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Etiologic Mastitis Agents of High Somatic Cell Count Dairy Herds in Tennessee

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Abstract

The objective of the study was to determine etiologic agent(s) of intramammary infections (IMI) in high somatic cell count (SCC) dairies in Tennessee. This was a prospective study utilizing 2,444 dairy cows from 20 high SCC dairy herds. Composite milk samples were aseptically collected from lactating cows in 20 dairy herds with a rolling herd average SCC > 400,000 cells/mL. Milk samples were cultured and mastitis pathogens identified following procedures recommended by the National Mastitis Council. The average herd percentage (average of the 20 herd averages) of culture-negative cows was 49%. The average herd percentages of positive-culture cows were 28% for coagulase-negative staphylococci (CNS), 15% for *Staphylococcus aureus,* 10% for environmental streptococci (ES), and ~ 2% for gram-negative organisms. Among cows with individual SCC > 1 million cells/mL, 30% of cultures yielded *Staph. aureus*, 23% CNS, 26% no growth, 26% ES, and 5% gram-negative organisms. Among cows with individual SCC > 400,000 cells/mL, cultures yielded 26% CNS, 30% no growth, 28% *Staph. aureus*, 21% ES, and 4% gram-negative organisms. *Staph. aureus* was the most common major pathogen, or equally common major pathogen, in 75% of high SCC herds. Environmental streptococci were the most common major pathogen, or equally common major pathogen, in 45% of high SCC herds. *Staph. aureus* is the major pathogen, followed by ES, among Tennessee dairy herds with high SCC. Coagulase-negative staphylococci are common, but their significance needs to be determined. Gram-negative organisms do not appear to be significant pathogens of Tennessee dairy herds with persistently high SCC.

Key words: dairy, mastitis, high SCC, etiologic agent, milk culture, *Staphylococcus aureus*

Résumé

L'objectif de cette étude était de déterminer les agents étiologiques de l'infection intramammaire dans les fermes laitières avec comptage de cellules somatiques élevé au Tennessee. Cette étude prospective comprenait 2444 vaches laitières provenant de 20 troupeaux laitiers avec comptage élevé. Des échantillons de lait composites ont été recueillis aseptiquement de vaches en lactation dans 20 fermes laitières avec une moyenne mobile du comptage de cellules somatiques au niveau du troupeau >400000 cellules/ml. Les échantillons de lait ont été mis en culture et les pathogènes de la mammite ont été identifiés suivant les recommandations du *National Mastitis Council*. Le pourcentage moyen au niveau du troupeau (la moyenne des 20 moyennes de troupeau) de vaches avec culture négative était de 49%. Le pourcentage moyen au niveau du troupeau de vaches avec culture positive était de 28% pour les staphylocoques à coagulase négative (SCN), de 15% pour *Staphylococcus aureus,* de 10% pour les streptocoques environnementaux (SE) et d'environ 2% pour les organismes a Gram négatif. Parmi les vaches avec des comptages de cellules somatiques > 1 million cellules/ml, la culture était positive dans 30% des cas pour *Staphylococcus aureus*, dans 23 % des cas pour les SCN, dans 26% des cas pour les SE, dans 5% des cas pour les organismes à Gram négatif alors qu'il n'y avait pas de croissance dans 26% des cas. Parmi les vaches avec des comptages de cellules somatiques > 400000 cellules/ml, la culture était positive dans 28% des cas pour *Staphylococcus aureus,* dans 26 % des cas pour les SCN, dans 21% des cas pour les SE, dans 4% des cas pour les organismes à Gram négatif alors qu'il n'y avait pas de croissance dans 30% des cas. Dans 75% des troupeaux à comptage élevé, *Staphylococcus aureus* était le pathogène majeur le plus commun ou a tout le moins aussi commun que les autres. Dans 45% des troupeaux à comptage

élevé, les SE étaient les pathogènes majeurs les plus communs ou à tout le moins aussi communs que les autres. Dans les troupeaux laitiers du Tennessee à comptage élevé, *Staphylococcus aureus* était le pathogène le plus commun suivi des SE. Les SCN étaient fréquentes mais leur rôle reste nébuleux. Les organismes à Gram négatif ne semblent pas être des pathogènes importants dans les fermes laitières du Tennessee avec des comptages fréquemment élevés.

Introduction

Mastitis is considered the most costly disease in the dairy industry.[30] The major cost is due to decreased milk production as a result of subclinical mastitis; however, both the clinical and subclinical forms result in increased antibiotic usage and reduced milk quality. Milk quality is primarily assessed by measurement of somatic cells and bacteria in bulk-tank milk. High bacterial counts suggest environmental contamination of milk, equipment failure, or milking system sanitizing mishaps, whereas somatic cell count (SCC) is a direct indication of the prevalence and incidence of mastitis in the herd. Tennessee ranks 50th among the 50 states in average SCC, and was the only state with a herd SCC average > 460,000 cells/mL.[20] It is a consistent trend for Tennessee dairies to be among the highest SCC states.[24] The maximum legal limit in which Grade A milk can be sold in the United States (US) is 750,000 cells/mL. Tennessee dairies have an average SCC of 463,000 cells/mL compared with the US average SCC of 288,000 cells/mL.[20] Clearly, there is substantial room for improvement and improvement is necessary to sustain the dairy industry in Tennessee. The National Mastitis Council (NMC) and American Association of Bovine Practitioners are advocates of lowering the legal limit of SCC in Grade A milk to 400,000 cells/mL, as has been done in other developed countries (European Union, New Zealand, Australia). The US SCC standard is by far the most lenient of any developed country. Most dairy industry personnel agree that this change will eventually occur either through regulatory enforcement or consumer demands and when it does, many dairies will face serious challenges to meet these higher standards of milk quality. In preparing for the possibility of future restrictions on SCC in milk, it is important to determine the etiologic agents likely responsible for intramammary infections (IMI) in high SCC cows within high SCC herds.

Mastitis pathogens may be divided into major and minor pathogens. The classic major mastitis pathogens are *Escherichia coli, Klebsiella* spp, *Staphylococcus aureus, Streptococcus* spp, and *Mycoplasma* spp. Minor mastitis pathogens, such as *Corynebacterium bovis* and coagulase-negative staphylococci (CNS), tend to result in relatively low SCC infections, although there are certainly exceptions to this rule. Many other pathogens can result in a high SCC mastitis, but their prevalence and incidence are usually less common than the major and minor pathogens listed above.

Mastitis pathogens may also be divided into contagious and environmental categories based on the source of the agent and means of transmission. Environmental pathogens, such as *E. coli* and *Klebsiella* spp (coliform bacteria), are controlled by maintaining a sanitary environment and keeping the cows clean, dry, and comfortable. Other environmental mastitis pathogens, such as *Strep. uberis* and *Strep. dysgalactiae*, are controlled using the same methods as for the coliforms, but in addition, pre- and post-milking teat disinfection with an effective germicide and dry-cow intramammary therapy are also recommended due to the potential contagious nature of these organisms. The primary source of contagious pathogens, such as *Strep. agalactiae*, *Staph. aureus*, and *Mycoplasma,* is the infected mammary gland. Contagious mastitis is typically controlled by milking-time hygiene, post-milking teat disinfection, dry cow intramammary therapy, and culling.

Numerous other microbes have been identified in clinical mastitis outbreaks. However, the tendency is to report the unusual cause of mastitis outbreaks rather than the "typical" cause.[3,11,17,33] Most mastitis experts would agree that dairy herds with a consistently high bulk tank SCC have a contagious mastitis problem. A study conducted at Washington State University indicated that coagulase-positive staphylococci (CPS) were the most prevalent (40%) IMI among nine herds with an average SCC of 421,000 cells/mL, whereas nine low SCC herds with an average SCC of 188,000 cells/mL had a 2% prevalence of CPS.[28] The majority of CPS are *Staph. aureus*.[27] Wilson and others reported that the prevalence of *Strep. agalactiae* and *Staph. aureus* mastitis was directly associated with bulk tank milk SCC.[38] Other studies have identified mastitis pathogens in high SCC herds.[12,13] In the aforementioned studies, individual cow IMI was not specifically associated with individual SCC. This association was examined in a small herd study where seven of the 10 highest SCC cows were infected with *Staph. aureus*.[26] Although studies of clinical mastitis (not specifically outbreaks) in multiple herds have been conducted, peer-reviewed/multi-herd studies that associated individual cow SCC with individual cow IMI in high SCC herds could not be found.[1,2]

Thus, there is a need to document the typical etiologic agents of high SCC cows in high SCC herds. Knowledge of the most common agents of mastitis in the chronically high SCC herd will help the veterinarian and producer reduce the herd SCC to acceptable levels. We hypothesize that the primary etiologic agent causing IMI in cows and herds with a chronically high SCC is *Staph. aureus*.

Materials and Methods

Cattle and Sample Collection

Dairy herds in eastern and middle Tennessee with a rolling herd average SCC > 400,000 cells/mL over the previous 12 months were identified via Dairy Herd Improvement Association (DHIA) records. Each herd meeting study criteria was assigned a consecutive number beginning with one. The RAND function of Excel® was used to generate a list of numbers that coincided with the number of herds. Beginning with the top of the list, owners of herds were contacted until 20 agreed to participate in the study. Composite milk samples were collected from all lactating cows, regardless of mastitis status or previous treatment, among the participating herds. The collection of one-time composite milk samples is a limitation to the study, but IMI can be accurately diagnosed with single milk samples.[35] Milk samples were collected after pre-milking preparation and just before applying the milking unit. Teats were cleaned with cotton pads moistened with isopropyl alcohol. A few streams of milk were expressed from each teat prior to collection in a sterile tube. Collection tubes were stored on ice immediately after collection and during transport to the laboratory. Collection tubes were stored frozen at 28°F (-2.2°C) for one to seven days until analysis occurred.

Milk Culturing Procedures

Culturing procedures and interpretation of culture results followed NMC guidelines.[a] Briefly, 50 µl of milk were plated on one-half of a blood agar plate. Plates were incubated at 98.6°F (37°C). After 18 to 24 hours' incubation, microbial colonies were presumptively identified by colony morphology and hemolysis. Additional tests were performed as needed (potassium hydroxide test, catalase test, gram stain, MacConkey agar, eosin methylene blue agar, esculin-CAMP agar, tube coagulase test). Microbial growth was ultimately identified by biochemical test strips (bioMerieux[b]). All plates, including those with no apparent growth at 18 to 24 hours, were re-evaluated 48 hours after plating. Contaminated samples (n = 29) were not included in culture result analysis. When two different mastitis organisms were present and significant in a culture result, both organisms were individually correlated with SCC. Some organisms, such as mycoplasma, that are known to create high SCC were not investigated in this study because of the special culturing methods required, and therefore represent a study limitation.

Descriptive Data

Collected data included SCC (individual cow and bulk tank), breed, and composite milk for culture. The individual cow SCC based on DHIA records closest to the sampling date were used for correlation purposes. The individual cow SCC data was typically within two weeks of the milk sampling date. Misclassification bias is inherent with this method of association, but based on the results did not significantly alter the conclusions. Milk samples from cows without any individual SCC data were excluded from association calculations, but not from overall prevalence. The most common etiologic agent(s) were determined by calculating proportionate etiologies among cows with cell counts < 400,000 cells/mL, > 400,000 (this includes > 1 million cells/mL), and > 1 million cells/mL milk.

Statistical Analysis

Data are presented as prevalence of specific intramammary pathogens among herds or all individual cows. When cow is used as the descriptor, calculations were computed by number of cows; when herd is the descriptor, calculations were computed by the average of herd averages. The differences between these two means of computing were slight (< 4 percentage points). The chi square test was used to distinguish among the prevalence of various pathogens and individual or herd SCC. Odds ratios and the associated 95% confidence intervals were calculated using a commercial software program.[c]

Results

Descriptive data for the 20 dairy herds studied are presented in Table 1. Rolling herd average SCC was 535,350 cells/mL (range 412,000 to 877,000). Rolling herd milk production average was 18,346 lb (8,339 kg) milk (range of 13,592 [6,178 kg] in a Jersey herd to 26,666 lb [12,121 kg] in a Holstein herd). Average number of cows per herd was 123 (range of 41 to 356). The percentage of cows with SCC > 1 million cells/mL was 13.7% (herd averages ranged from 5.5 to 27.5%; average of herd averages was 14.2%). The percentage of cows with SCC > 400,000 cells/mL was 28.1% (herd averages ranged from 16.2 to 42.9%; average of herd averages was 28.6%).

The average herd percentage of a pathogen or pathogen group within a SCC category is presented in Table 2 for all lactating cows, cows with SCC > 1 million cells/mL, cows with SCC > 400,000 cells/mL, and cows with SCC < 400,000 cells/mL. This table shows the tendency of a given culture result to occur in a given SCC category. No growth, CNS, and corynebacterium culture results were significantly more likely to be < 400,000 cells/mL than the major mastitis pathogens. No growth culture results were significantly more likely to be < 400,000 cells/mL than CNS. However, corynebacterium culture results were not significantly different from CNS culture results in any SCC category. Major mastitis pathogens and less common mastitis pathogens were significantly more likely to be cultured in cows with cell counts > 400,000 cells/mL and > 1 million cells/

Table 1. Farm characteristics including rolling herd average somatic cell count (RHAscc), rolling herd average milk (RHAmilk, lb), breed, number of cows lactating per farm when sampled, number of cows/farm with SCC data, percent of cows with SCC data in herd with SCC > 1 million cells/mL (%GMIL), and percent of cows with SCC data in herd with SCC > 400,000 (%GK).

Farm	RHAscc	RHAmilk (lb)	Breed	No. Cows	No. SCC	No. GMIL	%GMIL	No. GK	%GK
1	412,000	20,891	Holstein	181	156	13	8.3	31	19.9
2	417,000	18,786	Holstein	73	73	4	5.5	15	20.5
3	418,000	26,666	Holstein	61	58	9	15.5	18	31.0
4	420,000	18,390	Mixed	69	68	8	11.8	14	20.6
5	422,000	16,366	Mixed	117	111	10	9.0	18	16.2
6	431,000	25,670	Holstein	197	191	24	12.6	50	26.2
7	439,000	18,618	Holstein	356	342	45	13.2	93	27.5
8	453,000	17,545	Holstein	110	91	25	27.5	39	42.9
9	457,000	17,549	Holstein	43	43	5	11.6	10	23.3
10	460,000	17,303	Jersey	116	115	17	14.8	37	32.2
11	511,000	15,220	Holstein	89	83	13	15.7	31	37.3
12	514,000	14,212	Brown Swiss	235	228	24	10.5	70	30.7
13	556,000	21,564	Holstein	79	76	9	11.8	23	30.3
14	570,000	16,867	Holstein	94	66	9	13.6	22	33.3
15	577,000	15,413	Holstein	175	168	23	13.7	42	25.0
16	584,000	13,592	Jersey	77	73	9	12.3	14	19.2
17	625,000	16,007	Holstein	41	41	8	19.5	15	36.6
18	717,000	18,621	Holstein	133	128	21	16.4	33	25.8
19	847,000	22,431	Holstein	115	114	25	21.9	42	36.8
20	877,000	15,220	Holstein	91	80	15	18.8	30	37.5
TOTAL				2,452	2,305	316		647	
MEAN	535,350	18,346		123			14.2		28.6

Table 2. The average herd percentage of a pathogen or pathogen group within a SCC category (e.g., of all no growth cultures averaged by herd average, only 7% were > 1 million SCC, of all CNS cultures only 12% were > 1 million, etc).

Culture result	Herd prevalence Mean (Range[1]) (%)	< 400,000 SCC Mean (Range) (%)	> 400,000 SCC Mean (Range) (%)	> 1 million SCC Mean (Range) (%)
No growth	49 (24.4-67)	84[a] (70-100)	16[a] (0-30)	7[a] (0-20)
Coagulase-negative staphylococci	27.8 (12.3-42.5)	71[b] (52-89)	29[b] (11-48)	12[b] (0-31)
Staph. aureus	14.5 (4.3-36.6)	44[c] (20-80)	56[c] (20-80)	29[c] (0-50)
Environmental streptococci	10.2 (4.9-22)	41[c] (0-65)	59[c] (35-100)	34[c] (0-67)
Corynebacterium	2.6 (0-10.7)	73[ab] (0-100)	27[ab] (0-100)	15[ab] (0-100)
Coliforms (*E. coli* and *Klebsiella*)	1.7 (0-4.9)	50[c] (0-100)	50[c] (0-100)	28[c] (0-100)
Other[2]	1.2 (0-4.5)	27[c] (0-100)	73[c] (0-100)	38[c] (0-100)

*Superscripts that are different within a column are significantly different ($P < 0.05$).
[1]Ranges represent the widest average percentages by herds (e.g. for the no growth > 1 million SCC category, one herd had 0% no growth cultures in this category whereas another herd had 20% no growth).
[2]Others included: *Prototheca, Streptococcus agalactiae,* coagulase-positive *Staphylococcus hyicus,* and *Bacillus.*

mL than no growth, corynebacterium, and CNS. There was no significant difference among the major mastitis pathogens in regard to SCC category. Figures 1 through 3 indicate the frequency of a given culture result at three SCC levels. For cows with > 1 million SCC/mL (Figure 1), *Staph. aureus* was the most common mastitis pathogen (30%), followed by ES (26%), no growth (25%), and CNS (23%). The corynebacterium, coliforms and other mastitis pathogens were isolated < 5% among cows with SCC > 1 million cells/mL. No growth (30%) cultures were slightly more common among cows with SCC > 400,000 cells/mL (Figure 2) than *Staph. aureus*

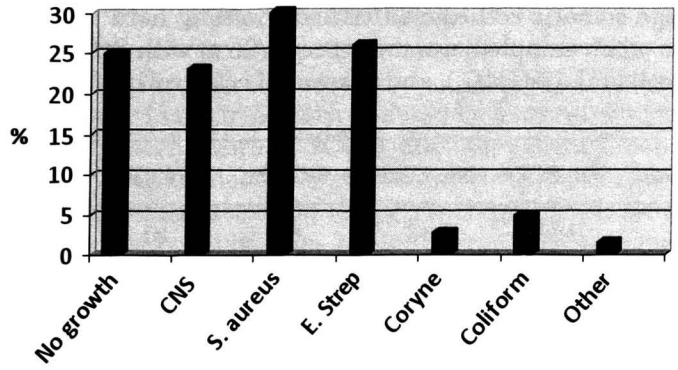

Figure 1. Culture results of cows with a somatic cell count > 1 million cells/mL (n = 313).

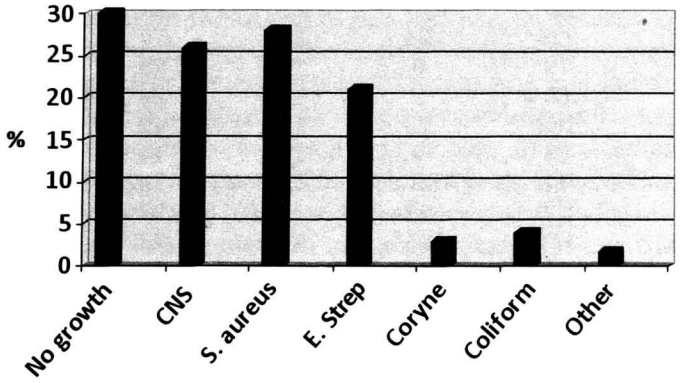

Figure 2. Culture results of cows with a somatic cell count > 400,000 cells/mL (n = 641).

(28%), CNS (26%), and ES (21%). For cows with SCC < 400,000 cells/mL (Figure 3), only no growth (55%) and CNS (25%) cultures were common; the frequency of the other mastitis pathogen categories was < 8%. Table 3 shows the odds of being a high SCC cow based on the organism cultured. All three of the major pathogens/pathogen groups (*Staph. aureus*, ES, and coliforms) were significantly associated with a high SCC at both cut-off points (1 million cells/mL and 400,000 cells/mL). Although > 25% of high SCC cows were culture-positive for CNS, the CNS were not associated with a high SCC. Only one herd had IMI due to *Strep. agalactiae.*

Staph. aureus was the most common or equally common major mastitis pathogen isolated from cows within individual herds with high SCC > 1 million cells/mL on 15 of 20 dairies. The environmental streptococci were the most common or equally common major mastitis pathogens isolated from cows with high SCC > 1 million cells/mL on nine of 20 dairies whereas coliform pathogens were never the most common and were only equally common on two dairies. Coliforms were not isolated frequently from any of the dairies studied (mean herd prevalence

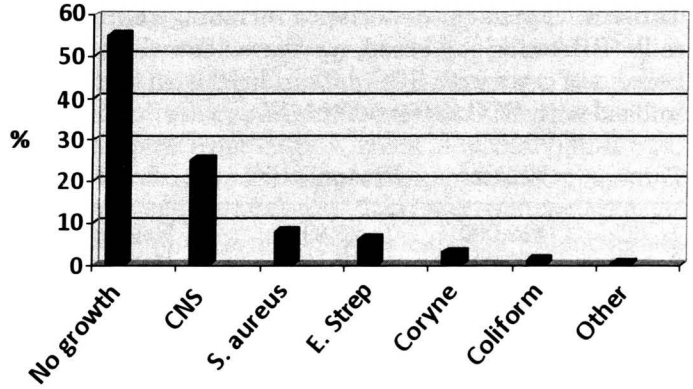

Figure 3. Culture results of cows with a somatic cell count < 400,000 cells/mL (n = 1,663).

1.7%). In three of the dairies studied, the prevalence of *Staph. aureus*-infected cows was relatively low among cows with a SCC > 1 million cells/mL and > 400,000 cells/mL (0%, 8%, 0%; 9.5%, 9.5%, 7.1%, respectively). The prevalence of ES-infected cows was low among cows with SCC > 1 million and > 400,000 cells/mL in two herds (0%, 0%; 8.2%, 9.5%, respectively).

The odds of isolating the two most prevalent pathogens among high SCC cows (*Staph. aureus* and ES) were compared to other mastitis pathogens (Tables 4 and 5). *Staph. aureus* and ES were significantly more likely to be isolated from high SCC cows (both > 400,000 and > 1 million cells/mL) than CNS, but were not significantly more likely to be recovered from high SCC cows than coliforms.

Discussion

In this study of high SCC herds, 13.7% of cows had a SCC > 1 million cells/mL. An Estonian study of four herds reported 7.8% of cows had > 1 million cells/mL, but samples were collected only from cows whose milk was being shipped to the milk plant.[15]

The purpose of this study was to determine the most prevalent pathogens associated with high SCC cows within high SCC dairy herds in Tennessee. To determine this, an organism would need to be significantly associated with high SCC IMI and be a prevalent pathogen. This study found that *Staph. aureus* was the most common IMI that was significantly associated with high SCC cows within high SCC herds. Goodger and Ferguson[14] found that *Staph. aureus* was the organism responsible for a large California dairy herd's persistently high SCC (> 600,000 cells/mL for several months).[14] Unfortunately, the aforementioned study did not reveal or mention other milk culture results. Although > 29% of *Staph. aureus*-positive cows had SCC > 1,000,000 cells/mL, it is important to note that 44% of *Staph. aureus*-positive

Table 3. Odds that a specific pathogen is associated with a high SCC versus a low SCC.

Culture result	SCC > 1 x 10^6			SCC > 4 x 10^5		
	Odds ratio	95% Confidence interval	*P*-value	Odds ratio	95% Confidence interval	*P*-value
Staph. aureus	2.549	2.05-3.11	< 0.001	3.15	2.58-3.84	< 0.001
Environmental streptococci	3.43	2.69-4.38	< 0.001	3.44	2.69-4.41	< 0.001
Coagulase-negative staphylococci	0.87	0.70-1.08	0.20	1.01	0.87-1.18	0.87
Coliform	2.87	1.61-5.12	< 0.001	2.45	1.43-4.21	< 0.001

Table 4. Odds that a high SCC cow was culture-positive for *Staphylococcus aureus* compared with other common mastitis pathogens.

Pathogen	SCC > 1 x 10^6			SCC > 4 x 10^5		
	Odds ratio	95% Confidence interval	*P*-value	Odds ratio	95% Confidence interval	*P*-value
Environmental streptococci	0.74	0.51-1.05	0.096	0.91	0.65-1.29	0.607
Coagulase-negative staphylococci	2.90	2.06-4.09	<0.0001	3.11	2.34-4.12	<0.0001
Coliform	0.88	0.47-1.67	0.70	1.28	0.71-2.32	0.407

Table 5. Odds that a high SCC cow was culture-positive for environmental streptococci compared with other common mastitis pathogens.

Pathogen	SCC > 1 x 10^6			SCC > 4 x 10^5		
	Odds ratio	95% Confidence interval	*P*-value	Odds ratio	95% Confidence interval	*P*-value
Staph. aureus	1.36	0.95-1.95	0.096	1.09	0.76-1.54	0.607
Coagulase-negative staphylococci	3.94	2.74-5.68	<0.0001	3.40	2.48-4.67	<0.0001
Coliform	1.20	0.62-2.29	0.59	1.40	0.76-2.58	0.273

cows had SCC < 400,000 cells/mL. This variability in SCC level in *Staph. aureus*-infected cows is well known. A Washington State study indicated that this variability is not due to pathogenicity of different *Staph. aureus* strains but more likely due to the mammary gland's response to IMI, possibly resulting in intermittent shedding.[19,31] Several management strategies may be used to decrease the prevalence of *Staph. aureus* IMI, which should result in lower bulk tank SCC levels. The value of segregation or use of separate milking units to reduce prevalence of *Staph. aureus* IMI and bulk tank SCC has been demonstrated.[38] A 1987 study demonstrated the positive benefits and costs associated with a successful *Staph. aureus* control program, resulting in a decrease of bulk tank SCC to < 400,000 cells/mL within four months of initiation of the program.[14] Dairy herds with persistently high bulk tank SCC should first consider culturing the herd to confirm *Staph. aureus* presence and prevalence within the herd, and then follow with control measures that have been demonstrated to be successful.

While finding that *Staph. aureus* was the most prevalent pathogen among high SCC herds was not surprising, some high SCC herds (three of 20) had a relatively low prevalence (< 7%) of *Staph. aureus.* In these herds, ES were most frequently associated with high SCC cows. Of ES IMI in this study, > 30% were associated with SCC > 1 million cells/mL and nearly 60% were associated with SCC > 400,000 cells/mL. In six of 20 herds, ES IMI were more commonly associated with SCC > 400,000 cells/mL than *Staph. aureus* IMI, and in four herds they were found

in equal proportions. In five of 20 herds, ES IMI was more commonly associated with SCC > 1 million cells/mL than *Staph. aureus* IMI, and in three herds they were found in equal proportions. Environmental streptococci may result in chronic IMI, similar to *Staph. aureus*, with an infection duration of up to nine months.[34] Control of ES is especially problematic. Historically, the ES were assumed to be transmitted via environmental sources.[18] However, there is new evidence that cow-to-cow transmission may occur.[7,39] Environmental cleanliness and milking time hygiene may not be sufficient to control ES, as New Zealand studies have shown that *Strep. uberis* is the primary pathogen of cows and heifers on pasture.[5,18] Most of the dairies in Tennessee pasture their cows and over 50% of the ES isolated in this study were *Strep. uberis*. A 2001 study suggested focusing on reducing new ES IMI during the dry period and identifying cows with persistent ES IMI during lactation for appropriate intervention.[22]

While coliform IMI was associated with high SCC cows, they were not significantly associated with a high herd SCC, probably because the prevalence was low (mean 1.7%, range 0-4.9%). This finding is consistent with findings from another study in which the prevalence of *E. coli* was not significantly associated with bulk tank milk SCC.[39] Chronic cases of *E. coli* have received press in recent years, but persistent infections tend to be the exception rather than the rule as < 5% of *E. coli* cases have been reported as chronic.[10] This low prevalence of chronic *E. coli* IMI may be increasing as a 2001 study found approximately 20% of clinical *E. coli* cases to be of the same genotype.[4] Authors of two recent studies have found differences in *E. coli* strains that result in chronic IMI, versus those strains that do not.[9,25] *Klebsiella* species are also known to result in chronic IMI.[40] Thus, while *E. coli* and other coliforms were not a significant reason for high SCC Tennessee herds, they may evolve into significant causes of high SCC in the future.

The CNS were among the most common IMI in each herd, yet CNS were not significantly associated with high SCC cows. Data in Table 2 are consistent with mastitis dogma that CNS and corynebacterium are minor mastitis pathogens. However, both CNS and corynebacterium have occasionally been reported to be associated with high SCC in individual cows, but would not be expected to be common reasons for a herd's high SCC.[6,21] The geometric mean SCC in quarters infected with *C. bovis* varied between 40 and 421 x 10^3/mL of milk in a meta-analysis study.[8]

Approximately 84% of milk samples from cows with a SCC of < 400,000 cells/mL were culture-negative, which is somewhat consistent with another study that found nearly 60% of quarters with a SCC < 260,000 cells/mL to be culture-negative.[16] Seven percent of cows with culture-negative results had an SCC > 1 million cells/mL. Reasons for no growth in high SCC cow milk cultures are a previously cured IMI, recent treatment with antibiotics, intermittent shedding, low concentration of the pathogen in the milk sample, pathogens that do not grow on standard culture media, and prolonged freezing. Even when microbial cures occur, in most cases, California mastitis test scores tend to remain elevated for an extended period of time.[29] It has been suggested that some of the no growth cultures from cows with clinical mastitis are due to *E. coli*, yet two separate studies found only a small percentage of clinical mastitis with no growth to be due to *E. coli*.[23,32,37] Because of the intermittent shedding nature of *Staph. aureus,* it is likely that some of the high SCC cows with no growth results were actually infected with *Staph. aureus*.[31] One theory that proved to be true for a few cows in one herd in this study was mycoplasma IMI. In this herd, only one cow of nine and four cows of 21 cultured *Staph. aureus* or ES; > 50% of cultures at these high SCC levels were no growth. Thus, mycoplasma mastitis should be strongly considered when more common bacteria associated with high SCC are infrequent.

Conclusions

The sustainability of the dairy industry in Tennessee is in jeopardy, as the number of dairy herds and number of dairy cows continue to decrease.[36] While milk production has increased, the average pounds of milk per cow per year for Tennessee (< 16,000 lb or 7,270 kg) is well below the national average (> 20,000 lb or 9,090 kg of milk/cow/year). Chronic mastitis, such as that caused by *Staph. aureus*, damages milk secretory cells resulting in decreased milk production. If Tennessee dairy herds are to survive and other high SCC herds in other states and countries, the high SCC needs to be addressed. Findings of this study strongly suggest that *Staph. aureus* should be considered first, and ES second, to help reduce herd SCC and thus help improve milk quality on dairy farms with high SCC.

Endnotes

[a]Laboratory Handbook on Bovine Mastitis, Revised Edition 1999, National Mastitis Council, Inc. Madison, WI
[b]bioMerieux Vitek, Inc., Hazelwood, MO
[c]SAS version 9.1.3, SAS Institute, Cary, NC

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