INTRODUCTION

Control of coliform mastitis remains a major challenge for mastitis research. While practical measures for control of *Staphylococcus aureus*, *Streptococcus agalactiae*, and to less extent, other streptococci have been developed and are being applied widely, these measures are ineffective against coliform mastitis. Additionally, trends toward larger herds and confinement housing systems may favor a higher incidence of mastitis from these organisms.

Present recommendations for prevention and therapy of coliform mastitis appear to be based largely on empirical observations. These recommendations, which include measures bearing on milking hygiene, milking systems and procedures, housing, bedding, sanitation, and therapeutic regimens are based on the best information available. However, we know of no practice or program that is unequivocally effective against coliform mastitis in carefully controlled trials. Current recommendations, therefore, require experimental verification. In addition, innovative approaches to control of coliform mastitis are needed.

In this review prepared by the Coliform Subcommittee of the National Mastitis Council, knowledge of coliform mastitis is summarized.

BACTERIOLOGY OF COLIFORM MASTITIS

“Coliform” is a general term for fermentative, gram-negative bacilli that inhabit the intestinal tract of man and other animals normally without causing disease and that are contained within the family Enterobacteriaceae. The term has not been defined strictly; some workers include in it all the enteric bacteria; others limit it to the lactose-fermenting members. In reference to bovine mastitis, the term usually has the latter meaning because the gram-negative bacteria commonly causing udder disease are lactose fermenters. The complexity of the group, the wide variations in biochemical reactivity, and the changing ecologic relationships have led to a confusing variety of names. Parr (91) in 1939 appropriately described the coliforms as a large, highly integrating, and somewhat unstable bacterial group that forms a wide gamut or continuum extending from the lactose-negative paracolon forms at one extreme to the highly reactive *Aerobacter aerogenes* at the other.

Certain members of this group — *Escherichia coli*, *Enterobacter aerogenes* and *cloacae*, *Klebsiella*, *Citrobacter* (previously *Escherichia freundii*) (11) and the paracolon bacteria — have caused mastitis in cows (109).

*E. coli*, a frequent cause of coliform mastitis (2, 38, 42, 75, 76, 108), is typically motile, a lactose fermenter, and forms either smooth or rough colonies on artificial media. It usually has a characteristic appearance on differential media used in identification of enteric organisms—MacConkey, eosin-methylene-blue, and brilliant green agars. Strains of this organism produce indole in media containing tryptophan and are methyl red positive but do not produce acetoin or use citrate as a sole source of carbon; thus, *E. coli* has the IMVIC reaction pattern “++--” (37).

*Klebsiella pneumoniae*, the *Klebsiella* species commonly associated with subclinical and clinical bovine mastitis (1, 3, 4, 13, 39, 43), produces large, moist, often mucoid colonies on artificial media as a result of production of polysaccharide capsules. This organism also ferments lactose rapidly, is nonmotile, can use citrate as a sole carbon source, decarboxylates...
lysine but not ornithine, and produces urease. Most strains do not form indole from tryptophan or yield a positive methyl red reaction; however, they typically produce acetoin causing a positive Voges-Proskauer reaction (37).

Another frequent cause of coliform mastitis in the cow is Enterobacter (aerogenes and cloacae) which is motile, exhibits less mucoid growth on artificial media than K. pneumoniae due to its smaller capsules, and ferments lactose rapidly. It also decarboxylates the amino acids lysine and ornithine, uses citrate as a sole carbon source, and produces acetoin. However, it is typically methyl-red negative and urease negative, and is incapable of forming indole from tryptophan (37).

The Citrobacter group is composed of Enterobacteriaceae designated as Escherichia freundii (11) and the Bethesda-Ballerup group of paracolon organisms, most of which ferment lactose, often slowly, and form H₂S (25). McDonald et al. (69) found 17.1% of 70 cultures of aerobic gram-negative bacteria isolated from bovine udder infections in a mastitis research herd were Citrobacter species. Other than in that report, Citrobacter rarely has been reported as a cause of mastitis in the cow.

In many reports in the literature the isolation of A. aerogenes from udder infections (30, 76, 102, 106) and the use of this organism in the study of experimental bovine mastitis (16, 21, 51) are described. This genus designation is becoming used less frequently. In Bergey's Manual of Determinative Bacteriology (8th ed.), all nonmotile A. aerogenes are categorized as K. pneumoniae and motile strains as E. aerogenes (11). By noting in a particular study whether the A. aerogenes is motile, the reader tentatively may classify it as either E. aerogenes or K. pneumoniae. As an example, the stock culture of A. aerogenes used by Schalm et al. (109) in several investigations was characterized as nonmotile, fimbriated, gram-negative, without demonstrable capsule, with colonies on blood agar being raised, convex, gray, circular, smooth, butyrous, glistening, and opaque; these characteristics, especially nonmotility, indicate that the organism was probably K. pneumoniae. The lack of a capsule does not warrant excluding this organism from the genus Klebsiella since certain growth media (high nitrogen, low polysaccharide) suppress capsule production.

Coliform organisms in the clinical laboratory generally are identified by inoculation of a part of a single isolated colony from an agar plate into each of a series of tests that have been selected not only for diagnostic specificity but also for reproducibility and high constancy within particular genera or species. For the highest accuracy of identification a large number of characteristics should be determined. However, on a practical basis, i.e., in the diagnostic laboratory, the use of such a system on a regular basis is not required and necessarily would pose difficulties because of the time and money required.

For epidemiological studies and also for the comprehensive characterization of strains in research, a sufficient number of tests must be selected to allow differentiation at least to the species category with minimal error and maximal reproducibility. An experimental technologist may use a few screening tests for the initial rough characterization of the organism, followed by a more extensive series of tests for the speciation of the isolate. As an example, McDonald et al. (69) used bovine blood agar for initial isolation of gram-negative bacilli from bovine udders. Colonies that had morphologic characteristics suggestive of coliforms and that gave positive results to the KOH tests later were streaked on tergitol-7 agar to insure purity. After storage, individual strains were reisolated on blood agar. For speciation, isolates were identified by procedures described by Edwards and Ewing (36) with a number of modifications involving the tests for motility, urease production, decarboxylation of amino-acids, and cytochrome oxidase production. Of the 70 cultures studied, only two failed to conform to the definition of Enterobacteriaceae. Serologic procedures also were applied to isolates identified as either E. coli or Salmonella species.

In many studies, systems in which conventional media were used for the identification of Enterobacteriaceae have proved successful. A number of these systems may be applicable to characterization of coliforms associated with bovine mastitis.

Johnson et al. (59) used a system based on Kligler's iron agar or triple-sugar iron (TSI) along with the indole test and lysine iron agar. Organisms isolated from stool cultures were inoculated into TSI while organisms from other sources were inoculated into Kligler's iron
COLIFORM MASTITIS

agar. Certain organisms could be identified presumptively on the reactions in these tests. Five series of additional tests were listed for organisms not fully identified in the initial test series. Tests common to most of these series included citrate, methyl-red, Voges-Proskauer, ornithine decarboxylase, motility, and fermentation of several carbohydrates.

Wolfe and Amsterdam (136) described the prompt identification of lactose fermenters using hydrogen sulfide production, ornithine decarboxylation, and citrate utilization (HOC system); this system was considered more valuable than a series including indole, methyl-red, Voges-Proskauer, citrate, and hydrogen sulfide tests. The HOC system later was supplemented with the motility test by Closs and Digraines (23) in an effort to minimize error due to occasional Enterobacter species that fail to decarboxylate ornithine promptly. Closs (22) also has combined the tests for hydrogen sulfide, ornithine decarboxylase, and motility into one tube that can be inoculated in parallel with a tube containing Simmon’s citrate.

Lindberg et al. (63), in an attempt to identify Enterobacteriaceae with a high degree of accuracy in a minimum number of tests, selected six that could be performed in five tubes: ornithine decarboxylase, β-D-galactosidase, acetoin (Voges-Proskauer), indole, urease, and hydrogen sulfide. For the identification of genera of the tribe Klebsiellaeae, they recommend the addition of these tests: lysine decarboxylase, malonate, deoxyribonuclease, sorbitol, raffinose, citrate, esculin hydrolysis, and motility.

Washington (131) has had much success inoculating an unknown strain in an initial series and known organisms in a parallel series. The series consists of TSI agar, ornithine-motility semi-solid agar, and indole test broth. Results with the unknown are compared with those of control organisms tested simultaneously.

Campbell and Roth (15) have developed three selective media combining methyl violet 2B as aids in the presumptive separation of K. pneumoniae from E. aerogenes. None of these media requires sterilization, and growth of K. pneumoniae is distinctive as compared with that of other gram-negative bacilli tested.

Fay and Barry (40) described a rapid ornithine decarboxylase test for the identification of species of the Klebsiella-Enterobacter-Serratia group and species of Proteus. The capability of the organism to decarboxylate ornithine was determined in 2 to 4 h; in standard tests the capability is determined in 1 to 4 days.

In the past few years diagnostic kits — Patho Tec, Enterotube, R/B Enteric Differential System, API System, Inolox Enteric I, and Minitek — for the rapid identification of Enterobacteriaceae have been marketed. Careful studies at large clinical laboratories of the accuracy of these kits have showed (87, 104, 122, 123, 127) a number of these systems might be useful for the bacteriological diagnosis of coliform mastitis.

Other techniques for the characterization of coliforms include the capsular serotyping of Klebsiella (4, 103); somatic, capsular, and flagellar serotyping of E. coli; bacteriocin typing (47, 119); determination of mouse lethality (117); determination of antibiotic sensitivity (29, 53); and sensitivity to serum (17).

A suggested scheme for laboratory identification of gram-negative bacteria isolated from mastitis is outlined in Table 1.

HOST-PARASITE RELATIONSHIPS

Time of Infection

In studying the epizootiology of a disease for developing control measures, it is useful to know when, by what portal, and under what circumstances the host is invaded by the parasite.

In coliform mastitis, the generally accepted hypothesis is that most, if not all, infections result from passage of the pathogen through the teat duct into the teat cistern. This pattern is not different from that of other pathogens. However, there is a major difference in the sequence of events leading to coliform infection which has been shown by indirect means. Infections caused by staphylococci and streptococci, which primarily are transferred from infected to uninfected glands during the milking process by the milker’s hands, udder towels, and milking clusters, are reduced by disinfectant teat dips applied immediately after milking. However, coliform infections are not reduced by postmilking teat dipping; this may
TABLE 1. Outline for laboratory identification of mastitis coliforms.

Typical gram-negative colony isolated on blood agar or other primary isolation medium

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<tr>
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<th>Eosin methylene blue (EMB) agar</th>
<th>MacConkey's agar</th>
<th>Triple Sugar (TSI) agar</th>
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- Strong lactose fermenters
- Weak or nonlactose fermenters

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<th>Motility</th>
<th>Simmon's citrate</th>
<th>Ornithine decarboxylase</th>
<th>Voges-Proskauer</th>
<th>Methyl red</th>
<th>Urease</th>
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Standard tests (11, 37) for Proteus, Salmonella, Shigella, Serratia, Edwardsiella and Citrobacter groups

d = different biochemical types.

± = most strains positive, some negative.
imply that coliform bacteria usually reach the teat end other than at milking time.

The major sources of coliform bacteria are in the cow's environment, e.g., bedding material, poorly sanitized teat cup liners, damp walkways, manure-covered exercise yards, and heavily contaminated water (9, 50, 85).

Jasper and Dellinger (55) studied a California dairy herd experiencing an above-average incidence of coliform mastitis to determine whether cows were prone to infection by dirty udders, teat erosions and sores, or temporary high populations of coliform bacteria on the teat apex. They found that high populations of coliform bacteria on the teat apex were usually transitory and possibly due to environmental contamination. Coliforms were isolated repeatedly only from the teats of chronically infected quarters. Teat swabs from the normal quarters of a cow with one chronically infected quarter were more frequently positive for coliforms than were those from the teats of uninfected udders. The teat apex of quarters shedding large numbers of coliforms tended to have above average coliform counts. Brander (9) also found nonhemolytic coliform teat end populations were highly transitory.

Under most circumstances the number of coliform organisms on the teat ends is much less than the number of staphylococci or streptococci (137). Likely, coliform bacteria are poorly adapted to survival on normal teat skin, and colonization of the teat skin or teat canal by these bacteria is uncommon. McDonald and Packer (70) repeatedly exposed the teats of 12 cows to equal numbers of both Str. agalactiae and A. aerogenes and observed approximately equal numbers of intramammary infections by each organism. They concluded that neither organism had an advantage in invasiveness over the other when numbers on the teat end were equal.

McDonald (67), using radiographic methods, examined the teat canals of cows after milking and found that at least 2 h were required for the teat ducts to constrict to their least diameter. He proposed that to minimize the number of bacteria entering the duct, there should be minimal contact between the teat apex and the environment during this 2 h.

Bramley and Neave (8) studied movement of K. pneumoniae through teat ducts of unmilked cows. Organisms suspended in litmus milk were placed 2 to 3 mm into the duct approximately 2 h after the last milking of a lactation. Milk samples were removed 48 h later by teat puncture from the teat sinus of the slaughtered animals and examined for the presence of the organism. Their results suggested that K. pneumoniae passed through the teat duct unaided by the milking machine and that the frequency with which they did so in 48 h depended upon the number of organisms inoculated. They proposed that this movement was most likely the result of bacterial growth and was consequently difficult to prevent. These data indicate that pathogens, after deposition on the teat orifice, may be able to pass either completely or partly through the duct between milkings.

There is also evidence that passage of coliform bacteria may be favored by milking or by the milking machine; these factors are considered under Milking Machines and Milking Hygiene.

The high proportion of peracute coliform mastitis at parturition is of considerable concern. Walser (130) studied 994 cows suffering from acute mastitis, 55% of which were attributed to coliforms. Of these coliform cases 15.2% occurred during the first 2 wk and 48.9% in the first 3 mo of lactation. Vergho (129) studied 108 cases of acute mastitis of which 62.0% were caused by coliforms; 66.6% of coliform mastitis was in the first 3 mo of lactation. Nonnecke (86) found that of 858 reported cases of clinical and subclinical Klebsiella mastitis, 1.5% occurred in the 2 wk before calving while 7.1, 3.2, 2.5, and 3.9% occurred in the 1st, 2nd, 3rd, and 4th wk after calving. Murphy and Hanson (76) reported that 39% of 70 new coliform infections were first detected in colostrum and had occurred during the dry period. Eberhart and Buckalew (35) reported that 35% of 84 new coliform infections were first detected in samples collected 5 to 11 days postpartum.

These data raise the question of when the gland actually is invaded in the immediately pre- or postparturient infections. One possibility is that coliforms invade the gland before, during, or just after drying off, remain dormant as the result of antibacterial systems in the dry secretion, and then multiply again when milk secretion begins before parturition. The other
posibility is that the bacteria invade the gland just before or during parturition, and infection is established almost immediately.

Bramley (6) infused E. coli into 17 lactating glands, of which 76% became infected; into 14 dry glands, of which 0 became infected; and into 2 glands 2 days precalving, of which both became infected. This result is similar to that in unpublished work by Newbould in which one quarter of a cow was infused with E. coli 30 days before expected calving with no apparent effect whereas one quarter of another cow, infused 5 days before expected calving, in a few hours incurred a peracute infection that resulted in an aborted fetus in 36 h. These data support the hypothesis that clinical signs may develop within a few hours when infection occurs close to the time of parturition. However, Schultze and Mercer (115) found that some new coliform infections were established during the 1st wk after cessation of regular milking and that some of these persisted throughout the dry period and were present at calving.

**Cow Response**

Infections of the mammary gland produced by organisms of the coliform group occur through the teat canal from the external environment (10, 14, 81, 93). If the coliform organism enters the gland and the habitat within the gland is hospitable, the organism multiplies rapidly and produces large amounts of endotoxin. Endotoxin is considered responsible for the systemic symptoms of peracute mastitis, which then is a severe endotoxemia.

That there is a fundamental difference in the initial stages of coliform infections and those caused by some gram-positive pathogens is suggested by the work of Frost (44), who showed that whereas Str. agalactiae and S. aureus adhered readily to ductal epithelial cells in vitro, E. coli and Corynebacterium bovis did not.

The characteristically sudden onset of the disease, i.e., normal at one milking and severely ill at the next, is due to rapid multiplication of bacteria followed by release and dissemination of endotoxin (96, 110). The endotoxin results in an influx of polymorphonuclear cells into the milk; e.g., Schalm et al. (110) found that the infusion of 50 colony-forming units (CFU) of A. aerogenes into a single gland of a cow resulted in a peak somatic cell count of 32 \times 10^6/ml of foremilk. Schalm et al. (112) postulated that destruction of the bacteria by these phagocytes causes release of the endotoxin, increases permeability, and leads to an influx of serum factors into the milk. Jain et al. (52) concluded that one function of the neutrophil leukocyte is to initiate the events leading to vascular permeability and that these cells and their lysosomes mediate the production of the cardinal signs of inflammation, pain, swelling, and hyperthermia.

A number of researchers (58, 107) have suggested that the accompanying severe systemic reaction of peracute coliform mastitis, which may appear before the clinical manifestation of the disease in the udder, may be due to anaphylactic shock in response to the endotoxin. Salajka et al. (107) postulated that cows become hypersensitive to specific antigens of certain bacterial serotypes and that when the particular serotype enters the sensitized gland in high numbers, a local reaction of the Arthus type is initiated and results in extensive gland necrosis. Said (105) experimentally induced coliform mastitis in normal bovine lactating glands by infusing E. coli or purified endotoxin. In both situations, an early drop in milk histamine was observed followed by a rise to above normal which persisted for 36 h. The author suggests that these results add credence to the concept that peracute coliform mastitis is an allergic response to the liberated endotoxin.

There is evidence that the severity of response to coliform infection may be associated with the cow. Nonnecke (86) found a higher incidence of Klebsiella infections in three of eight areas in a barn housing a large research herd than in the other five. Each area contained 16 cows, and in each of the three areas with high incidence most clinical infections were in two or three cows. Two cows, one in each of two areas, were responsible for 26.9% of all clinical Klebsiella infections in the herd during 15 mo. Five cows, including three in the three high-incidence areas, were responsible for 46% of all clinical Klebsiella infections in the herd despite the fact that all 16 cows in any one area were exposed to the same environmental and management conditions.
Antibacterial Mechanisms in the Host

Serum or humoral factors. The "O" or somatic antigen of the gram-negative bacterium exists as part of the lipopolysaccharide (LPS) component of the cell wall. This complex molecule, often referred to as endotoxin, can be divided into two distinct parts—a lipid component referred to as lipid A, which is responsible for the endotoxigenic activities associated with gram-negative infections, and the polysaccharide portion which has two recognized regions referred to as the basal core polysaccharide and the "O"-specific polysaccharide, the carrier of the "O"-specific antigen (45). An organism with a complete "O" antigen often is referred to as a smooth organism. The structure and composition of the core are similar among genera of Enterobacteriaceae and identical within a genus. Rough "R" mutants may be isolated from many genera of gram-negative bacteria. These occur when a biosynthetic defect prevents complete synthesis of an LPS molecule. These mutants lack the complete "O" specific polysaccharide and may exist with their "O" polysaccharides in various degrees of completion.

The "O" antigen appears to be responsible for eliciting the production of natural antibodies. These complement-dependent antibodies are perhaps the most important antibodies in mastitis, and relative susceptibility of organisms to them may be one of the governing factors in the ability of different coliform organisms to grow in the gland and produce mastitis (109).

The "R" mutants are much more susceptible to the bactericidal activity of serum than the smooth forms. This increased sensitivity may be due to the rough mutant's lack of "O" specific antigen and its exposed basal core that allows nonspecific and prevalent natural antibodies to be effective (74). Characteristics of natural antibody are effectiveness against the gram-negative bacteria, heat lability (destroyed at 56°C for 30 min), requirement for complement to be effective, site of activity which is the basal core of the LPS fraction, and, hence, its lack of specificity, and the class of antibody (IgM) to which it belongs. These antibodies are probably produced by low-level infection or in response to absorption of LPS antigens from the gut (72, 109).

Collins et al. (24) showed that Klebsiella and E. coli from the environment of the cow and from mastitic mammary glands varied greatly in their susceptibility to the bactericidal action of bovine serum and complement. They also found that individual strains varied in their susceptibility to sera from different cows. Klebsiella strains were more susceptible to serum, both normal and immune, than either E. coli or Pseudomonas. The authors attributed the bactericidal activity of bovine sera for Klebsiella to antibody and complement factors; lysozyme was also essential for activity.

Carroll (17) studied the bactericidal activity of bovine serum against six strains of Klebsiella isolated from the mouth, udder skin, and milk of mastitic udders of cows. He also tested the susceptibility of a stock strain of A. aerogenes against the serum dilutions. Bactericidal activity against all strains of Klebsiella was low as compared with that against the stock A. aerogenes. Of the Klebsiella strains tested, the mouth isolate was most susceptible while one isolate from mastitic milk was less susceptible, and the remaining four organisms (one from udder skin and three from mastitic milk) were highly resistant. In testing whether serum had activity against an organism, sera from cows in their first lactation more frequently had no activity than did sera from cows in their third and fourth lactation. Carroll proposed that bactericidal activity of serum was directed against related antigens common to all coliforms tested and, being heat labile, was due to natural antibodies. He suggested that during the critical phase of mastitis when serum factors enter milk, serum-susceptible bacteria are less likely to establish an infection than the serum-resistant types and that it is likely that highly resistant coliforms always will cause mastitis in a normal gland. The stock strain of A. aerogenes which was susceptible to bovine serum was incapable of causing mastitis when small doses, 10^3 CFU, were placed into the normal mammary gland (21).

To test the hypothesis that serum-sensitive and serum-resistant coliforms differ in their ability to cause mastitis, Carroll et al. (19) inoculated udders of cows with serum-sensitive and serum-resistant organisms. Normal quarters with stripping somatic cell counts of less than 200,000 cells/ml were considered suitable for experimentation. The serum-resistant organ-
isms tested were a strain of *A. aerogenes*, two strains of *Klebsiella*, and a strain of *E. coli*. The serum-sensitive organisms were a strain of *A. aerogenes*, a strain of *Klebsiella*, and two strains of *E. coli*. The serum-resistant organisms usually were administered in doses of less than $10^3$ CFU of inoculum whereas doses of the serum-sensitive organisms ranged from less than $10^5$ CFU to more than $10^6$ CFU. Acute or peracute mastitis developed in 15 of 15 normal lactating quarters of cows given serum-resistant bacteria. The somatic cell counts of the stripping for each of these quarters was less than 240,000 cells/ml before inoculation. In quarters with stripping cell counts greater than 300,000/ml, the serum-resistant *Klebsiella* did not grow, or grew but did not change cell counts in the quarters significantly. However, the serum-resistant *A. aerogenes* caused peracute mastitis in quarters with stripping cell counts as high as $1.2 \times 10^6$/ml. The serum-sensitive organisms did not grow in 12 of 16 quarters having stripping cell counts of less than 40,000/ml, and only mild signs of inflammation developed in the other four quarters. Massive doses of serum-sensitive organisms, greater than $10^6$ CFU, resulted in inflammation that likely occurred in response to the endotoxin produced by such a large inoculum. The authors considered growth of the inoculum in the gland triggered influx of natural antibodies and complement components that aided in opsonization of bacteria but did not kill the serum-resistant organism.

Carroll (18) tested the bactericidal activity of sera and milk obtained before and after inoculation of the udder against selected coliforms. Serum-resistant organisms — two *Klebsiella*, one *A. aerogenes*, and one *E. coli* — were resistant to killing by bovine sera, but the serum-sensitive organisms, one *Klebsiella*, one *A. aerogenes*, and two *E. coli* were not. In the pre-inoculation milks, the serum-resistant organisms generally grew, but the serum-sensitive organisms either did not grow or were killed. Development of mastitis in inoculated glands preceded the appearance in milk of bacteriocidins which were always lethal for the serum-sensitive organisms. Bactericidal activities of postinoculation milks peaked when the milks appeared serous, and, as shown by analysis of whey samples, when concentrations of serum albumin and immunoglobulins equal to those in blood were reached in the whey. Carroll found that absorption of milks by homologous and heterologous organisms removed bactericidal activity and allowed the milk to support growth of serum-sensitive bacteria. Nonantibody factors such as lysozyme were considered to play some role in the bactericidal activity of blood and milk. Lysozyme, when added to serum, potentiated lysis of *E. coli*. However, lysozyme alone or complement and lysozyme in the absence of antibody were ineffective.

Mittal and Ingram (74) studied the bacteriolytic activity of normal sheep serum against nine smooth and four rough strains of gram-negative bacteria. They showed that smooth organisms, including one *Klebsiella*, were resistant or only moderately sensitive to the bactericidal activity of normal sheep serum whereas the rough organisms were extremely sensitive. These workers postulated that the smooth organisms were resistant to the effects of sheep serum because the target antigen for the serum antibody was covered completely by the surface "O" antigen. Lack of activity of serum, even though antibodies against these organisms were demonstrable in the serum, was possibly due to lack of specific antibodies or to the inability of sheep complement to react with surface antigens of these cells. The latter possibility was considered doubtful because these strains were also resistant when serum complement from other animal species was added. The moderately susceptible smooth organism was thought not to have the "R" antigen completely internal to the "O" antigen and that possibly a small but variable portion of the "R" antigen exposed on the surface was susceptible to natural antibodies of the serum. The sensitivity of rough strains was likely due to the effectiveness of natural antibodies and complement in the normal serum.

Zinner and McCabe (138), from studies designed to evaluate the possible protective effect of specific IgG and IgM antibody in patients suffering from gram-negative bacteremia, feel that in man there are two classes of immunoglobulins. These are complementary to each other, but each has a separate type of protective ability that acts during different phases of development of the gram-negative infection. The IgM may act as the initial protective mechanism by preventing infection with serum-sensitive strains, but once infection with
serum-resistant strains becomes established, IgG may become the major protective immunoglobulin as a result of its greater opsonizing capacity. High blood titer of antibody to the cross-reactive Re antigen from a rough strain produced a significant decrease in the severity and lethality of gram-negative bacteremias, and because of this Zinner and McCabe (138) proposed that a rough mutant might prove useful as an immunogen against complications due to gram-negative bacteria.

Reiter and Sharpe (100) found that infusion into the cow's udder of .01 - .1 μg/quarter of purified endotoxin produced a leukocyte invasion after 2 to 3 h, and they expected that the membrane permeability also would change to permit the transfer of immunoglobulins and other blood protein into milk. However, blood serum immunoglobulins, IgG and IgM, and complement appeared in milk only after infusion of 1 μg of endotoxin. Therefore, leukocyte invasion of the udder is not accompanied necessarily by the appearance of blood proteins in the gland secretions. This finding indicated a differential movement of blood components into the mammary gland during inflammation. Consequently, if opsonins, plasma proteins that promote phagocytosis of bacteria and other particles, are in the blood, they would appear in the milk only after the organisms had multiplied sufficiently to elicit an inflammatory response (97).

Reiter and Bramley (97) showed that infusion of .1 or 1.0 μg of endotoxin resulted after 1 day in increased concentration of IgG but not IgM in milk; β-lactoglobulin, α-lactalbumin, and complement concentrations remained unchanged. Bovine serum albumin increased after infusion of 1 μg/quarter and possibly after .1 μg/quarter.

Normally, in milk, complement is either absent or in small amounts. Colostrum can contain high concentrations of specific antibodies, but these high concentrations inhibit complement-binding activity and bactericidal effect. Such colostrum, however, becomes bactericidal when diluted in milk containing complement; a similar dilution occurs naturally during the transition from colostrum to milk after parturition (98).

Lactoperoxidase: thiocyanate: hydrogen peroxide system. This inhibitory system in milk, originally called Lactenin 2, was first demonstrated against some lactic streptococci that produce their own H₂O₂; it also killed E. coli and Pseudomonas aeruginosa but only when an exogenous source of H₂O₂ was available (97). Preliminary in vivo experiments have shown that H₂O₂ produced after infusion of glucose and glucose oxidase can delay E. coli infection in the udder. Thiocyanate concentration of milk in the udder varies depending on type of feed and its thiocyanate content.

Lactoferrin. Bovine milk contains an inhibitor, lactoferrin, a protein that inhibits multiplication of bacteria that have a high iron requirement by binding the iron. Smith et al. (120, 121) and others (133) showed that bovine dry secretion contained up to 100 times the amount of lactoferrin in normal milk. Harmon et al. (48) reported that during infection of the bovine mammary gland, lactoferrin in whey increased as much as 30-fold.

Lactoferrin is bacteriostatic in vitro for S. aureus, Ps. aeruginosa, Staphylococcus albus (65), Bacillus stearothermophilus, and Bacillus subtilis (98). Bullen et al. (12) showed that a combination of lactoferrin and specific E. coli antibody had a greater bacteriostatic effect against E. coli in vitro than either component alone. E. coli antibody alone only slightly reduced the viable count whereas lactoferrin alone reduced the viable E. coli count to 2% of that of the control.

Reiter et al. (99) reported that two strains of E. coli were not inhibited by undiluted bovine colostrum, but that colostrum became bacteriostatic after dialysis or dilution and addition of lactoferrin. They suggested that citrate in undiluted whey competes with lactoferrin for iron, thus making the iron available to bacteria.

Smith et al. (120) showed that bovine apolactoferrin inhibited growth of eight coliform strains and was bactericidal for one strain of E. coli.

The absorption of lactoferrin in bovine colostrum to E. coli has been reported by Steel (124). Such binding of this protein to untreated bacteria has not been reported previously. The inability to dissociate lactoferrin from E. coli by .1 M sodium acetate suggested that the absorption was specific.

Reiter and Bramley (97) reported that of the nonantibody, noncellular defense systems only lactoferrin has been active against E. coli in the
dry bovine mammary gland. Further, lactoferrin can play a part only in the defense of the nonlactating gland because the low lactoferrin and high citrate concentrations in milk render lactoferrin useless in the lactating gland.

Cellular factors. One of the first results of the multiplication of coliforms in the mammary gland is an influx of somatic cells, chiefly neutrophilic polymorphonuclear leukocytes (PMN). These are considered the body's prime line of defense against any invading pathogen by processes of phagocytosis and intracellular killing. When there is a lack of PMN in the milk, invading bacteria multiply unhindered. Katsube and Blobel (62) infused saline into two glands of six cows, and the resulting phagocytosis protected the glands against 10^6 CFU A. aerogenes. Schalm et al. (111) showed a protective effect of a pre-existing leukocytosis against A. aerogenes and concluded that 2 x 10^5 to 5 x 10^5 cells/ml of foremilk act to protect the gland against infection. Katsube and Blobel (62) found that PMN from milk readily ingested E. coli and A. aerogenes in vitro and that although most A. aerogenes survived, there was killing of the E. coli.

Despite these demonstrations, to be effective, the phagocyte to bacteria ratio in vivo must be high (83). In many intramammary infections, both bacteria and PMN are shed in large numbers indicating that the PMN are not eliminating the bacteria (97) although they well may act as a means of keeping the bacteria in check. Additionally there can be variations in different cows in efficiency of PMN against other organisms (82, 90), and we have no reason to believe that this variation would not apply against coliform bacteria.

CLINICAL ASPECTS OF COLIFORM MASTITIS

Coliform mastitis may range in severity from fatal, peracute cases to subclinical infections detectable only on cultural examination. While the peracute forms attract much attention, such cases probably constitute only a small part of all coliform mastitis. In a series of 31 E. coli infections, Lotan (64) classified 15% as severe. In a large herd in which coliforms were the predominant cause of clinical mastitis, Bushnell (14) estimated that 10% were of the peracute toxic form.

In this discussion clinical forms are categorized as peracute, acute, chronic, and subclinical. The distinction among these classes are arbitrary, and a single infection may at various times present different clinical pictures.

Peracute Coliform Mastitis

An excellent summary of peracute coliform mastitis was given by Radostits (96). It occurs commonly soon after parturition but may develop at any time during lactation. Rarely, peracute mastitis is seen before calving (14). The disease is usually sudden in onset; the cow may appear normal at one milking and at the next milking show pronounced signs including anorexia, fever to 42 C, depression, shivering, and rumen stasis. Inflammatory signs in the udder may be minimal at this time, and swelling may be detectable only after the udder is milked out. Later, the quarter is swollen and hard, and the teat may be thickened, edematous, and sensitive. Collateral edema extending over unaffected quarters may suggest that they also are involved. In some instances, the quarter may be smaller than the others due to hypogalactia (96). Rarely, peracute coliform mastitis may become gangrenous (14) although gangrene is not produced by coliform bacilli alone (60).

Signs of endotoxic shock may appear within a few hours of onset. At this time, the prognosis is guarded to poor. Recumbency, marked depression, progressive dehydration, diarrhea, and normal or below-normal rectal temperatures are associated with endotoxic shock. The disease at this stage may appear similar to parturient hypocalcemia, and hypocalcemia may be present. Blood calcium has been low in both naturally occurring (96) and experimental coliform (46) mastitis.

Changes in the milk may be sufficiently characteristic to permit a tentative diagnosis of coliform infection (96). In the early stages, the milk may appear normal or faintly watery. Subsequently it may be serous and contain tiny particles. In very severe cases the mammary secretion may become bloody. In some instances, usually when the milk has not become serous, large amorphous clots may be present. The affected quarter may become agalactic, and milk yield from unaffected quarters also may be reduced substantially. Active infection
often is limited to one mammary gland, but additional quarters may be involved.

If the animal does not die quickly, a protracted illness with poor appetite and progressive weight loss may ensue (113). Swelling of hock and fetlock joints may develop in such cases (31, 113). Bushnell (14) estimated that of cows with peracute coliform mastitis, 10% died, 70% became agalactic, and 20% returned to milk. Cows that became agalactic often return to normal production in subsequent lactations (14, 84, 113).

**Acute Coliform Mastitis**

This is probably the most common form of coliform infection. It differs from the peracute form in that the signs are less severe and the course of the disease is usually short. Both the systemic signs and the inflammatory response in the udder are short-lived, and recovery is rapid. Fever is usually present but may persist for only a few hours. The affected quarter is swollen, hard, and sensitive, but this phase does not persist. Some residual swelling of the gland, most noticeable after milking, may remain for several days. The milk may be watery or serous, and clots may be present, especially in foremilk. Clots and increased somatic cell counts may persist for days after the organism can no longer be isolated.

Cows with acute coliform mastitis usually receive antibiotic therapy, and recovery usually is attributed to treatment. However, many of these cases likely would recover spontaneously. Lotan (64) reported that in 24 of 31 cases of clinical *E. coli* mastitis in which no treatment was given, the organism was not recovered 8 days later. Similarly, in experimental *A. aerogenes* infections the organism usually was cleared from the gland in 4 to 9 days (110).

**Chronic Coliform Mastitis**

This term is used to denote coliform infections of relatively long duration with one or more clinical episodes. In the intervals between exacerbations, the glands may secrete normal-appearing milk although biochemical characteristics and somatic cell counts may indicate infection (76). During clinical attacks, signs may be limited to secretion of abnormal milk, but in some instances the gland may swell, and systemic signs may include anorexia and fever. In clinical remission bacterial numbers in the milk may be low, and the bacterium may be difficult to demonstrate with common cultural techniques (76, 110). Clinical episodes are preceded by marked increases in bacterial numbers. Schalm et al. (110) suggested that rapid bacterial multiplication becomes possible when the inflammatory response, including leukocytic infiltration, is reduced. Chronic infections may end spontaneously, usually after an acute clinical attack (76).

**Subclinical Coliform Mastitis**

Several authors have reported the isolation of coliform organisms from milk in the absence of obvious inflammatory signs (56, 85, 113). In a herd in which coliform mastitis has become enzootic, such subclinical infections may be common. Schalm and Woods (113) found that in one herd, 40% of all coliform infections were of this latent type. Newman and Kowalski (85) reported that in a herd in which *Klebsiella* mastitis had occurred, similar organisms were isolated from the milk of 29 of 54 cows although many of these cows gave no evidence of clinical or subclinical infection as determined by strip plate examination. Bushnell (14) reported that streak canal infections, low grade transient infections, and low grade chronic infections often go unobserved.

Many subclinical and short-lived infections may be present at calving but are not detected. Murphy and Hanson (76) detected 31 coliform infections in precalving colostrum; of these, 12 caused no clinical signs and were no longer present 7 to 10 days after calving.

**Role of Endotoxin**

The acute changes in coliform mastitis are caused by bacterial endotoxin. Schalm et al. (110) suggested that both systemic signs and local inflammatory changes were induced by endotoxin released from lysed bacterial cells. It later was shown that intramammary infusion of endotoxin from *A. aerogenes* (20) or *E. coli* (89, 114) mimicked the natural disease. However, the response was more rapid in onset and regressed more quickly than that elicited by infusion of viable organisms. Absorption of endotoxin into blood after infusion into the mammary gland has been demonstrated (139); endotoxin was detected in blood only in low
concentration and was cleared more quickly from blood than from milk. This result suggests that endotoxin is detoxified rapidly after absorption into the circulation.

Although toxemia is a prominent feature of acute forms of coliform mastitis, less attention has been directed to whether bacteremia sometimes may be present. Several authors have referred to joint involvement, particularly of the hocks and fetlocks, as a late development in acute coliform mastitis (31, 54, 113). One explanation of these arthritic changes might be that the joints become the site of metastatic infections established during a brief bacteremic phase.

Clinico-Pathologic Changes

Leukopenia appeared in the early stages of acute coliform mastitis in both natural (96) and experimental infection (46). Reduction in neutrophil numbers was most marked, but lymphopenia also was seen (46). Similar changes in blood leukocytes after intramammary infusion of endotoxin also have been reported (20, 89). In these studies reductions in numbers of segmented neutrophils, lymphocytes, monocytes, and eosinophils were marked. The decline in circulating neutrophils was attributed to massive migration of these cells into the affected gland. However, endotoxin administered i.v. produced similar changes in the leukocyte picture (46), and endotoxin likely acts in other ways as well to induce leukopenia. A normal leukogram was restored quickly in experimental E. coli infections as the acute signs were reversed (46).

Other clinico-pathologic changes in acute coliform mastitis may include hemoconcentration (96), increased serum cortisol (46), and increased serum glutamic-oxaloacetic transaminase (SGOT) (46, 96). Hyperglycemia (20, 89) and increased corticosteroid in blood (89) were observed in endotoxin-induced mastitis.

As noted earlier, the severe systemic and local reactions in coliform infections may include allergic responses (94, 105, 106).

Pathology of Coliform Mastitis

The inflammatory reaction in the mammary gland may be predominantly serous with edema or hemorrhagic (49). Inflammation is centered in the ducts while the alveoli are filled with serous fluid and vacuolated epithelial cells, but leukocytes may be few or absent in the alveoli. If the course of the disease is prolonged, necrosis may be extensive. There is often severe edema of the subcutis. The supramammary lymph nodes draining the affected quarter often are enlarged and edematous, and necrotic foci may be disseminated widely in the nodes (49).

THERAPY OF COLIFORM MASTITIS

Few, if any, controlled studies on therapy of coliform mastitis are available. Most recommendations appear to be based on clinical observations and on general principles of antimicrobial and supportive therapy. Recommended therapeutic measures for peracute and acute coliform mastitis include the following:

1. Removal of bacteria and toxins from the mammary gland (14, 84, 96). The affected quarter should be milked out as thoroughly and as often as practical. Oxytocin given i.v. may facilitate removal of bacteria, toxin, and inflammatory exudate.

2. Antibacterial therapy. Systemic administration of appropriate antibacterial drugs supplemented by intramammary infusion is usually recommended (14, 84, 96). Several authors have stressed the need for early antibacterial therapy if it is to be effective. The problem of selecting antibiotics for treatment of coliform mastitis is discussed below.

3. Fluid therapy. The need for supportive therapy depends on the severity of the disease and usually is required only in the peracute form. The administration of large amounts of balanced electrolyte solutions will counteract dehydration and cause forced diuresis to maintain renal function for the excretion of toxic metabolites (96). Administration of 5 to 10 liters in 1 h may be sufficient; the cow may show marked improvement within 12 h. When shock is advanced, much larger volumes (60 to 110 ml/kg) of body weight (BW) may be needed with half the calculated dose given in the first 2 to 4 h and the remainder in the next 6 h (96).

4. Systemic glucocorticoids. These are recommended in severe cases as part of the supportive therapy. The rationale for their use rests on their protective action in endotoxic shock as in other species (73). A high dosage, e.g., 44 mg/100 kg body weight of dexamethasone, is recommended (66); this dosage should be administered once or may be repeated once after 8 to 12 h. While severe coliform mastitis is not common in cows in late pregnancy, this dose of corticosteroid likely would cause abortion at that time.

5. Antihistamines. Drugs of this type sometimes are used in treatment of acute coliform mastitis, but little information on their usefulness is available (96).

6. Calcium therapy. Hypocalcemia may be present in some cases of acute coliform mastitis. Calcium i.v. reversed some of the signs induced by i.v. endotoxin (46), but both Radostits (96) and Bushnell (14) have warned that such therapy may have damaging effects on the heart in animals that are intoxicated or in shock. If calcium is administered, extreme caution should be used.

The preceding discussion has concerned the acute form of coliform mastitis. Little information on therapy of chronic form is available. Schalm and Woods (113) had good results with streptomycin given intramammarily. Schultze and Mercer (115) noted that some chronic gram-negative infections were refractory to repeated antibiotic therapy during lactation.

Selection of appropriate antibiotics for the treatment of coliform mastitis poses several problems for the clinician. When a case first is seen, an etiologic diagnosis is usually not available. Although a coliform infection may be suspected, other bacteria may be involved. In this situation, antibiotic selection will be on the basis of previous antibiotic sensitivity determinations and clinical experience (96). A different antibiotic may be used subsequently, depending on clinical response and results of sensitivity testing of the organism.

A second problem is that only a few antibiotics may be used appropriately and confidently for coliform infections. In a recent study of in vitro activity of various antibiotics against gram-negative organisms isolated from bovine mastitis, McDonald et al. (68) found that only gentamicin, chloramphenicol, and polymyxin B were effective against more than 90% of these bacteria. Streptomycin, nitrofurazone, neomycin, tetracycline, and cephalothin were active against fewer strains; and complete resistance was shown to penicillin, cloxacillin, erythromycin, and others. In addition to these in vitro studies clinical reports have indicated good results with chloramphenicol (96) and gentamicin (84). At present gentamicin, chloramphenicol, and polymyxin B are not cleared by the Food and Drug Administration (FDA) for systemic or intramammary use in dairy cattle. In the absence of data on these antibiotics for milk and tissue residue, the clinician either must forego their use or direct excessively long withholding.

Finally, the role that antibiotics play in the therapy of coliform mastitis is doubtful. Many coliform infections are self-limiting and will be resolved without therapy. On the other hand, some cows will die or become fit only for salvage despite early and intensive antibiotic therapy. Although investigators at the Food and Drug Administration recently have noted little interest in development of products for the treatment of acute mastitis (71), the need for proven regimens for therapy of coliform mastitis is urgent.

ENVIRONMENTAL AND MANAGEMENT FACTORS

The natural flora of the intestine includes a large population of coliform bacteria, especially *E. coli*. Feces are an important source of these organisms in the environment. Additionally, certain coliforms, notably *Klebsiella* and *Enterobacter* species, are common in many environmental situations independent of fecal contamination. Duncan and Razzell (28) isolated these bacteria from water, tree needles, bark, and soil in a remote forest setting and obtained many similar isolates from fruits and vegetables in a supermarket.

In addition to their common occurrence, it also appears that many strains of these bacteria also may be in the environment, and perhaps most of these are pathogenic when they gain entrance to the bovine udder. Saran-Rosenzuaig and Cohen (108) serotyped 60 strains of *E. coli* isolated from cows with mastitis in two Israeli dairy herds. In one herd, 4 of 39 isolates had a common antigenic pattern, 4 other serotypes were each identified twice, and the other 27
strains were all antigenically distinct. Among 21 isolates from the second herd, 4 serotypes were identified twice, and all other strains were antigenically distinct. Braman et al. (4) reported a similar diversity of *K. pneumoniae* capsular types among isolates from mastitic milk within individual herds. Because no reports of herd problems associated with a single strain are available, it is assumed that coliform bacteria are not transferred commonly between cows. Therefore, coliform mastitis is not highly contagious but is more likely to result from environmental contamination.

Recent trends toward increasing herd size, increasing confinement feeding and housing, and decreasing cow time on pasture probably have concentrated coliform bacteria in the cow’s environment. Thus, in investigating factors which affect the incidence of coliform mastitis, the following should be considered: type, amount and cleanliness of bedding, design and maintenance of housing facilities, and possible effects of weather.

**Bedding**

In recent years, the role of bedding materials, especially sawdust, in coliform mastitis has been studied. In British studies (8), the importance of coliforms in the bedding was stressed; coliform infections occurred most often when coliform populations exceeded $10^6$ CFU/g of wet bedding. In a recent Cornell study (80), green sawdust was seeded at $10^6$ CFU/g to simulate dirty conditions. Ten cows were maintained on this contaminated bedding, and 10 were held on dry shavings; all other conditions were the same for both groups. Somatic cell counts, determined weekly, were higher in the shavings-bedding group than in the treatment group. The treatment group had consistently more contamination of teat ends. However, no new coliform infections occurred in either group, and the investigators concluded that the occurrence of coliform infection is multi-factorial and does not depend on degree of contamination alone.

In a bacteriological survey of a sawdust-bedded herd with a high incidence of clinical mastitis, Newman and Kowalski (85) found that 29 of 54 cows were positive for *Klebsiella*. Subsequently, 2 cows died, and 5 became agalactic, but the other 22 positive cows had no clinical signs, and screening tests did not indicate subclinical mastitis. After sawdust was discontinued as bedding, most of these cows became negative for *Klebsiella*. These observations suggest that sawdust was a source of the infecting organisms and that the organism in the milk is not associated always with an observable inflammatory response.

Rendos et al. (101) showed effects of different bedding types on coliform populations of teat ends. Three groups of five cows each were bedded for successive 3 wk on sawdust, wood shavings, and wheat straw. Both fresh and used bedding were sampled for total coliform and *Klebsiella* numbers. For each bedding type, coliform numbers were higher in used than in fresh bedding. Sawdust supported the highest coliform populations, straw the lowest. Coliform populations in bedding were reflected in contamination of teat ends; cows bedded on sawdust had higher populations of total coliforms and of *Klebsiella* on teat ends than cows bedded on other materials.

Free stalls bedded with sawdust are hollowed out easily and provide sites for accumulation of feces and urine with possible rapid multiplication of coliforms. Under these conditions the risk of new coliform infection is increased (14).

Sawdust is a good bedding for cow comfort and for availability. Several attempts have been made to reduce coliform concentrations by kiln drying the sawdust before use and/or by chemical means (5, 7, 9). Drying the sawdust is 90 to 99% effective in destroying coliforms (9) but is expensive. Sterilized sawdust becomes contaminated soon after it is put into use; hence, the economic practicality of this measure is questionable. Chemical treatment with 5% wt/vol paraformaldehyde is also effective (5) but dangerous (7) due to possible contamination of milk, irritation of lungs, and drying of teat skin. Also, high coliforms may be reestablished as early as 7 days after treatment (5).

**Housing**

As the available area per cow decreases, the incidence of coliform mastitis increases (5, 14, 50). Decreasing the area per cow from 7.5 to 5 m² in a concrete based sawdust yard increased the percentage of *E. coli* isolated from the bedding (5). Exposure for long times to coliform sources, such as contaminated bedding
or water, increases the new coliform infection rate (5, 14, 50, 95).

Cows housed in free stalls or cow sheds have the highest incidence of coliform mastitis. This high incidence may be due to bacterial build up in the stalls and alleyways. Cows on pasture or “out-wintered” have the fewest infections (5, 50, 95). Housing factors such as proper lighting, humidity, temperature, and air circulation in the barn environment may minimize stress and risk of infection to the cow (5, 32, 33, 50, 118, 128). Cows will adapt to almost any type of management practices if allowed to do so slowly. Sudden changes in daily routine, feed, and/or climate increase the risk of mastitis and other diseases (118, 130).

Weather

Little work has been published on the effects of weather on mastitis incidence. Under laboratory conditions *Klebsiella* serotypes survived best when relative humidity was 33% or lower (128). There is agreement that sudden changes in weather (118, 130) such as thunderstorms predispose to coliform outbreaks. Also, cold moist air currents in summer and warm moist air currents in winter are likely conditions for coliform outbreaks. The season does not seem to affect the incidence as much as sudden changes in weather conditions.

In a Norwegian study (118), a relationship was significant between the incidence of mastitis and precipitation. As precipitation increased, clinical cases of mastitis increased. The author also suggested that air temperature was a primary factor because mastitis flare-ups increased as the temperature rose in July.

**MILKING MACHINES AND MILKING HYGIENE**

Milking machines affect the likelihood of coliform infection in three interrelated ways. Their use may lead to deterioration of the physical condition and resistance to infection of the udder and teats, they may play a role in the transport of organisms into and at least part way through the streak canal, and the milking system may serve as a reservoir of coliform organisms.

Bramley (5) reported elevated incidence of coliform teat apex population in cows with eroded teats and in older cows generally; part of the increasing erosion and generalized deterioration with age may be due to long-term effects of the milking machine. For example, Peterson (92) showed that overmilking of either nonlactating or lactating cows could produce damage to the interior of the teats. Lesions included hyperemia, edema, hemorrhage, and necrosis of the epithelial membrane and teat wall, and structural damage and loss of keratin in the streak canal. Damage was less severe in teats overmilked many times for short periods than in teats overmilked 15 or 20 min at only four milkings. Katona and Meszaros (61) reported that machine-milked cows often (70 to 80% of the time) have thick (2.5 to 3.0 mm) and fasciculate teat ducts; 75 to 80% of hand-milked cows have thin (1.5 mm), soft ducts.

The second possible role of the milking machine in coliform mastitis is in mechanisms of the delivery of bacteria, including coliforms, to or through the streak canal during milking.

DeHart et al. (26) sought to answer the question of when coliform infections occur. Forty cows were divided into three treatment groups and one control group of 10 cows each. The udders of each group were saturated in a 10⁹ CFU/ml *E. coli* broth in different ways or at different times to simulate different barn or premilking conditions. All cows were milked in the same manner. The treated groups had more infections than the control group, but differences among the treatment groups were not significant. The conclusion was that exposure to the coliform broth increased the infection rate, but time of exposure was unimportant.

Bushnell (14) found a high incidence of coliform mastitis in dairy herds in which excessive water was used to wash cows in preparation for milking. He proposed that the excessive water on the udder transmits coliforms to the end of the teat before, during, and after the milking process. During milking, this contaminated water may be forced against the teat end and possibly into the gland by vacuum fluctuations produced by the milking machines. To illustrate the necessity of avoiding excessive water on the udder during milking he cited two large California dairy herds maintained under average corral conditions. Both had reduced coliform infection rates after a program was introduced in which all udders were sanitized and thoroughly dried before milking. Bramley
and Neave (8) studied the new coliform infection rate in two groups of cows. The first group was milked with a machine designed to produce high cyclic and irregular vacuum fluctuations, and the second group was milked with a machine designed to diminish the production of these impact forces during milking. During 1 wk of exposure to high E. coli, 6 of 16 quarters in the first test group became infected, and 5 of 80 quarters in the second group became infected. The authors concluded that under certain conditions, a milking machine may influence the rate of coliform infection in quarters exposed to high contamination.

There are, undoubtedly, other effects than impacts which relate milking machine to implantation of bacteria into the streak canal. Although not specific to coliforms, Jasper and Whittlestone's study of the pulsationless PME milking system (57) demonstrated transfer of tracer bacteria from cup to cup, teat to teat, and cow to cow. Because there is no mechanism for droplet impact in this system, pathogens may have been borne by aerosols within the system. Another report of infection not attributable to impact is that of Fell (41), who found that slow milking as a result of choked squeeze pulsation and reduced airline vacuum increased CMT scores over the course of one lactation in 16 cows as compared with those in a control group of 16 cows. He reported that this milking system did not exhibit significant vacuum fluctuation, so impact should not have been an important effect.

The third potential role at the milking machine as a factor in coliform infection may be as reservoir of pathogens. Jasper (54) reported that in three of eight pastured herds with excessive clinical mastitis Klebsiella was the dominant organism causing mastitis. In each herd, inadequate premilking sanitization of the teat cups was felt to play a role in the transmission of infection. Inadequate sanitization was accompanied by contaminated teat salve in one herd and by contamination during administration of antibiotics in another herd. One conclusion of the study was that maintenance of a herd on pasture does not eliminate the possibility of coliform exposure from a variety of environmental sources.

Weight and Ferking (132) described a herd that suffered from an abnormally high incidence of peracute coliform mastitis. They thought that inadequately cleansed milking machines were the source of infection. Rinsing of inflations with disinfectant and cleansing solution failed to alleviate the problem as a result of poor penetration into the cracks of the rubber. Only when the machine was dismantled and thoroughly cleansed weekly, and all parts, including tubing, boiled in 2% sodium carbonate for 10 min monthly did the coliform mastitis problem disappear.

Jasper and Dellinger (55) found that teat end contamination was, in fact, highly correlated with infected quarters in a high-producing string of about 90 cows. They found that high teat end populations from environmental sources were transitory and often did not persist even throughout a milking. For this reason, they consider that infected quarters are a source of contamination of teat skin. Skin contamination from one teat may lead to infections of other quarters of the same cow, so teat infections must be viewed as a possible source of organisms for transmission via the milking machine.

In summary, the milking machine may cause deterioration of the teat's resistance to infection and enhancement of its suitability to harbor coliform organisms. The machine also plays a role in physical transmission of organisms through the streak canal, both by the so-called "impact" mechanism and by other mechanisms not defined as well. Although the initial source of coliforms is environmental, another source for milking-related infections appears to be infected quarters. The machine can become contaminated with the organisms shed and then serves as a secondary reservoir for future infection.

**CONTROL OF COLIFORM MASTITIS**

**General Considerations**

Clinical aspects of coliform infections are sufficiently different from those of staphylococcal and streptococcal infections that the herd survey, the traditional method of measuring incidence of mastitis, is probably not appropriate to measure the impact of coliform mastitis. Herd surveys usually will reveal coliform infection between .1 and 1.0% of quarters even though clinical mastitis due to coliforms may be common. For example, in a
herd studied for 2 yr coliform infection as determined by herd survey varied from .9 to 1.2% of quarters (35). In the same period there were 24 new coliform infections and 31 causes of clinical coliform mastitis per 100 cow-years. Coliform mastitis was of more importance than the herd survey indicated. In view of the propensity of coliform infection to cause clinical mastitis (35) and its typically short duration, the importance of coliform mastitis probably is estimated best in cultural data from clinical mastitis.

In general, a control program for any mastitis pathogen must either reduce the rate at which new infection occurs and/or shorten the duration of infection (27). The duration of infection can be shortened by spontaneous recovery, therapy during lactation, dry cow therapy, and culling. Results from a 3-yr study of a teat dip, dry cow therapy program show the importance of each of these routes of elimination for the major pathogens and the contrast between coliforms and other infections (Table 2) (77). Most coliform infections (58%) were eliminated following treatment of clinical cases during lactation. These data imply high efficacy for antibiotic therapy of coliform mastitis, but it is uncertain how many of these infections would have been eliminated without treatment. Relatively few coliform infections become chronic; therefore, dry cow therapy and culling can have little effect in reducing infection. Any measures intended to reduce the duration of infection are likely to be of little value. In developing coliform control measures, prevention of new infection must be emphasized.

Effect of Control Programs

Mastitis control programs based on teat dipping and dry cow therapy are highly effective in controlling staphylococcal and streptococcal mastitis but have little effect on coliform infections. A 3-yr mastitis control program was conducted in 30 herds in England (135) and 27 herds in New York (78). Primary aspects of the program were use of a 4% hypochlorite teat dip after each milking and infusion of all quarters with antibiotics at drying off. The percentage of quarters infected was reduced from 28 to 7% with the major reduction in staphylococcal and streptococcal infections. The percentage of quarters infected with coliforms was .2 in both

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<th>TABLE 2. Relative importance of routes of elimination of organisms in a mastitis control program (77).</th>
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<td><strong>Infectious eliminated</strong></td>
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the initial and final bacteriological surveys in the British herds. The initial coliform frequency was higher in the New York herds than in the British herds and decreased slightly during the experiment. The percentage of quarters with clinical coliform mastitis did not increase in either study. The New York study was continued for an additional 3 yr to determine if reduction of gram-positive infections with consequent reduction in milk leukocytes would make the cows more susceptible to gram-negative infection (79). The number of quarters infected with coliforms remained constant.

Eberhart and Buckalew (34) conducted two trials of a teat dipping and dry cow therapy program. In trial 1 (120 cows) they reported more new coliform infection in the treated and dipped group than in the controls. In trial 2 (80 cows) the program appeared to reduce the infection rate of new coliforms.

The lack of efficacy of this program in controlling coliform mastitis is due largely to the failure of teat dipping to prevent new coliform infections. Using 125 cows for 13 m in a half udder experiment, Wesen and Schultz (134) found a 53% reduction in total new infections in quarters dipped with 1% iodophor; however, new coliform infections were not reduced. Similarly, in a trial with a chlorhexidine teat dip, Schultz and Smith (116) reported reductions in new staphylococcal and streptococcal infection but no effect on gram-negative infections.

Although gram-positive bacteria often colonize the teat end before penetration into the teat cistern, there is little evidence to show that coliforms do so despite constant contamination of the teat end by these environmental bacteria (33). Unlike those bacteria that are transferred from quarter to quarter among cows during milking, coliforms can reach that teat end at any time (5, 26, 32). Most teat dips are not effective for the duration of the interval between milkings (5). Thus, coliforms and the host may contact each other when the germicidal activity of the dip is reduced or absent (5, 32, 34). More work is needed to prolong the germicidal effectiveness of teat dips.

**Other Control Methods**

No control methods effectively have reduced coliform mastitis in carefully controlled trials under field conditions. The best recommendations available are derived from empirical observations and from attempts to control coliform mastitis in the field. Although some of these measures are probably valid, their usefulness needs to be verified in controlled studies.

Several of the recommendations are summarized here; they are discussed more elsewhere in the paper.

1. Bedding materials, especially sawdust, may be a source of contamination of the teat ends with coliform bacteria (8, 85, 101). Changing to alternate bedding materials sometimes has halted outbreaks of coliform mastitis (5, 85). Although efforts have been made to reduce coliform populations in bedding, these methods are not recommended for routine use (7).

2. Increasing the space allotted per cow, keeping stalls clean, and reducing the amount of housing time may decrease the exposure to coliform bacteria and reduce new infections (5, 9, 50).

3. Careful drying of udders and teats to avoid milking cows with wet udders may reduce coliform mastitis (14).

4. Attention to milking machines and milking procedures is required to assure that the machines are not reservoirs of coliform bacteria (54, 132), that machine action does not assist bacterial passage through the teat canal (125, 126), and that action of the machine does not damage the teat and reduce its ability to prevent the passage of bacteria (61, 92).

**ACKNOWLEDGMENTS**

The authors are indebted to the National Mastitis Council, Inc., for financial support and to D. E. Jasper and L. E. Newman for critical review and Fay Eggers for editorial review of the manuscript.

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**Coliform Mastitis**


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