci in the family Streptococcaceae (OIE, 2005). They vary from spherical to short bacillary cells, about 1 μm in diameter. Division occurs in one plane, producing pairs and chains (fig. 1). Chain formation is variable, though some species (*S. equi*) are consistent chain formers. Young cultures are gram positive; in exudates and old cultures (> 18 hours) often stain gram negative. The cell envelope and cell wall resemble those of staphylococci. Capsules if present, are polysaccharide and antiphagocytic. Those of *S. pyogenes* and *S. equi* consists of nonantigenic hyaluronic acid. *S. pneumonia* and *S. agalactiae* have antigenically diverse capsules, which provide a basis for serotyping (Dwight and Yuan, 1999).

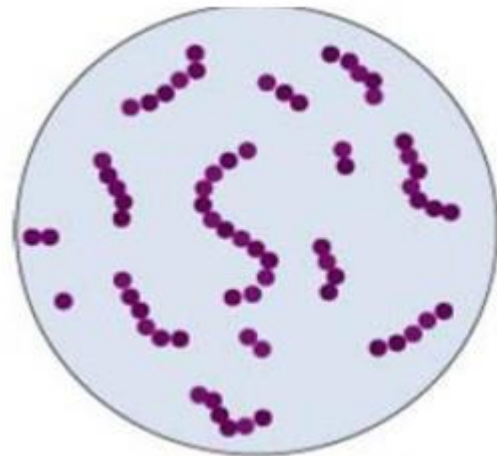


Figure 1: Streptococcus showing chain arrangement (Kerorsa, 2020)

Cells of streptococci are normally spherical or ovoid in shape, but some species may appear as short rods under certain cultural conditions. They are typically arranged in chains or pairs, chain formation being seen best in broth cultures. Individual cells are usually 0.8-1.2 μm in diameter and chains lengths vary from a few cells to over 50, depending on the strain and the growth conditions. It is not unusual for cells in older cultures to appear Gram-variable, whilst some strains may be highly pleomorphic on initial isolation. Some species produce capsules, either of hyaluronic acid or a variety of type-specific polysaccharides, but this is not a regular feature throughout the genus as a whole. Several species produce extracellular polysaccharides when grown in the presence of sucrose, including both glucans and fructans (Hardie and Marsh, 1978a).

Two characteristics of major importance in the identification of Streptococci are the Lancefield groups and kind of haemolysis produced. Lancefield groups are

designated by the capital letters A-V. This grouping is based on serological differences in a carbohydrate substance in the cell wall called component C; the antigenic determinants are amino sugars. A precipitation test is employed using extracts containing component C and specific grouping sera that are usually prepared in rabbits. Other serological procedures, including latex agglutination, coagglutination, and fluorescent antibody tests are also used to identify Lancefield groups (Carter and Wise, 2004).

The majority of pathogenic Streptococci possess a serologically active carbohydrate antigenically different from one species to another species. These cell wall antigens, designated as A-H and K - V, are the basis of the Lancefield grouping system and are widely used by clinical laboratories for serogrouping. Groups B, C, D, E, G, L, U, and V contain the pyogenic Streptococci responsible for pus - producing (suppurative) infections in a variety of hosts. Some pathogenic Streptococci, notably *Streptococcus uberis*, *Streptococcus parauberis*, and *Streptococcus pneumoniae*, are not groupable in the Lancefield grouping scheme and are identified by features such as fermentation behavior, ability to grow at different temperatures, salt tolerance, optochin sensitivity, bile solubility (Gyles *et al.*, 2010). *Streptococcus agalactiae* is a gram-positive coccus or round bacterium with a tendency to form chains as reflected by the genus name Streptococcus. It is beta-hemolytic, catalase-negative, and facultative anaerobes. GBS is surrounded by a capsule composed of polysaccharides (exopolysaccharide). The species is subclassified into ten serotypes (Ia, Ib, II- IX) depending on the immunologic reactivity of their polysaccharide capsule. This is why the plural term group B Streptococci (referring the serotypes) and the singular term group B Streptococcus (referring to the single serotype) are both commonly encountered (Slotved *et al.*, 2007).

2.2. Epidemiology of *Streptococcus agalactiae*

Streptococcus agalactiae strains isolated from udder mastitis and from human infections were shown to have 58% similarity, and clustering of the isolates showed they shared 70% genetic similarity. Infections from human strains are more likely to spontaneously heal than those originating from strains infecting udders of other animals. The self-cure rate is very low in animal-to-animal transmitted strains. Indirect evidence shows that younger cows in their first lactation period are more resistant to contagious causative agents. The pathogen can survive for a relatively long time and persist, undetected, in the udder. These animals serve as infection reservoirs and sources for spreading the disease. Unlike *S. aureus* and other contagious pathogens, *S. agalactiae* usually cannot multiply or grow outside the udder but can survive for a short duration on the hands of milking personnel, milking

machines, and teat surfaces. This may be sufficient for its spread to healthy cows during milking. Even if herd hygiene is adequate, some risk is associated with buying new cows if they are infected and not held in quarantine before integration into the herd. *S. agalactiae* is known for its high infectivity, rapid spread, and silent nature. The prevalence of the organism demonstrates that this bacterium is a significant cause of mastitis, especially in herds that are not well managed and have poor hygiene. Studies have shown that the main causative agents of mastitis in less-developed countries such as Ethiopia are the contagious ones and that the most prevalent mastitis cases are those of contagious origin. This could be associated with unhygienic milking practices and poor herd management (Cobirka *et al.*, 2020).

2.3. Transmission

In dairy cows, *S. agalactiae* is considered contagious bacterium of the udder in dairy cows, and it was considered an obligate pathogen. However, recent research in Norway has found that the bovine gastrointestinal tract and the dairy cow environment were reservoirs of *S. agalactiae*, and showing the existence of two transmission cycles: a contagious transmission cycle via milking machine and a fecal-oral cycle, via drinking water. The cow-to-cow transmission in the same herd is due to insufficient hygiene in the milking parlour, which permits multiple animals to become exposed to potentially contaminated equipment, hands and other milking utensils from an infected cow. Therefore, transmission more often occurs during the milking operation (Reyes, 2016). Generally, the pathogen in dairy cows is often spread by contaminated milking equipment, unskilled attempts at intramammary medication and unsanitary milking practices (Mohammed, 2006).

2.4. Disease caused by *Streptococcus agalactiae*

Among streptococci species *S. agalactiae* causes a number of diseases in different animals. It is an important cause of mastitis which continues to be a major cause of subclinical mastitis in dairy cattle and is a source of economic loss for the industry (Lucia *et al.*, 2017). Its significance as a veterinary pathogen capable of causing economic loss from high fish mortality in aquaculture is also well documented. In addition to the cause of mastitis in dairy cattle, it is an invasive human pathogen responsible for neonatal sepsis, meningitis and maternal sepsis while also being an opportunistic pathogen in immunocompromised adults and the elderly. The significance of GBS as a food borne pathogen has only been recognized recently due to a large unprecedented outbreak associated with raw fish consumption in Singapore in 2015. Severe symptoms reported in the outbreak included meningoencephalitis, bacteremia, and septic arthritis (Zwel *et al.*, 2018).

2.5. Virulence Factors of *Streptococcus* Species

Streptococcal virulence factors can be the categories of surface proteins, extracellular toxins which include Streptolysin-S, Streptolysin-O, streptokinase, and pyrogenic exotoxins. Streptolysin-O cause lysis of leukocytes and erythrocytes. Capsules protect the pathogen from the host's defenses and also from the effects of antibiotics and other chemicals. Streptococcal pyrogenic exotoxins A, B, and C are believed to be responsible for the rashes in scarlet fever and for the symptoms of toxic shock syndrome; arise in the distribution of streptococcal pyrogenic toxin producing strains is believed to be associated with the rise in Group A streptococcal invasive infections. The others are cell-associated factors that include M protein, help the bacterium in evading host's immune system, and lipoteichoic acid which, is mostly associated with *S. pneumoniae*, facilitates adherence (Mishra and Agrawal, 2013).

2.6. Virulence Factors of *Streptococcus agalactiae*

Streptococcus agalactiae produces several virulence factors, including haemolysins, capsule polysaccharide, C5a peptidase (only human pathogenic strains), hyaluronidase (not all strains), and various surface proteins that bind human IgA and serve as adhesins. Nine different types of the capsular polysaccharide have been identified (Ia, Ib, and II-VIII). The serotype most frequently associated with neonatal infections is type III, whereas infections in adults are more evenly distributed over the different serotypes. Among the haemolysins produced by *S. agalactiae*, one, known as the CAMP factor (so-called because it was originally described by Christie, Atkins and Munch-Petersen), plays an important role in the recognition of this species in the laboratory (Seid and Demeke, 2018).

2.7. Pathogenesis of *Streptococcus agalactiae*

When the primary barrier of the streak canal is passed, if bacteria are not flushed out by the physical act of milking they proliferate and invasion of the udder tissue follows. There is considerable variation between cows in the developments that occur at each of the three stages of invasion, infection and inflammation. The reasons for this variation are not clear but resistance appears to depend largely on the integrity of the lining of the teat canal. After the introduction of infection into the teat, the invasion, if it occurs, takes 1-4 days and the appearance of inflammation 3-5 days. Again there is much variation between cows in the response to tissue invasion, and a balance may be set up between the virulence of the organism and undefined defense mechanisms of the host so that very little clinically detectable inflammation may develop despite the persistence of a permanent bacterial flora. The development of mastitis associated with *S. agalactiae* is essentially a process of invasion and inflammation of lobules of mammary tissue in a series of crises,

particularly during the first month after infection, each crisis developing in the same general pattern. Initially there is a rapid multiplication of the organism in the lactiferous ducts, followed by passage of the bacteria through the duct walls into lymphatic vessels and to the supramammary lymph nodes, and an outpouring of neutrophils into the milk ducts. At this stage of initial tissue invasion, a short lived systemic reaction occurs and the milk yield falls sharply as a result of inhibition and stasis of secretion caused by damage to acinar and ductal epithelium. Fibrosis of the interalveolar tissue and involution of acini result even though the tissue invasion is quickly cleared. Subsequently, similar crises develop and more lobules are affected in the same way, resulting in a stepwise loss of secretory function with increasing fibrosis of the quarter and eventual atrophy (Radostits *et al.*, 2006).

2.8. Diagnosis

There are two stages of disease diagnosis: first is an indicator of disease status if it is present or not, and in the second stage, the causative agent is detected. Disease status is indicated by the visible appearance of the udder and milk in the case of clinical mastitis. For subclinical mastitis, on-farm screening tests are used traditionally, such as somatic cell count (SCC) and California mastitis test. None of the abovementioned methods indicate causative agent and quantitative results for level of severity (Viguier *et al.*, 2009).

Mastitis diagnosis begins with the observation of any apparent change in the mammary gland or milk followed by other clinical signs such as fever, weakness, or loss of appetite. SCC is often considered as most widely used biomarker for detection of bovine mastitis and is linked with infection status in terms of severity and stage. California mastitis test (CMT) is very simple and can be performed on farm for SCC estimation; the reagent used in CMT is sodium alkyl aryl sulfonate, and It is based on the principle that nucleic acids and other cell constituents are released in the presence of high SCC and a gel is formed which can be easily detected (Muhammad *et al.*, 2010). Bacterial culturing based on detection of pathogens is still considered as a gold standard despite many discrepancies and inconvenience. The major discrepancies associated with culturing method are false negative results, as well as being a time-consuming and labor-intensive activity (Ashraf and Imran, 2018).

2.8.1. Physical Examination

Based on physical examination, animals can be classified as clinically affected by bovine mastitis and apparently healthy. The cardinal signs of inflammation such as hotness, pain, swelling, redness and loss of function of teats and its associated structures through visual inspection and palpation have significance importance during physical examination. Furthermore, abnormal changes in the milk including its color and

presence/absence of clotting materials are part of physical examination of clinical mastitis (Dereje *et al.*, 2018).

2.8.2. California mastitis test

The CMT is a screening test which is useful in indicating and controlling mastitis since it focuses attention on the individual quarters that are secreting milk with high number of leukocytes. The CMT is a cow-side test, so the results are available immediately. Although it does not identify the type of bacteria that cause mastitis, the CMT is useful in identifying quarters that have high SCC. The degree of reaction between a reagent and the DNA of cell nuclei indicates the number of somatic cells in a milk sample, however, the relationship between SCC values and CMT is not precise because of the high degree of variability in SCC values within each CMT score. The test is very simple, can be performed at milking time, gives instant results and is economical. It will be carried out as screening test for subclinical mastitis and for selection of samples for culture. The reaction will be interpreted based on the thickness of the gel formed by CMT reagent and milk mixture, and the test result will be scored as negative (0), trace (T), + (weak positive), ++ (distinctive positive) and +++ (strong positive) according to Sadashiv and Kaliwal (2014). The CMT reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time. The degree of reaction between the detergent and the DNA of nuclei is a measure of the numbers of somatic cells in milk (Larsen, 2000).

2.8.3. Cultural and biochemical characteristics

In vitro culture regarded as gold standard test for mastitis and milk samples can be taken for bacterial culture in a specific media and further microbiological or biochemical tests applied for specific detection of bacteria. The main draw back with bacterial culturing is that, they need specific medium and time consuming. It is important to note that culture is capable of detecting only viable cells and thus the clinical relevance of culture negative results require further study (Rajeev *et al.*, 2009). Culturing can be used in a targeted fashion for specific control programs such as segregation plans for contagious mastitis or for surveillance to detect the presence of new or emerging pathogen. Culturing is also used to evaluate treatment efficacy and to establish susceptibility patterns to aid in the development of rational treatment strategies (Larsen, 2000).

Most of the bacterial pathogens causing mastitis grow on ox or sheep blood agar due to their nutritional content. A MacConkey agar plate is streaked in parallel to detect *Enterococcus faecalis* and any gram-negative bacteria that are able to grow on the medium. Edwards medium is highly selective for Streptococci and also act as an indicator medium for haemolysis and for the hydrolysis of aesculin (Quinn *et al.*, 2004). Identification of Mastitis-producing Streptococci as

described by Quinn *et al.* (2004) has small, translucent colonies at 24 hours' incubation on blood agar with alpha-haemolysis, beta-haemolysis or gamma-haemolysis. Growth on Edwards medium: all the Streptococci are able to grow on this selective medium. *S. uberis* and *Enterococcus faecalis* hydrolyze aesculin but *S. agalactiae* and *S. dysgalactiae* do not. Aesculin hydrolysis on Edwards medium is indicated by a darkening of the medium and colonies. Gram-stained smear: scattered gram-positive cocci. Streptococci are not usually seen in chains from colonies on a solid medium. Catalase test: Streptococci are catalase-negative. CAMP test: only *S. agalactiae* gives a sharp arrow-head enhancement of haemolysis caused by the beta-haemolysin of *Staphylococcus aureus*.

2.9. Treatment of *Streptococcus agalactiae* infection

Therapeutic response of the cows can be monitored using individual somatic cell count data if available, or using the California Mastitis Test, and with bacteriological samples in herds with contagious mastitis. In general, the use of narrow-spectrum antimicrobials is preferable. Prudent use guidelines have been developed which also include antimicrobial treatment of mastitis (Passantino, 2007). First choice antimicrobials for treating mastitis caused by streptococci and penicillin susceptible staphylococci are β -lactam antimicrobials, particularly penicillin G. Broad-spectrum antimicrobials such as third or fourth generation cephalosporin should not be used as first alternatives for mastitis, as they may increase emergence of broad spectrum β -lactam resistance. Too short a duration of standard treatment is probably an important reason for poor cure rates in mastitis therapy (Deluyker *et al.*, 2005). Clinical mastitis should be treated for at least three days; this recommended treatment duration is longer than label treatments in many countries. All mastitis treatment should be evidence based i.e., the efficacy of each product and treatment length should be demonstrated by scientific studies (Cockcroft and Holmes, 2003).

Treating subclinical mastitis with antimicrobials is generally not economical during lactation because of high treatment costs and poor efficacy. Treatment of subclinical mastitis will not affect the incidence of mastitis in the herd unless other preventive measures are taken. Studies on treating cows based on high somatic cell counts have generally shown that no effect on milk production has been achieved (Hallen *et al.*, 2008). In herd problems caused by very contagious bacteria such as *S. aureus* or *S. agalactiae* treatment of subclinical mastitis is advised. Antimicrobial treatment of dairy cows creates residues into milk, and therefore residue avoidance is an important aspect of mastitis treatment (Wagner and Erskine, 2006). Selecting a substance with a low minimum inhibitory concentration value for the target pathogen is preferable, particularly when the

antimicrobial is administered systemically. The antimicrobial should have bactericidal rather than bacteriostatic action, because phagocytosis is impaired in the mammary gland (Kehrli and Harp, 2001). Antimicrobial susceptibility determined in vitro has been considered as a prerequisite for treatment. However, activity in vitro does not guarantee efficacy in vivo when treating bovine mastitis (Olsen *et al.*, 2006).

2.10. Control and prevention of diseases caused by *Streptococcus agalactiae*

A reduction in the prevalence of *S. agalactiae* can be achieved through a combination of treatment and culling programs. Similarly, the reduction of incidence via milking practices can also help to decrease the pool of infected animals. Good hygienic practices and better animal husbandry way of animal handling can reduce the chances of mastitis. Most of the cases of mastitis are due to the injury of the udder followed by microbial infection and these can be avoided by rapid and regular treatment. Timely and routinely practices of disinfectants in the shed and paddocks always reduce the incidence of mastitis. Regular screening of milk and milk samples always reduce the number of infected animals. As such no effective vaccine is available against all possible pathogens due to multiple etiologies; however, various vaccines have been attempted against bacterial pathogens with mixed success. Measures aiming at preventing new cases of mastitis include fly control, optimal nutrition, improvement of milking hygiene, avoidance of inter-sucking among young ones, implementation of post-milking teat disinfection, regular control of the milking equipments and implementation of milking order (Deb *et al.*, 2013).

CONCLUSION

Bovine mastitis is one of the most economically important diseases in the dairy industry. It could be caused by bacterial, viral or fungal agents. Among bacterial agents *Streptococcus agalactiae* causes contagious mastitis. It is a gram-positive coccus or round bacterium with a tendency to form chains as reflected by the genus name *Streptococcus*. Mastitis diagnosis begins with the observation of any apparent change in the mammary gland or milk followed by other clinical signs such as fever, weakness, or loss of appetite. Somatic cell count is often considered as most widely used biomarker for detection of bovine mastitis and is linked with infection status. California mastitis test is very simple and can be performed on farm for somatic cell count estimation. The pathogen is beta-hemolytic, fastidious, CAMP test positive, catalase-negative, and facultative anaerobes. Identification of the bacteria is performed by using antimicrobial susceptibility tests and molecular techniques. Reduction of the cases due to *Streptococcus agalactiae* should be

achieved through a combination of treatment and culling programs.

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