Common Mammary Pathogens and Factors in Infection and Mastitis

N. C. JAIN
Department of Clinical Pathology
School of Veterinary Medicine
University of California
Davis 95616

ABSTRACT

Inflammation of the mammary gland, commonly known as mastitis, is considered a complex disease in view of its complexities of etiology, pathogenesis, sequelae, therapy, and related aspects. The disease of most concern is the one produced by pathogenic microorganisms. However, as a prerequisite the pathogen must not only enter the mammary gland but also be able to survive and multiply in numbers sufficient to produce pathogenic effects. The normal bovine mammary gland is protected from such attacks by several anatomic and biologic armamenta which must be overcome by the invading pathogens. Organisms involved in causation of mastitis vary in their habitat, virulence, and susceptibility to host barriers. Similarly cows vary in their resistance to microbial entry into the mammary gland and subsequent response to overcome the infection. Only a few organisms, however, need to survive in the milieu of the mammary gland to produce mastitis. This paper is a general discussion of these aspects concerning bovine mastitis in relation to common mammary pathogens.

INTRODUCTION

Mastitis (from the Greek word *mastos* meaning breast and the suffix *itis* meaning inflammation) classically is defined as inflammation of the mammary gland. Similarly, inflammation is defined simply as a reaction to injury. Hence, injury of any type to mammary tissue may be expected to induce an inflammatory response or mastitis. However, the udder disease of major concern is the one associated with microbial infection. Bovine mastitis is a complex disease in view of the complexity of its causes, pathogenesis, intensity, duration, residual effects, immunity, therapy, and eradication.

The internal environment of a normal mammary gland is ideally sterile, but saprophytic bacteria may be found as commensals in some "normal" mammary glands. However, mastitis begins with penetration of pathogenic bacteria through the teat canal (streak canal) into the interior of the gland. If the internal environment of the gland is favorable to survival and multiplication of the invading bacteria, the by-products of bacterial growth and metabolism may irritate the delicate mammary tissue and induce an inflammatory reaction. The clinical signs of mastitis are, in reality, an expression of the host defense intended to destroy the invader and to make way for repair to regain normalcy. Occurrence of mastitis is enhanced by the managerial factors that favor spread of pathogenic bacteria among cows and weaken the natural resistance of the teat orifice to bacterial invasion. The environment of the cow is rich in mammary pathogens and poses a constant threat to udder health. The severity of mastitis primarily is determined by the nature of the invading pathogen and natural mechanisms of resistance available to the cow and to some extent by stresses placed on the mammary gland by milking practices and environmental factors.

FORMS OF MASTITIS

The characteristic features of an inflammatory response are swelling, heat, redness, pain, and disturbed function. *Peracute* mastitis exhibits all the signs of inflammation along with systemic signs of fever, depression, shivering, loss of appetite, and rapid weight loss. *Acute* mastitis also is characterized by all gross signs of inflammation and some signs of systemic disturbance such as fever and mild depression. In *subacute* mastitis, the cardinal signs of mastitis are less pronounced, and there are no systemic signs. The existence of inflammation in the absence of gross signs is referred to as
subclinical mastitis. Mastitis is chronic when the inflammatory process persists for months. It may remain subclinical indefinitely or, in some cases, may have temporary exacerbations of subacute or acute nature. The existence of a pathogen within the mammary gland without any evidence of mastitis is referred to as latent infection. Such glands seem to be particularly sensitive to trauma or environmental stress. Detection of subclinical and chronic mastitis and of latent infection may require repeated determination of milk constituents and culturing of milk, particularly of incubated milk samples. Diagnosis of mastitis or infection in the absence of gross clinical signs generally is based on laboratory findings. Infection and inflammation of the mammary gland could occur independently, but inflammation without infection is rare. Mastitis is said to exist when milk contains somatic cells, primarily neutrophils, in excess of 500,000/ml.

COMMON TYPES OF INFECTIOUS MASTITIS

A variety of bacteria produce mastitis. Individual cows and herds vary in susceptibility, extent, types, and duration of infection. Although some of the mammary pathogens can be found in the environment of the cow, the primary reservoir of organisms within a herd is the cow itself, i.e., the infected cow. The common mammary pathogens are streptococci, staphylococci, and coliform organisms. In general, staphylococcal and streptococcal infections are more common than coliform infections; however, in some herds, coliforms may be the predominant cause of mastitis. A brief description of staphylococcal, streptococcal, and coliform mastitis follows.

Streptococcal Mastitis

Streptococcal species frequently reported to cause mastitis are *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*, with the former being more prevalent. The incidence of infection varies from herd to herd and may exceed staphylococcal mastitis.

*Streptococcus agalactiae* was a major cause of chronic mastitis in pre-antibiotic era and is still a serious cause of chronic mastitis in some herds, although it can be eradicated readily by proper antibiotic therapy and management. *Streptococcus agalactiae* multiplies in the milk and on the mammary epithelial surfaces, generally causing a subacute or chronic inflammatory reaction with periodic acute flareups. The affected tissue eventually is destroyed resulting in reduced milk production or agalactia. The incidence of *Str. agalactiae* mastitis seems to increase with the lactation age of cows in an infected herd, fourth or more lactations showing a 75% or higher infection rate (38).

*Streptococcus agalactiae* is an obligate parasite of the udder and, unlike coliform organisms, it does not survive in the environment of the cow; hence, it is relatively easy to eradicate. New infections in a *Str. agalactiae*-free herd generally are attributed to introduction of infected cows, to transmission of the organism through milking personnel as a carrier, and to heifer calves raised on raw milk containing *Str. agalactiae* and permitted to suckle the teats of penmates (38). Incomplete milking aggravates *Str. agalactiae* mastitis (36).

*Streptococcus uberis* and *Str. dysgalactiae* are not obligate udder pathogens. They can survive for long periods in the environment of the cow and may be cultured from skin and other parts of the cow; hence, these organisms are difficult to eradicate. However, new infections can be reduced by applying rigid herd management practices. These infections are not contagious as is *Str. agalactiae* (38). These organisms invade the udder when conditions become favorable and may cause acute or chronic mastitis but more commonly a subclinical reaction.

Although udder and teat surfaces seem to be the most common reservoirs of *Str. uberis*, mastitis due to this organism is less frequent. It was isolated frequently from herds eradicated of *Str. agalactiae* (25). In problem herds *Str. uberis* infections showed a seasonal trend and a relationship with lactation cycle and age. Higher infections were in older cows in winter or during early lactation period, and least in summer or during the dry period (42).

Staphylococcal Mastitis

Staphylococcal mastitis is associated with udder infection by *Staphylococcus aureus*. It became prominent as herds were freed of *Str. agalactiae* infection. *Staphylococcus aureus* mainly produces subclinical and chronic mastitis, but it also may cause peracute mastitis and...
lead to gangrene of the quarters. Bacterial toxins and toxic products are thought to be involved in the causation of mastitis and gangrene. The α-toxin is potentially the most damaging, because it causes vasoconstriction leading to ischemic necrosis of affected tissues and gangrene (7). Gangrenous mastitis is most often in young cows and after calving. Injection of small amounts (10 IU) of α-toxin induces acute mastitis within a few hours with recovery within 2 days (45). Combinations of β- and δ-toxins are more irritating than either toxin alone (43). Coagulase and other bacterial products are thought to enhance infection, allowing bacterial growth in the face of host defense mechanisms like phagocytosis (1). Delayed type hypersensitivity is considered an important part of the pathogenesis of staphylococcal mastitis and is related to peptidoglycan fraction of the cell wall (22).

The principal reservoirs of \textit{S. aureus} are the udder and teat skin and the milk of infected glands (12, 44). Infection can be spread during milking, and the infection rate increases with age (37). As few as 10 CFU (colony forming units) of \textit{S. aureus} can infect the udder (31). The organism has the capacity to penetrate tissues producing deep seated foci; hence, intramammary antibiotic therapy is not completely successful in eradication of staphylococcal mastitis. Leukocytosis in milk is not successful in disposing of virulent \textit{S. aureus} because a) certain bacterial products like α-toxin and leucocidin are damaging to neutrophils, b) protein A is antiphagocytic, and c) certain bacterial products protect them from intracellular killing (1). However, leukocytosis in the milk seems to keep bacterial growth under control for a chronic staphylococcal infection in a cow changed in acute gangrenous mastitis, within 4 days, during neutropenic state (40). The incidence of staphylococcal mastitis in a herd could be reduced and possibly eliminated by periodic bacteriologic examination, segregation of infected and noninfected cows, treatment of infected cows, removal of nontreatable cows, and strict hygienic measures. Treatment at drying off is more effective than in lactation. Vaccination has not been generally successful in preventing udder infection (5).

Glands harboring nonpathogenic staphylococci (micrococci) commonly exhibit mild leukocytosis in milk without clinical mastitis. This leukocytosis was protective against coliform organisms (35, 39). Indiscriminate treatment of cows for staphylococcal infections will lead to removal of the nonpathogenic staphylococci and may predispose the herd to infection by coliform organisms or other mammary pathogens. Similarly, mild inflammatory response associated with \textit{Corynebacterium bovis} infection is thought to exert a protective effect against invasion of mammary pathogens (2).

Intramammary infections with \textit{S. epidermidis} are being recognized increasingly (24), but it is still not considered a serious mammary pathogen since most infections are eliminated spontaneously (6).

**Coliform Mastitis**

The coliform organisms commonly involved in mastitis are \textit{Escherichia coli}, \textit{Klebsiella} sp., and \textit{Enterobacter aerogenes}, the former being most prevalent. Their pathogenic effect is an attribute of endotoxin contained in bacterial cell wall. Coliform bacteria are ubiquitous in the environment of the dairy cow; manure and infected quarters are the primary source of \textit{E. coli} (10, 38). Sawdust bedding was a source of \textit{Klebsiella} sp. in several herds with mastitis due to this organism (4, 33) while in some herds the source of infection remained unknown (19).

Coliform mastitis is typically acute or peracute, but chronic and subclinical infections accompanied with periodic acute flareups also occur. Cows suffering from peracute mastitis may die within a few days due to endotoxemia but usually overcome acute mastitis. Coliform mastitis is generally self-limiting and does not cause extensive damage to the mammary parenchyma; hence, milk production does not decrease significantly following recovery. Herds and cows vary in types of coliform organisms causing mastitis (20, 27). Generally, multiple serotypes are isolated. Infections usually are acquired from the environment via the teat canal and not transmitted directly from cow to cow, as is the case with most streptococcal and staphylococcal infections. Coliform infection may occur at any stage of lactation; however, cows shortly after parturition or within 2 to 3 mo thereafter and the best producers and the easiest milkers seem more susceptible (13, 18).

Although coliforms are widespread in the environment of the cow and frequently are
isolated from teat skin (27), coliform mastitis is relatively uncommon. This is perhaps due to relative susceptibility of most coliform bacteria to humoral and cellular factors in milk. Natural antibodies to coliform organisms in milk and leukocytosis in the udder generally prevent establishment of udder infection with most of these organisms (38). Hence, glands infected with more common udder pathogens and experiencing mild leukocytosis generally remain free of coliform infection. Therefore, coliform mastitis is considered to be a disease of the gland uninfected with other pathogens. Herds maintained on milking hygiene and dry cow treatment and eradicated of other mammary pathogens such as streptococci and staphylococci, may become increasingly susceptible to coliform infections (20). Chronic coliform infections are difficult to eliminate (13). Teat dipping and dry-cow therapy are not effective against coliforms although they are effective against streptococci and staphylococci (20).

FACTORS INVOLVED IN INFECTION AND MASTITIS

To induce mastitis, a pathogen must first gain entrance into the mammary gland, survive the intramammary bacteriostatic and bactericidal agents, and then multiply in significant numbers. Resistance to bacterial invasion of a mammary quarter is determined, for the most part, by the structure and function of the teat canal. It remains to be demonstrated how bacteria actually pass through the teat canal under normal conditions. Ecological balance of organisms upon and within the mammary gland and within the herd may influence the pathogens involved in causation of a major mastitis problem in the herd. The occurrence of infection in many instances can be related to the state of host and its environment. High bacterial numbers near the teat orifice will increase the probability of some organisms gaining access into the gland and causing mastitis. The response varies among quarters for a multitude of reasons such as the type of organisms, stage of infection, host reaction, etc. Mere isolation of organisms from milk does not constitute udder infection. It needs to be determined whether it represents contamination of milk or infection of the teat canal or the udder.

The Teat Canal

The teat canal is a narrow passage at the tip of the teat. It measures about 5 to 14 mm in length and about .4 to .77 mm in diameter, being narrower at the tip of the teat and wider at the base (38). Teat canals lengthen and dilate with increase in lactational age of cows (23).

The normal teat canal has several anatomic features that act as barriers to penetration of bacteria. These features are most effective in the first lactation and tend to decrease with increasing lactational age, as indicated by the increase in incidence of udder infections with age (38). The lining of the teat canal consists of a stratified squamous epithelium, like the skin of the teat, and its surface continually undergoes keratinization to form sebum-like material which fills the lumen of the canal. This material is rich in long-chain fatty acids having a bacteriostatic effect on certain bacteria, e.g., Str. agalactiae. Removal of this material from the teat canal caused a loss of resistance to Str. agalactiae infection (29). Also, infection readily occurred when small numbers of streptococci were placed directly into the teat cistern of normal glands (28).

The teat canal is surrounded by a true sphincter of smooth muscle fibers which function in maintaining a tight closure of the canal. Quarters having patent teat canals (lack of tight closure) have a greater incidence of infection (38). The wear and tear of milkings, especially when the milking act is performed carelessly, may reduce the effectiveness of the teat canal as a barrier to bacteria.

Milk Factors

Humoral and cellular factors inhibiting bacterial growth are in normal milk and in greater concentration in mastitic milk. Cell-free normal milk possesses a variable degree of growth inhibitory activity for streptococci, staphylococci, and coliform organisms (38). Lactoferrin is bacteriostatic in vitro for a variety of bacteria because of its iron-chelating ability which makes iron nonavailable for bacterial growth. Lactoferrin is in normal milk, neutrophils, and in higher concentrations in dry secretion and mastitic milk. Thus, it is conjectured to play a role in protecting the mammary gland from infections during dry period and in controlling bacterial growth during mastitis (16).
A potent bactericidal system for coliform organisms consists of antibody and complement and is in serum, milk, and colostrum (9, 38). The concentration or activity of this system in milk is dependent upon the degree of vascular permeability of the udder, i.e., the activity increases with the severity of mastitis. Individual coliform organisms vary in their susceptibility to the bactericidal system. The bactericidal activity in the serum varies among cows irrespective of their history of coliform mastitis or vaccination status (8). The pathogenicity of coliform organisms to cause mastitis is related to their relative susceptibility or resistance to this bactericidal system, i.e., the susceptible organisms are less able or mostly unable to induce mastitis when inoculated in small numbers into normal glands while serum-resistant organisms regularly induce mastitis (9). However, the latter may not be able to induce mastitis if neutrophil count of the milk exceeds 250,000/ml at the time of inoculation. The serum bactericidal activity against coliform bacteria resides primarily in IgM and to a lesser extent in IgG1 but not in IgG2 (11).

Neutrophil leukocytes enter the mammary gland and appear in milk during mastitis. Their main role at this site is to engulf and kill invading pathogens (17) although there are reports demonstrating in vitro growth promoting activity of leukocytes for bacteria (32, 47). Normal milk opsonizes bacteria for easy phagocytosis, and mastitic milk is superior in this respect because of its content of serum opsonins. Bovine colostrum contains IgM antibodies and heat labile factor(s) which opsonize E. coli (41). Bovine neutrophils from blood and milk can engulf and kill a variety of bacteria in vitro (17). However, cells from milk seem to be less efficient phagocytes because of their a) reduced glycogen content which is thought to provide energy for phagocytosis, b) surface coating of milk casein which seems to inhibit phagocytosis, and c) ingestion of milk fat globules which makes the neutrophils less accommodative for bacteria (17, 34). Nonetheless, in vivo experiments have demonstrated amply the significance of neutrophil leukocytes in prevention and control of mammary infections (17). About 200,000 to 500,000 neutrophils/ml of milk have been effective in preventing infection with small numbers of Str. agalactiae, E. coli, E. aerogenes, and staphylococci (38). Hence, it is necessary to count leukocytes in fresh milk and strippings milk before performing experimental inoculations of the udder whether to test infectivity of an organism or effect of vaccination, to avoid misinterpretation of results. Phagocytic activity of neutrophils varies among cows and at different stages of lactation (15, 30, 34).

Mononuclear cells are generally numerous in milk from noninfected glands and in dry secretion. A part of the cells designated as "epithelial cells" in such secretions are macrophages (21) and may, therefore, appear to provide another cellular barrier to infection.

Dry Period

Cows entering the dry period tend to have a higher rate of new infections during the first few weeks after the last milking. The number of dry period infections increases with the age of the cow and in cows already infected in one or more quarters (38). Fast milkers may be involved more frequently. New infections at drying off can be increased by exposing the udder artificially to large numbers of pathogens. Hence, teat dipping and antibiotic therapy at drying-off have been considered effective in reducing new infections at this time. However, success varies with the type of mammary pathogens.

In a comparative study of the susceptibility of milked and unmilked glands to infections (46), factors such as intramammary pressure, leakage of milk, concentration of bacterial inhibitors, and composition and cell counts of secretion did not appear to influence rate of infection. Yield at drying-off, milking rates, and teat patency were not related to the differences in susceptibility between animals. It was suggested that the main reason for the lower rate of new infection in the milked quarters is that pathogens entering the teat canal or teat sinus usually are flushed out with the secretion during milking.

Number of Pathogenic Bacteria

Rate of udder infections may be related to the number of potential pathogenic bacteria gaining entry through the teat orifice. Results of experimental studies support this contention. The incidence of intramammary infections seems to be related to the number of organisms...
on the teat skin and teat end (24). Streptococci and staphylococci are in high numbers on teat skin; hence, they are the cause of most intramammary infections. The incidence of Str. agalactiae and E. aerogenes infections was directly proportional to the number of organisms applied to the teat-end skin (26). A high incidence of Klebsiella mastitis was associated with a build up of Klebsiella organisms in sawdust and was controlled by switching to other beddings (4, 33). In another study (3), higher population (10^7/gm) of coliform bacteria in sawdust bedding was related to increased incidence of coliform infections.

Selective Adherence of Bacteria to Mammary Epithelium

Adherence of bacteria commonly associated with mastitis to epithelial cells of different regions of the mammary gland was studied recently (14). Adherence increased from the teat sinus to lacteferous sinus to the large ducts. Cells of the ductal epithelium underwent dynamic changes probably resulting in secretion and/or desquamation, and adherence of organisms could be demonstrated only at a late stage of these changes. There was no difference between cells from different quarters of the same cow, but differences were noted among cows. Adherence of many pathogens generally paralleled their prevalence as cause of mastitis. For example, S. aureus and Str. agalactiae adhered best while E. coli and Klebsiella sps. adhered poorly. Strains of Str. agalactiae, Str. uberis and Str. dysagalactiae varied in their ability to adhere, and, hence, their virulence was related to adherence, i.e., those adhering well were pathogenic. Corynebacterium bovis adhered to squamous epithelium around the teat canal and not to the ductular epithelium.

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