

# Subclinical Mastitis: A case history

by David Ohman\*

## INTRODUCTION

A study was conducted to determine the incidence of subclinical mastitis infections in one herd of 32 Guernsey cows. The California Mastitis Test (CMT) was used to determine the level of mammary gland inflammation. Individual quarter milk samples were collected for the isolation and identification of mammary gland pathogens. By correlating the incidence of pathogen isolation with the results of the CMT, an attempt was made to accurately evaluate the level of subclinical mastitis due to bacterial infection.

By definition, subclinical mastitis lacks the visual signs associated with clinical mastitis, ie: the production of abnormal milk and, in some cases, noticeable udder inflammation.<sup>1,16</sup> However, in subclinical mastitis, subtle changes such as increased numbers of leukocytes and sloughed epithelial cells occur in response to various types of trauma, including intramammary infection.<sup>1,7</sup>

The focus of this study was subclinical mastitis caused by bacterial agents. A quarter was considered to have subclinical mastitis if it had a positive culture and a CMT reaction of 2, 3, or 4.

Subclinical mastitis often goes unnoticed and therefore, its prevalence and economic importance is not fully realized. In the US, at least 40% of all cows have either clinical or subclinical mastitis in at least one quarter; with the subclinical form being 15 to 40 times more prevalent.<sup>11,12</sup> The economic importance of this can be appreciated by the fact that 70% of the total cost of mastitis is due to the imperceptible decrease in potential milk production caused by the subclinical infection.<sup>12</sup>

In the herd of cattle under study, little

attention was paid to udder health, for the operators followed practices inconsistent with those suggested by experts<sup>1</sup> to reduce the incidence of intramammary infections. The cow's udders were washed with a common cloth, instead of single service paper towels, and teats were not dried following the washing-off procedure. Also the udders were machine stripped for a longer period than recommended by the National Mastitis Council.<sup>7,10</sup> The excessive machine stripping causes teat and udder damage, and reduces the natural ability of the gland to resist bacterial infection.<sup>7,12,17</sup> Teats were not dipped with a bacteriocidal solution following milking and cows were not routinely treated at drying off. During lactation, clinical cases of mastitis were not treated unless the inflammation was severe.

Some reasonable goals for mastitis management are:

- (1) no more than 15% of the cows or 8% of the quarters infected with mastitis pathogens
- (2) CMT composite scores:

0 - 1	88%	<sup>12</sup> (negative—trace)
2	7%	(weak positive)
3 - 4	5%	(distinct—strong positive)

Considering the poor management of the study herd, a high infection rate and high CMT scores were expected.

Approximately 90 bacteria can cause mastitis. Of these organisms, 4 can be held accountable for 90-95% of all cases of mastitis: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*.<sup>12</sup>

## MATERIALS AND METHODS\*

*California Mastitis Test*<sup>15</sup> A white paddle with 4 shallow cups, one for each quarter, was used to collect milk samples of approximately

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2 ml per cup. An equal amount of CMT reagent was then added.\*\* Since the sample cups are affixed to the paddle, it is possible to simultaneously mix the samples and reagent by gently oscillating the paddle to swirl the mixture. The reaction is scored within 10 seconds after mixing. The CMT was graded from 0 (negative) to 4 (strong positive) based on degree of precipitation and viscosity.

**Collection of Samples**—127 milk samples from the functional quarters of 32 cows were collected in Hotis tubes (sterile glass tubes with screw tops containing .5 ml of .5% Brom cresol purple). Careful collection procedures were followed to reduce contamination of the samples with barn dust and skin debris. Before collection the udder and teats were scrubbed with cotton pads soaked in 91% isopropyl alcohol. The first 3 or 4 strips of milk from each quarter were discarded.<sup>4</sup>

**Culturing**—Once collected, the samples were incubated in Hotis tubes for 3 hours at 37° C, and then streaked for isolation on 5% bovine blood agar plates. Both Hotis tubes and blood agar plates were incubated aerobically for 24 hours.

**Bacteriological Identification**—Colony morphology, Gram stain, hemolytic pattern, and catalase reaction were the tests used to tentatively identify the isolated organisms. Final identification was made with the aid of carbohydrate utilization tests (salicin, sorbitol, insulin, and trehalose) and the free coagulase test. Hemolysis and carbohydrate utilization tests were recorded after 24 hours of incubation. The coagulase test was read after 3 hours.

RESULTS

When the CMT scores of 2, 3, or 4 and positive pathogen isolation data were correlated, the incidence of subclinical mastitis due to bacterial infection was determined to involve 23% of the quarters and 50% of the cattle.

**Bacteriological results**—Two gram positive udder pathogens, *Staphylococcus aureus* and *Streptococcus uberis* were isolated and identified from the individual quarter milk samples.

*Staphylococcus aureus* was isolated from 52

of 127 quarters (41%) involving 21 of the 32 cows tested (66%). Two different strains were identified, one with the characteristic *Staphylococcus* double zone of beta hemolysis. The other strain showed total clearing. Both *Staphylococcus aureus* strains were coagulase positive.

An alpha hemolytic *Streptococcus* was isolated from two quarter samples (1.5%) of one cow (3.1% of the cattle tested). The organism was identified as *Streptococcus uberis*.<sup>5</sup>

**California Mastitis Test (CMT)**—The distribution of CMT scores were as follows: CMT 0 and 1, 65%; CMT 2, 15%; and CMT 3 and 4, 20%.

Table 1 shows the distribution of quarters as to CMT score and positive cultures. The percentage of positive cultures per CMT score is also indicated.

TABLE 1. Correlation of CMT score with cultural results.

CMT Score	Total	# Culturally Positive	% Positive
0	70	14	20
1	11	6	55
2	18	13	72
3	17	13	76
4	8	7	88
	124	53	43

DISCUSSION

The bacteriological study of the milk samples from the herd analyzed showed a very high isolation rate for hemolytic, coagulase positive *Staphylococcus aureus*. Of the quarters tested, 41% yielded positive cultures, representing 60% of the cows. The strains isolated were determined to be pathogenic as they were beta hemolytic and coagulase positive.

Three hemolysins are produced by *Staph aureus*: alpha, beta and delta. Some strains of *Staph aureus* can produce both alpha and beta hemolysins.<sup>5</sup> When both are produced, the alpha hemolysin causes total hemolysis immediately around the colony and the beta hemolysin causes partial clearing at the outer perimeter of the total clearing. The final result is a double zoned hemolytic pattern.<sup>5</sup> When only the alpha hemolysin is produced, only the total clearing is seen; no double zone is present.<sup>2</sup> The production of beta hemolysin is characteristic of animal strains of *Staph aureus*, however, the *Staph aureus*

\* Media and materials were supplied by Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University.

\*\*0.3% Sodium alkylaryl sulfonate with .01% Brom cresol purple indicator in liquid solution.

isolated from 5 quarter samples in this study failed to produce beta hemolysin. In a study conducted by Minett, only 5 of 97 animal strains he studied failed to produce beta hemolysin.<sup>5</sup> The *Staph aureus* isolated in the present study may be of human origin as alpha hemolysin is characteristic of human strains.

Beta hemolysis and the ability to produce coagulase are used to determine if an isolate is pathogenic.<sup>5</sup> Coagulase causes the coagulation of plasma which is damaging to the host, and is protective for the bacteria since they would be coated with a fibrin film which increases their resistance to the killing action of the blood.<sup>5,14</sup>

In addition to coagulase, *Staph aureus* produces several other toxins which cause sequential events in the host leading to pathological alterations in infected mammary glands, and in some cases can cause death to the cow.<sup>5,12</sup>

Some strains of *Staph aureus* produce all the toxins while some only produce a portion of them.<sup>5</sup> Because the virulence of the strain is dependent upon which of the toxins are produced, a whole spectrum of pathogenicity is exhibited, ranging from mild subclinical mastitis to peracute mastitis. Acute clinical cases left untreated often progress to the chronic state, at which time they respond poorly to treatment due to walling off by scar tissue. In some cases, virulent strains can cause peracute mastitis and death may result.<sup>1,12</sup> In the herd studied, the strains of *Staph aureus* present seemed to be of lower virulence, as no cases of peracute mastitis due to *Staph aureus* had been recorded.

The presence of *Staph aureus* in the udder causes a marked leukocytosis.<sup>3</sup> However, the increased leukocyte numbers often are not successful in killing the invader as virulent *Staph aureus* produces alpha toxin and leucocidin which destroy leukocytes. Protein A and coagulase also help to deter phagocytosis.<sup>5</sup> In less virulent strains, the influx of PMNs suppresses the bacterial multiplication. When the pathogen (*Staph aureus*) is decreased in number, the rate of PMN entrance into the milk decreases and the bacteria begin to multiply again. This cycle is repeated, yielding a chronic pattern of remission and flareups of mastitis.<sup>14</sup>

At one time, *Staph aureus* was considered to be widespread in the cow's environment.

Findings now indicate that the main reservoir of infection is the udder and teats in the form of both intramammary and skin infections.<sup>7,12</sup> *Staph aureus* does not readily persist on healthy teat skin, but will readily infect lesions. Lesions at the teat ends are of primary importance as these septic lesions aid colonization of the streak canal, an ideal location for transfer into the udder.<sup>12,13</sup> Humans can also act as a reservoir of infection when milking cattle. Both animal and human strains of *Staph aureus* can be transferred in this way.<sup>12</sup>

The spread of *Staph aureus* primarily occurs during the milking process.<sup>12</sup> Common rags and sponges act as reservoirs and means of transfer. *Staph aureus* has been reisolated from contaminated rags and sponges after several minutes of soaking in disinfectants. The longest period of survival of *Staph aureus* on soaked rags was one week.<sup>12</sup> Teat cups and the hands of milkers can also act as modes of pathogen transfer. These organisms in water droplets can be drawn into the teat cups, and in times of vacuum fluctuation propelled into the teat canal and cistern via milk droplets.<sup>7</sup>

Control of *Staph aureus* involves stopping cow to cow transmission.<sup>1</sup> This can be done by using single service paper towels, and drying teats before and after milking. A bacteriocidal teat dip is required to kill those organisms that have been transferred during the milking process. The teat dip remains on the teat as a residue which decreases the number of bacteria on the teat at the next milking.<sup>8,12,16</sup> Routine dry cow treatment is also recommended to eradicate existing infections and to provide a protective level of antibiotics during the first 2 weeks of the dry period. This is a period when the mammary gland is highly susceptible to infection.<sup>1</sup>

Methods for control of *Strep agalactiae* are generally the same as those previously described for *Staph aureus*. Since these control measures were not employed, it was felt that *Strep agalactiae* would also be isolated from this herd. Samples were taken for the Hotis test, but no *Strep agalactiae* was found. It is possible that *Strep agalactiae* was present and not isolated because only one sample per quarter was cultured.

Failure to isolate *Strep agalactiae* could have been due to its inability to compete with *Staph aureus*. Today, *Staph aureus* is the most common pathogen of the bovine udder

in advanced dairy countries.<sup>18</sup> This is due to the fact that *Staph aureus* has relatively flexible growth requirements and is resistant to antibiotic therapy once it becomes established in the udder. This is in direct contrast to the extremely specific growth requirements and susceptibility to antibiotics exhibited by *Strep agalactiae*.<sup>18</sup> Perhaps these factors led to a take over in the herd by *Staph aureus*.

Besides having very flexible growth requirements and antibiotic resistance, *Staph aureus* has the ability to withstand a hostile onslaught of leukocytes within the udder. *Staph aureus* has coagulase and protein A to protect it from phagocytosis. It also produces alpha toxin and leucocidin which can kill surrounding leukocytes. These toxins shield the organism from host defenses. If phagocytized by PMNs, *Staph aureus* can survive within these cells. Survival within the PMN is due to catalase production and the presence of fat globules within the PMN. Both of these factors inhibit the killing power of the PMN.<sup>14,18</sup>

The CMT was used in this study as a screening test to determine the degree of inflammation of the udders surveyed. It is a very easy test to run, and offers additional data for the diagnosis of mastitis. It should not, however, be used as the sole criterion for the diagnosis of mastitis infections as it only detects somatic cell numbers and not the presence of bacteria.<sup>1</sup> In some cases the somatic cell elevations may be due to damage by bacterial agents, but a somatic cell increase is not pathognomonic for bacterial infection. Almost any other irritant can cause a similar reaction.<sup>1</sup> This is illustrated in the data where many quarters were CMT positive, but no bacterial pathogens were isolated (although in some cases the pathogens may have been missed). The CMT is a good indicator for bacterial infection as is shown in table 1. As the CMT scores increased, the probability of organism isolation also increased.

As was expected, CMT scores were found to be considerably in excess of those values given earlier for goals.<sup>12</sup> The high somatic cell counts can probably be attributed to bacterial inflammation and mechanical injury resulting from overmilking. A very high percentage of quarters yielded pathogens. The cultural results correlated well with high CMT scores as shown in table 1.

The reason for including the CMT as a measure of udder inflammation in the analysis of subclinical mastitis was to exclude from consideration those organisms inhabiting the streak canal and teat orifice. These organisms were probably quite numerous since the teats were not routinely dipped.<sup>9,13</sup> It was hoped a more accurate evaluation could be made of bacterial infection rather than bacterial isolation.<sup>9</sup>

The high number of subclinical infections in the herd under study can be directly related to the poor udder health practices followed. These practices enhanced the spread and proliferation of *Staph aureus*. Three characteristics of *Staph aureus* led to its prevalence in this herd: 1) ability to infect teat lesions resulting from overmilking, 2) ability to survive for long periods on common rags used to wash udders, and 3) ability to survive the host leukocytic assault.

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