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Investigation of elevated bulk tank milk bacteria counts associated with cow infections

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Cow udder infections are rarely the source of bacteria that result in elevated, bulk-tank milk bacteria counts. Even if elevated somatic-cell counts are prevalent among cows in a herd, or the incidence of mastitis in a given time period is high, the number of bacteria shed by infected quarters of the udder is low, compared to numbers that are of concern in cases of elevated bulk tank milk bacteria counts. Additionally, the duration of high rates of bacterial shedding from udder infections is short. Addressing issues related to milking equipment hygiene usually reduces elevated bacterial counts in bulk milk. In conventional terms, this means improving the cleaning of milking and milk storage equipment, remedying deficiencies in cooling so that milk is cooled appropriately to below 4°C after every milking, and maintaining milk at that temperature during farm storage and transport to the processing plant. Occasionally, however, udder infections can affect milk quality.

Ontario's milk purchaser, the Dairy Farmers of Ontario organization, has made milk quality a priority for the Ontario industry, from producers and veterinary practitioners, through processing, to the finished product. The organization has sponsored intense, raw-milk, quality testing and on-farm educational programs to improve producer practices related to milk quality. Within this broader framework, reducing the frequency of high bacteria counts, and improving the overall bacterial quality of raw milk, is a current industry focus. To help lower milk bacteria counts, three years ago, the provincial regulatory lab adopted continuous epifluorescent microscopy to enumerate bacterial cells in milk stained with acridine orange. The results are reported as BSN units. A culture is not required, but counts are believed to be more representative of the overall quality of the milk, and samples can be processed in a few hours. Ontario is currently the only jurisdiction in North America using this technology in a regulatory program. In January 2000, the Ontario regulatory limit for bacteria in milk was lowered from a plate loop count (PLC) of 100,000 colony-forming units/ml to 110,000 BSN units (which is approximately equivalent to a PLC of 50,000 colony-forming units/ml).

Industry programs emphasizing the need to lower milk bacteria counts and the introduction of this new machine have resulted in more frequent and detailed field investigations of the on-farm causes of high milk bacteria counts. As a result, veterinary practitioners in Ontario and in other jurisdictions are facing more questions from producers about the importance of cow infections leading to high milk bacteria counts. While it is still rare that cow infections are the cause of high counts, there are two situations, described below, where cows are the source of the high counts. These case examples are ones that veterinary practitioners and other field staff should be aware of, and tactics for identifying these situations should be incorporated into farm investigations once conventional causes of high bacteria counts are ruled out.



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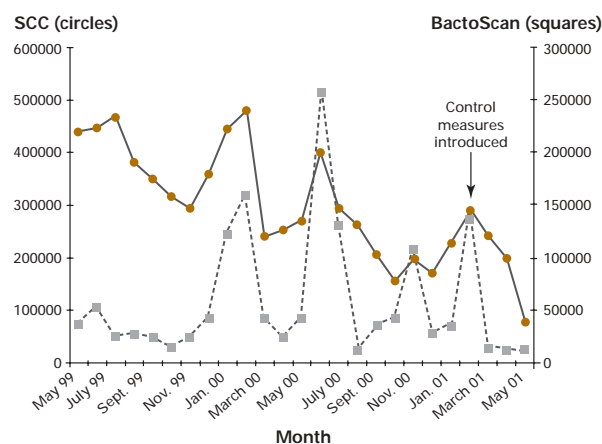
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Figure 1: Monthly somatic cell and BSN count test results from May 1999 to May 2001.



Case 1

Over 12 months, a closed herd of 140 milking cows had repeated fluctuations of the monthly bulk tank milk bacteria count from 11,000 to 257,000 BSN units per milliliter. During the same time period, the monthly bulk tank milk somatic cell count ranged from 78,000 to 445,000 cells/ml (Figure 1). Cows were housed in a free-stall barn and milked three times daily in a milking parlor system. The barn had natural ventilation, a concrete floor, and sand for bedding. Mastitis control procedures included antibiotic therapy of all cows at the end of lactation, cleaning the teats with single-service germicide towels prior to milking and 1% iodine teat disinfectant applied post-milking by spraying. Thirty-five non-lactating cows were housed in the same barn as the milking cows, but in a separate pen.

Historically, *Streptococcus agalactiae* and *Staphylococcus aureus* (*S aureus*) had been the most consistent bacteria isolated from samples of bulk tank milk on this farm (Table 1). A detailed inspection of the milking system by the manufacturer did not reveal any abnormalities. During the previous year, 50 milking cows had been culled for high somatic cell count, but no milk cultures had been performed on these cows.

| Date | Culture Results |
|----------------|---|
| September 2000 | <i>Staphylococcus aureus</i> <i>Streptococcus agalactiae</i> |
| March 2001 | <i>Staphylococcus aureus</i> <i>Streptococcus agalactiae</i> <i>Corynebacterium bovis</i> |
| April 2001 | <i>Staphylococcus aureus</i> <i>Streptococcus agalactiae</i> <i>Corynebacterium bovis</i> |
| May 2001 | <i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Pseudomonas</i> |

At the beginning of the investigation, the producer collected composite samples (4 quarters in one bottle) from each milking cow for BSN and somatic cell count determinations (Table 2). Subsequently, it was recommended that a milk culture program be initiated for cows with a somatic cell count >150,000 cells/ml, based on Dairy Herd Improvement records.

Of 26 cows cultured, 2 were positive for *Streptococcus agalactiae* and 2 were positive for *S aureus*. One cow had a somatic cell count greater than 5×10^6 cells/ml and a BSN count greater than 20×10^6 . *Streptococcus agalactiae* was found in the composite milk sample from this cow. However, in the entire herd, there was a poor correlation between the somatic cell count and the BSN value (Figure 2).

The producer was convinced that a few cows in the herd could be responsible for the high BSN count in the bulk tank milk. To determine the impact of the suspect cows, the producer proposed excluding their milk from the bulk tank and re-testing the bulk tank milk. After removing the cow with the BSN count greater than 20×10^6 units, the bulk tank count declined from 270,000 to 150,000 BSN units.

Discussion

Bacteria can enter a herd's bulk tank milk from many sources, including:

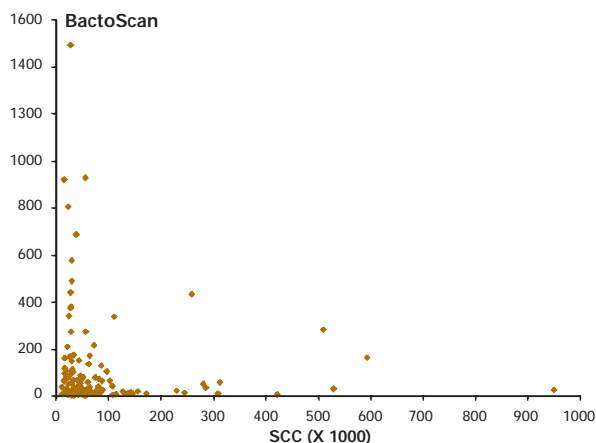
- inadequate cleaning of milking equipment
- poor cooling
- poor teat cleaning
- in the milk from cows with infected udders.

In this herd, the milking equipment was found to be exceptionally clean and well maintained. Hot water supply was adequate and appropriate equipment cleaning protocols had routinely been followed. In addition, stored milk had

| Lactation group | n | BSN | n |
|-----------------|----|-----------------|----|
| 1 | 70 | < 29,000 | 84 |
| 2 | 47 | 30,000-109,000 | 51 |
| 3 | 13 | 110,000-499,000 | 3 |
| 4 | 8 | > 500,000 | 2 |
| 5 | 1 | | |
| 6 | 1 | | |

| Somatic cell count | n |
|--------------------|-----|
| <149,000 | 114 |
| 150,000-249,000 | 11 |
| 250,000-349,000 | 7 |
| 350,000-449,000 | 1 |
| 450,000-549,000 | 2 |
| >550,000 | 5 |

Figure 2: Relationship between somatic cell count and BSN obtained from 140 milking cows. One cow with high somatic cell count (5,187,000) and BSN (20,779,000) has been excluded from this graph.



been kept at the correct temperature all the time. Bacteria that do not cause mastitis had rarely been isolated from the bulk tank in high numbers.

Satisfied that the milking equipment was unlikely to be the source of the elevated levels of bacteria in the bulk tank milk, and with evidence of elevated cow and bulk tank milk somatic cell counts, it became important to evaluate mastitis as a cause of both problems. All the events of the past year with the potential to negatively impact milk quality were examined. *Corynebacterium bovis*, an organism that originates from cows, is considered a mildly pathogenic udder infection, but it has been shown to spread rapidly from cow to cow in the absence of adequate post-milking teat dipping. The isolation of *Corynebacterium bovis* from the bulk tank milk suggested that an ineffective germicidal teat dip had been used and/or an ineffective post-milking teat dipping protocol had been practiced at some time. The post-milking application of 1% iodine teat disinfectant was changed from *spraying* to *dipping* to improve teat coverage. Additionally, in order to improve general teat-end hygiene, a change in the pre-milking preparation to a 0.5% iodine licensed pre-milking teat dip and removal of the disinfectant after a contact time of 30 to 45 seconds with a single-service paper towel, was recommended.

Cows identified with *S aureus* were leg banded. All four quarters of these cows were treated with an antibiotic at 24-hour intervals, as directed on the selected product label. If one course of treatment did not result in a cure, the owner was advised that further antibiotic treatment had a very low probability of curing the chronically infected, high somatic cell count cows. Cows identified as infected with *Streptococcus agalactiae* were treated either during lactation or dried off and treated with a dry-cow intra-mammary product. Quarter cure rates by either method would be expected to exceed 90% for this pathogen.

Mastitis caused by *S aureus* usually results in the shedding of only a few bacteria in milk, while *Streptococcus agalactiae*

are present in very high numbers. Cows infected with the highly contagious *Streptococcus agalactiae* are usually infected in multiple quarters and frequently, the infection spreads rapidly and extensively among milking herd mates. This results in constant high rates of bacterial shedding of this organism by infected cows. *Streptococcus agalactiae* was previously identified as a rare, but possible cause of high bulk tank milk bacteria counts using plate loop count techniques.

In this herd, it appears likely that the high bulk tank milk bacteria counts were caused by cows infected with *Streptococcus agalactiae* that shed large numbers of organisms. It is unfortunate that so many cows were culled prior to the investigation for high somatic cell counts without a culture for pathogen identification. While culling effectively lowered both BSN and somatic cell counts, it is likely that many of the cows infected with *Streptococcus agalactiae* were removed that could have been successfully treated.

One month after the producer implemented the recommendations, the bulk tank BSN count was found to be within the normal range. Bulk milk somatic cell count was <90,000 cells/ml.

Interpreting cow BSN test results

The usefulness of cow somatic cell count tests to identify cows that are likely to be infected with mastitis bacteria is well established. Milk meters are used to collect a sample that is representative of the entire volume of milk in the quarter and of the udder at a milking. The somatic cells are counted using Foss technology and the resulting individual cow somatic cell count results are then compared to benchmarks or "cut points" that research has established, through comparison to milk cultures, for the probability of infection.

Although cow BSN counts have been done in Ontario in the course of similar investigations, there appears to be little research on the determination of a protocol for sample collection and interpretation of the results of counts done on composite cow samples. It is likely inappropriate to use the same collection protocol for samples in BSN determinations as that used for somatic cell counts. The best methodology for sample collection with the two tests may differ because, while cells can only come from the cow, bacteria can enter the milk sample from the milk meter, contamination of teat ends, or sample mishandling. It is well established that culture results obtained from milk samples collected via milk meters are not interpretable. So, while it may be necessary to obtain a representative sample of the entire volume of milk produced by the cow for BSN testing, collection through milk meters is unlikely to be appropriate because of the risk of contamination from non-cow sources. Additionally, since bacteria are not evenly distributed throughout the milk produced by the cow, obtaining pre-milking or post-milking samples directly from the cow has the potential to produce both false positive and false negative results. Ultimately, all that bacterial counts can do is enumerate the bacteria in a milk sample. The test result

gives no indication of the bacterial types that are present, how long they have been there, or where they came from.

Results from cows tested concurrently in Ontario by both BSN and culture, demonstrate that there is no consistent relationship overall between bacterial culture and BSN results. The usefulness of the somatic cell count test, however, in identifying the cows likely contributing to persistently high herd BSN results, improves considerably in herds where *Streptococcus agalactiae* and *Prototheca* infections have been identified previously.

Case 2

Over a 3-month period, a closed herd of 80 milking cows had monthly bulk tank milk BSN counts >109,000 BSN units, while the monthly bulk tank milk somatic cell count ranged from 90,000 to 150,000 cells/ml. Cows were housed in a tie-stall barn and milked twice daily. The barn had tunnel ventilation, a concrete floor, and straw for bedding. Mastitis control procedures included antibiotic therapy of all cows at the end of lactation, pre-milking preparation with 0.5% iodine teat dip, removal of the disinfectant with a single-service paper towel, and post-milking teat dipping with 1% iodine. Thirty-five non-lactating cows were housed in a different barn from the milking cows.

Prototheca organisms had been the most consistent pathogen isolated from the bulk tank. A detailed inspection of the milking system by the milking equipment dealer did not reveal any abnormalities. At the beginning of the investigation, the producer collected composite samples from each milking cow for milk culture. A *Prototheca* species was found in the composite milk sample from only one milking cow and the somatic cell count was <150,000 cells/ml. No treatment was recommended and the infected cow with *Prototheca* was culled. The bulk tank BSN count was found to be within the normal range as soon as milk from the infected cow was withheld from the bulk tank.

Discussion

Culturing milk samples from lactating cows when persistent, high bacteria (BSN), bulk tank counts occur is warranted for potentially identifying not only *Streptococcus agalactiae*, but also *Prototheca* species infected cows. Quarters infected with *Prototheca* organisms may continually shed large numbers of organisms in milk and have been shown previously to increase bacterial numbers in the bulk tank.

Prototheca species are achlorophyllic algae that are ubiquitous in wet manure-contaminated areas. *Prototheca zopfii*, and to a lesser extent *Prototheca wickerhamii*,

have been reported to be etiologic agents in intramammary infections in cows. *Prototheca* organisms are environmental mastitis pathogens, but they may possibly spread from cow to cow during milking. Slowly progressive and often subclinical, protothecal intramammary infection occurs because of the lack of an effective treatment, as well as the absence of spontaneous recovery. Progressive agalactia, leading to the complete cessation of quarter milk production with only mild clinical signs, appears to be a feature of protothecal mastitis infections.

Although protothecal mastitis infections in a herd can cause high bulk tank bacteria counts, a protothecal diagnosis is not always associated with extremely high individual cow somatic cell counts. *Prototheca* infected cows, identified in Ontario over the last 2 years, have almost always had cow composite somatic cell counts >200,000 cells/ml, but rarely have they exceeded 1 million cells/ml. However, quarters infected with *Prototheca* organisms react on the California Mastitis test paddle. One hypothesis is that this apparent contradiction occurs because milk production and the contribution from chronically infected quarters are reduced. Dilution with milk from healthy, uninfected quarters reduces cow composite somatic cell counts. Therefore, Dairy Herd Improvement's cow somatic cell count reports can be used to identify protothecal-suspect cows, provided sufficiently stringent cutpoints are used to select cows to culture. However, as illustrated in the case herd, even with a cut-point of 150,000 cells/ml, some infected cows may be missed. Follow-up evaluations with CMT or whole herd cultures, as was done in this case, may become necessary when the index of suspicion is very high.

Between July 1999 and December 2000, 65 Ontario herds had a *Prototheca* species identified by a culture of milk samples obtained from either a cow or a bulk tank at the Animal Health Laboratory in Guelph. Of these 65 herds:

- 31% never had a monthly herd BSN test result >109,000
- 22% had 1 monthly herd BSN test result >109,000
- 48% had 2 to 9 herd BSN counts >109,000
- Of the 31 farms that had 2 to 9 herd BSN counts >109,000, 65% were in violation of the penalty range. In Ontario, a penalty is applied when the official monthly count exceeds 109,000 BSN units in 2 out of 3 consecutive official tests.

Prevention of mastitis caused by *Prototheca* organisms

Prototheca is a relatively rare and sporadic infection of cows. Herd outbreaks, where large numbers of cows

are infected over a short time, are even less frequent. There are no large-scale studies of numbers of afflicted herds. In case studies of herds where protothecal mastitis has occurred, the organism has at some time been cultured from virtually every area of the dairy farm, including pasture soil, milk parlor wash water, cow drinking troughs, forage, calf manure, and cow manure. In an examination of the environment on farms without protothecal mastitis, the organism was not found at all, found in very low numbers, or found only at a few sites. So, while not all farms have the algae in their environments, on farms where it is present, the *Prototheca* organism is widely disseminated.

Prototheca species have been shown to survive and pass through the intestines of single-stomached animals without multiplying and it is likely that the organism can survive in the gastrointestinal tracts of cows and calves too. After ingesting the organism, the cow passes manure and contaminates her own bedding (as well as that of the herd mates), the alleyways, and the stalls. A factor common to all *Prototheca* infected areas on farms is that they are contaminated with cow manure. In warm weather, or in humid farm sites, protothecal organisms survive and multiply outside the cow. When numbers are high, there is greater probability of teat-end contact and, if conditions are right, mastitis begins.

Veterinarians should recommend producers take the same approach to prevent *Prototheca* species infections as for any manure-borne, environmental, mastitis-causing organism. Teats must always be clean and dry before the milking unit is applied. All herds, but especially those at risk of protothecal mastitis, must adhere to excellent pre-milking preparation to ensure all contaminating environmental bacteria and *Prototheca* organisms are removed from the teat skin. Preparation should involve either washing and drying with individual paper or cloth towels, or pre-dipping with a licensed iodine product and drying the teats with an individual paper or cloth towel.

Bedding can be contaminated by manure or by cows with dirty feet from poorly cleaned alleyways. In tie-stall barns, cows repeatedly forced to stand in gutters contaminate bedding and the lying area under the udder with manure. *Prototheca* organisms thrive in areas where humidity is high. Lowering the humidity reduces the ability of *Prototheca* species to survive and multiply. During the time of greatest seasonal risk of environmental mastitis (July to September in Ontario), producers need to adapt housing and calving areas to increase air movement and remove moisture.

Recommendations to reduce the impact of protothecal mastitis on bulk tank bacterial counts

- Prevent new protothecal infections by adjusting the milking order. Infected cows shedding large numbers of organisms in their milk may be contagious to herd mates via teat liners. Milk the known infected cows last.
- Prevent mastitis infections through teat preparation. Cows may become newly infected when organisms on teat skin get a chance to enter the teat end during milking. Improve milk preparation to ensure teat skin is always clean and dry before milking. Use either a wash and dry preparation with individual paper or cloth towels, or predip with an approved iodine product and dry with a separate paper or cloth towel.
- Stop milking infected quarters. This immediately prevents this milk causing high bacteria counts from entering the bulk tank, and allows the producer to retain the cow and her uninfected milk production. No quarter treatments should be given.
- Examine the infected cow's records of clinical mastitis and cow somatic cell counts to determine if the start of infection can be identified. Unless shown otherwise, suspect that new cases are beginning in the peri-parturient or early lactation period.
- Frequently monitor the remaining quarters of known infected cows being milked into the bulk tank using the California Mastitis Test paddle. If new quarters show reaction, culture them individually to confirm new infections.
- Do not feed milk from protothecal-infected quarters to calves. Calves pass the organisms into the environment via manure and increase the general contamination level on the farm.

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Abstract of Interest

Herd Prevalence and Incidence of *Streptococcus agalactiae* in the Dairy Industry of Prince Edward Island

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Herd Prevalence and incidence of mastitis caused by *Streptococcus agalactiae* was determined for dairy cattle on Prince Edward Island during December 1992 and June 1994. For each census, bulk tank milk samples from all dairy herds (n = 452) in the province were tested on two occasions, and the results were interpreted in parallel. The combined sensitivity of the testing protocol was estimated to be 91%. The confirmatory latex agglutination test had previously reported specificities approaching 100%. Therefore, the estimated specificity of the testing protocol was assumed to be 100%. The apparent prevalence of *S agalactiae* in December 1992 and in June 1994 was 17.7 and 13.1%, respectively. Based on the characteristics of the test, the estimated true prevalence was 18.9 % in December 1992 and 14.4% in June 1994. Infection with *S agalactiae* was associated with elevated bulk tank somatic cell count (SCC) and elevated standard plate counts. Economic losses associated with *S agalactiae* were attributed to production losses (associated with bulk tank SCC), milk quality penalties (associated with bulk tank SCC and standard plate count), and decreases in milk quality (associated with bulk tank SCC). For herds that had been negative for *S agalactiae* in December 1992, evaluation in June 1994 yielded an incidence of new infections of 3.51 per 100 herds per year.

J Dairy Sci 1997;80:464-470.

Popular Websites

<http://www.nmconline.org>

National Mastitis Council

<http://www.managingmilkquality.com>

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