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# Results of Milk Samples Submitted for Microbiological Examination in Wisconsin from 1994 to 2001

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## ABSTRACT

The objective of this study was to examine the characteristics of milk samples submitted for microbiological examination at the Wisconsin Veterinary Diagnostic Laboratory between 1994 and 2001. Results (n = 83,650) of microbiological testing of milk samples (n = 77,172) submitted to the Wisconsin Veterinary Diagnostic Laboratory from January 1994 until June 2001 were analyzed. Submissions included milk samples obtained from cases of clinical and subclinical mastitis as well as samples obtained for mastitis surveillance programs. Results were recorded as no growth, contaminated, or identified as specific bacterial pathogens. Statistical analysis was performed to determine trends in the isolation of mastitis pathogens. The proportion of samples identified as contaminated decreased from 20.6 (1997) to 9.5% (2001). The proportion of samples coded as no growth increased from 22.6 (1994) to 49.7% (2001). Isolation of *Staphylococcus aureus* decreased from 17.7% (1994) of isolates to 9.7% (2001), while isolation of *Streptococcus agalactiae* decreased from 8.1 (1994) to 3.0% (2001). Coagulase-negative *Staphylococcus* spp. were isolated from 12.7 to 17.5%, environmental *Streptococcus* spp. were isolated from 11.6 to 20.1%, and *Escherichia coli* were isolated from 3.1 to 6.7% of all isolates. No growth and contaminated samples comprised almost 50% of total submissions, and it is important that producers have proper expectations when submitting milk samples. The proportion of isolates identified as *Staph. aureus* and *Strep. agalactiae* decreased, suggesting the proportion of contagious bacteria causing mastitis has decreased. Environmental and contagious pathogens demonstrated characteristic differences by season.
(**Key words:** dairy cow, mastitis, milk quality, milk sample)

## INTRODUCTION

Mastitis is a costly disease of dairy cattle resulting in reductions in milk yield and milk quality. Losses attributed to mastitis include reduced milk yield, milk discard, premature culling, treatment costs, and increased labor (Fetrow, 2000). Bacterial pathogens that cause mastitis are generally classified as either contagious or environmental based upon their primary reservoir and mode of transmission.

The primary reservoir of contagious mastitis pathogens is the udder of the cow, and they are commonly transmitted among cows by contact with infected milk. The most important contagious mastitis pathogens include *Streptococcus agalactiae*, *Staphylococcus aureus*, *Corynebacterium bovis*, and *Mycoplasma* spp. *Staphylococcus aureus* is usually considered the most common contagious pathogen and has been reported to infect 7 to 40% of all cows (Fox and Gay, 1993). Historically, *Strep. agalactiae* was the most common contagious mastitis pathogen, but successful control efforts have reduced its prevalence. The use of dry cow therapy, postmilking teat disinfectants, and effective premilking hygiene are effective control procedures for most contagious mastitis pathogens.

Exposure to environmental mastitis pathogens may occur continuously because the primary route of exposure is contact with moisture, mud, and manure. Unlike mastitis caused by contagious pathogens, mastitis caused by environmental pathogens cannot be eradicated from a dairy herd (Smith and Hogan, 1993). The most important environmental mastitis pathogens include gram-negative bacteria (such as *E. coli* and *Klebsiella* spp.) and *Streptococcus* spp. (such as *Strep. uberis* and *Strep. dysgalactia*). Mastitis caused by environmental pathogens can be controlled by reducing exposure and by increasing immune resistance of the cow.

Prevalence and seasonal trends of mastitis pathogens have been examined in a limited number of herds (Bishop et al., 1980; Oliver and Mitchell, 1984; Wilson et al., 1997; Myllys et al., 1998). A survey of a university dairy herd conducted from 1977 to 1979 concluded that mastitis cases were more prevalent in the summer and winter (compared with spring and fall) re-

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gardless of the cow's age or stage of lactation (Bishop et al., 1980). The prevalence of mastitis pathogens has been compared between herds that practiced mastitis control (1088 cows from 17 herds) and herds that did not (1105 cows from 17 herds) (Oliver and Mitchell, 1984). The study concluded that control measures were more effective for contagious mastitis pathogens as compared to environmental mastitis pathogens.

Results of quarter milk samples obtained from 4495 cows in 1988 and 2648 cows in 1995 were analyzed to assess the prevalence of mastitis in Finland (Myllys et al., 1998). The authors reported an overall decrease in the prevalence of mastitis in Finland during the study period. The proportion of coagulase-negative *Staphylococcus* spp. isolated from milk samples increased while the proportion of *Staph. aureus* decreased.

Milk samples collected from 108,312 dairy cows during 1601 farm visits that occurred between January 1991 and June 1995 were used to estimate the prevalence of mastitis in New York and Pennsylvania (Wilson et al., 1997). The overall prevalence of intramammary infection was 48.5%. *Staphylococcus* spp. (including *Staph. aureus*) and *Streptococcus* spp. (including *Strep. agalactiae*) were isolated from 38% of cows.

Milk samples are often submitted for microbiological examination as part of the diagnostic process for mastitis control programs. Information about the prevalence of specific mastitis pathogens is useful for implementation of preventive strategies. The objective of this study was to examine the results of milk samples obtained from dairy cows and submitted for microbiological examination at the Wisconsin Veterinary Diagnostic Laboratory between 1994 and 2001.

## MATERIALS AND METHODS

Records from all milk samples submitted to the Wisconsin Veterinary Diagnostic Laboratory (WVDL) for microbiological examination from January 1994 until June 2001 were retrieved. Test results were copied from original paper records into a computer spreadsheet for analysis.

Milk samples submitted to the laboratory were cultured using standard microbiologic methods. Briefly, 0.01 ml of milk was streaked on a portion of a blood agar plate, an eosin methylene blue agar plate (Becton-Dickson Microbiology, Franklin Lakes, NJ) and Thallium-Krystal Violet-Toxin plate (TKT; Becton-Dickson Microbiology, Franklin Lakes, NJ) and incubated at 35 to 37°C overnight in a $CO_2$ incubator. Plates were examined for growth at 24 and 48 h. Bacteria were identified by colony morphology and Gram stain. For gram-positive cocci, catalase tests were performed to distinguish catalase-negative *Streptococcus* spp. from catalase-positive *Staphylococcus* spp. The CAMP test and growth on bile esculin agar were used to differentiate *Strep. agalactiae* from other streptococci. Catalase-positive gram-positive cocci were further identified using a coagulase test, hemolysis patterns, and mannitol salt agar (Becton-Dickson Microbiology). Gram-positive bacilli were further identified using the catalase test and biochemical reactions as needed. Gram-negative bacilli were identified by the oxidase test, motility, indole and ornithine decarboxylase, and Simmons citrate. Contaminated samples were defined as a mixture of at least two environmental type organisms without isolation of a major mastitis pathogen.

### Statistical Analysis

Statistical analysis was performed for selected major mastitis pathogens. Chi-squared analysis (Proc FREQ; SAS, 1999) was performed to determine if the proportion of individual pathogens isolated was independent of year. Logistic regression (Proc LOGISTIC; SAS, 1999) was performed to evaluate the probability of isolation of bacteria as year increased. The logistic regression model for the proportion of bacteria isolated by year included isolation as a response variable and year as a continuous variable. Significance was evaluated at $P < 0.05$. Goodness of fit of the logistic regression model was evaluated at $P > 0.05$. Logistic regression (Proc LOGISTIC; SAS, 1999) was performed to identify seasonal differences in the isolation of specific mastitis pathogens. Data were analyzed in a model that included isolation of specified pathogen (*Staph. aureus*, *Staphylococcus* spp., *Strep. agalactiae*, *Streptococcus* spp., *E. coli*, *Klebsiella* spp., and *Corynebacterium bovis*) as the dependent variable. Independent variables included in the model were season (winter, spring, summer, fall), year, and season × year.

## RESULTS

Submitted milk samples (n = 77,172) were characterized as no growth (n = 25,655), contaminated (n = 12,792), or containing bacterial pathogens (n = 38,725). The total number of bacteriological results (n = 45,203) included mastitis pathogens isolated in pure culture (n = 31,926) and mastitis pathogens (n = 13,277) isolated from milk samples (n = 6799) that yielded multiple mastitis pathogens. The number of bacteriological results per year ranged from 6,301 to

**Table 1.** Number of results[1] of results of microbiological analysis of milk samples by year.

| Year | Number of results |
|---|---|
| 1994 | 8,668 |
| 1995 | 6,301 |
| 1996 | 10,428 |
| 1997 | 8,659 |
| 1998 | 8,981 |
| 1999 | 16,505 |
| 2000 | 14,129 |
| 2001[2] | 9,979 |
| Total | 83,650 |

[1]Results include samples coded as no growth, contaminated and mastitis pathogens.

[2] January through June only.

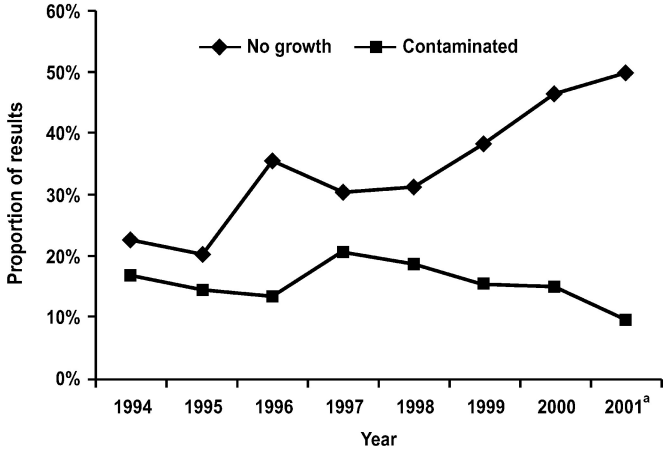


**Figure 1**. Proportion of results characterized as no growth or contaminated by year. [a]January to June only.

16,505 (Table 1). A large variety of bacterial species (n = 61) were isolated from milk samples, but only a few isolates of some species were found. The diagnostic usefulness of almost half of the milk samples submitted was limited because the samples were contaminated or no bacteria could be isolated from the sample (Table 2).

The proportion of samples that did not result in bacterial growth increased with time ($P < 0.001$), while the number of samples characterized as contaminated slightly decreased with time ($P < 0.001$) (Figure 1). There was no significant relationship between isolation of *E. coli* and *Klebsiella* spp. and year (Table 3). Isolation of other mastitis pathogens significantly decreased with year (Table 3). During the period of study, the proportion of results attributed to *Staph.* spp. decreased from 15.9 to 12.8%, *Streptococcus* spp. decreased from 19.3 to 11.6% and *C. bovis* decreased from 3.2 to 1.6%. Isolation of *Strep. agalactiae* and *Staph. aureus* decreased the most dramatically (Figure 2).

Season, year, and season × year ($P < 0.001$) significantly affected the proportion of samples characterized as no growth and contaminated (Table 4). Milk samples were less likely to be characterized as no growth in the winter compared with spring (odds ratio = 0.74; $P < 0.001$), summer (odds ratio = 0.89; $P < 0.001$) or fall (odds ratio = 0.93; $P = 0.004$). A larger proportion of samples were characterized as no growth in the spring as compared to summer (odds ratio = 1.2; $P < 0.001$) and fall (odds ratio = 1.3; $P = 0.004$). Milk samples were less likely to be characterized as contaminated in the winter as compared to summer (odds ratio = 0.84; $P < 0.001$) or fall (odds ratio = 0.82; $P < 0.001$) and in the spring as compared to summer (odds ratio = 0.80; $P < 0.001$) or fall (0.78; $P < 0.001$).

Season, year, and season × year significantly affected the proportion of *Staph. aureus* ($P < 0.004$), *Staphylococcus* spp. ($P < 0.02$), *Strep. agalactiae* ($P < 0.001$), and *Streptococcus* spp. ($P < 0.001$)(Table 4).

**Table 2.** Results of milk samples submitted from 1994 to 2001.

| Isolate | Number of isolates | % of results |
|---|---|---|
| *Staphylococcus aureus* | 8,114 | 9.70 |
| *Staphylococcus* spp.[1] | 11,062 | 13.22 |
| *Streptococcus agalactiae* | 3,483 | 4.16 |
| *Streptococcus* spp.[2] | 10,231 | 12.23 |
| *E. coli* | 3,377 | 4.04 |
| *Klebsiella* spp. | 1,001 | 1.20 |
| *C. bovis* | 2,281 | 2.73 |
| Other bacteria | 5,654 | 6.76 |
| No growth | 25,655 | 30.67 |
| Contaminated[3] | 12,792 | 15.29 |

[1]Coagulase-negative *Staphylococcus* spp.

[2]Not including *Strep agalactiae*.

[3]Defined as a mixture of at least 2 environmental type organisms without isolation of a major mastitis pathogen.

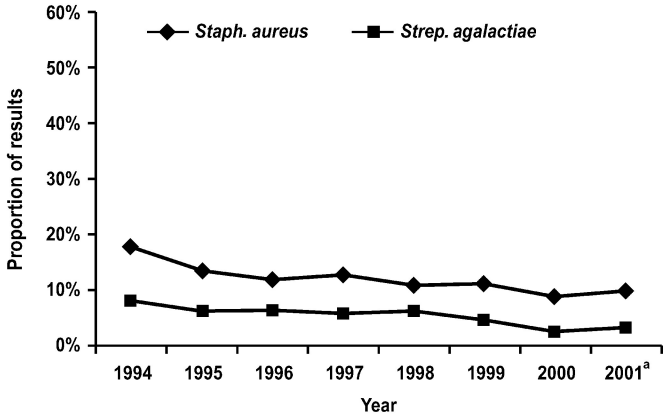


**Figure 2**. Proportion of results attributed to *Staphylococcus aureus* and *Streptococcus agalactiae* by year. [a]January to June only.

**Table 3.** Results of logistic regression model of proportion of isolates by year for bacterial pathogens.

| Bacterial pathogen | Odds ratio | 95% confidence interval | *P* Value | Pearson GOF[1] |
|---|---|---|---|---|
| *Staphylococcus aureus* | 0.91 | 0.90-0.92 | <0.0001 | <0.0001 |
| *Staphylococcus* spp. | 0.98 | 0.97-0.99 | <0.0001 | <0.0001 |
| *Streptococcus agalactiae* | 0.87 | 0.84-0.87 | <0.0001 | <0.0001 |
| *Streptococcus* spp. | 0.92 | 0.91-0.92 | <0.0001 | <0.0001 |
| *E. coli* | 1.00 | 0.98-1.01 | 0.8537 | <0.0001 |
| *Klebsiella* spp. | 0.98 | 0.95-1.01 | 0.1452 | 0.0001 |
| *C. bovis* | 0.93 | 0.92-0.95 | <0.0001 | <0.0001 |

[1]Goodness of fit.

*Staph. aureus* was less likely to be isolated in summer as compared to fall (odds ratio = 0.85; $P < 0.001$). *Staph. aureus* were more likely to be isolated in the winter as compared to spring (odds ratio = 1.3; $P < 0.001$), summer (odds ratio = 1.6; $P < 0.001$) or fall (odds ratio = 1.4; $P < 0.001$) and in the spring compared with summer (odds ratio = 1.3; $P < 0.001$) or fall (odds ratio = 1.1; $P = 0.011$). *Staphylococcus* spp. were less likely to be isolated in winter as compared to summer (odds ratio = 0.82; $P < 0.001$) or fall (0.90; $P = 0.002$) and in the spring as compared to summer (odds ratio = 0.86; $P < 0.001$). Isolation of *Staphylococcus* spp. was more likely in the summer as compared to fall (odds ratio = 1.1; $P = 0.005$).

**Table 4.** Proportion of results by season and year.

| Characteristic | Season | Overall % | Year (%) 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 |
|---|---|---|---|---|---|---|---|---|---|---|
| No Growth | Win. | 33.1[a] | 24.3 | 17.2 | 39.5 | 26.2 | 21.1 | 28.3 | 43.3 | 42.0 |
| | Spr. | 40.3[b] | 17.0 | 26.1 | 25.1 | 26.7 | 34.6 | 42.4 | 51.8 | 54.8 |
| | Sum. | 35.7[c] | 26.6 | 18.4 | 37.9 | 36.7 | 37.1 | 39.4 | 44.2 | |
| | Fall | 34.7[c] | 21.6 | 20.2 | 39.9 | 33.1 | 30.0 | 38.4 | 45.7 | |
| Contaminated | Win. | 14.5[a] | 16.6 | 13.9 | 10.8 | 25.3 | 19.3 | 15.4 | 10.7 | 10.1 |
| | Spr. | 13.9[a] | 23.7 | 11.7 | 16.2 | 12.5 | 16.9 | 13.4 | 14.2 | 09.1 |
| | Sum. | 16.8[b] | 13.0 | 13.9 | 13.6 | 23.9 | 15.2 | 16.5 | 21.0 | |
| | Fall | 17.2[b] | 10.2 | 17.9 | 12.0 | 20.8 | 24.1 | 16.9 | 17.1 | |
| *Staphylococcus aureus* | Win. | 14.0[a] | 20.4 | 18.0 | 13.3 | 16.2 | 14.6 | 15.4 | 10.1 | 10.5 |
| | Spr. | 11.4[b] | 21.0 | 12.9 | 18.5 | 12.8 | 09.6 | 10.0 | 06.7 | 09.1 |
| | Sum. | 09.1[c] | 13.3 | 09.8 | 06.2 | 08.7 | 07.4 | 09.8 | 09.4 | |
| | Fall | 10.5[c] | 13.4 | 13.8 | 08.4 | 11.6 | 11.3 | 10.0 | 07.7 | |
| *Staphylococcus* spp.[b] | Win. | 14.6[a] | 13.6 | 16.7 | 10.7 | 51.6 | 15.6 | 15.3 | 16.1 | 13.8 |
| | Spr. | 15.2[ab] | 16.2 | 12.9 | 15.9 | 17.8 | 16.2 | 18.0 | 14.3 | 12.1 |
| | Sum. | 17.2[c] | 17.0 | 19.7 | 18.7 | 18.3 | 17.8 | 16.1 | 14.1 | |
| | Fall | 15.9[b] | 18.7 | 14.9 | 13.0 | 18.6 | 15.9 | 16.5 | 14.8 | |
| *Streptococcus agalactia* | Win. | 06.2[a] | 10.6 | 06.0 | 07.2 | 09.4 | 08.9 | 05.6 | 03.8 | 02.5 |
| | Spr. | 03.7[b] | 06.0 | 07.0 | 03.6 | 04.2 | 06.3 | 03.3 | 01.2 | 03.3 |
| | Sum. | 04.8[c] | 06.9 | 05.4 | 05.7 | 04.2 | 05.4 | 04.5 | 02.0 | |
| | Fall | 05.2[c] | 08.2 | 06.4 | 08.7 | 05.5 | 03.2 | 04.7 | 01.9 | |
| *Streptococcus* spp. | Win. | 17.1[a] | 20.6 | 23.6 | 16.0 | 18.6 | 18.7 | 28.8 | 13.1 | 15.7 |
| | Spr. | 13.9[b] | 20.8 | 22.2 | 17.9 | 17.7 | 13.9 | 09.1 | 08.7 | 08.8 |
| | Sum. | 12.6[c] | 15.5 | 17.9 | 11.6 | 12.1 | 11.6 | 12.6 | 10.9 | |
| | Fall | 13.4[bc] | 20.1 | 16.6 | 09.5 | 13.7 | 14.6 | 12.7 | 07.3 | |
| *E. coli* | Win. | 03.5[a] | 02.6 | 04.1 | 02.1 | 03.2 | 04.1 | 04.0 | 03.9 | 04.0 |
| | Spr. | 04.2[b] | 04.0 | 06.1 | 02.6 | 05.9 | 06.5 | 03.6 | 04.9 | 03.2 |
| | Sum. | 06.9[c] | 07.5 | 07.5 | 04.2 | 08.4 | 08.3 | 05.1 | 09.5 | |
| | Fall | 05.0[d] | 03.6 | 05.4 | 03.5 | 05.2 | 08.0 | 03.8 | 06.6 | |
| *Klebsiella* spp. | Win. | 01.2[a] | 01.3 | 01.3 | 00.9 | 00.6 | 01.5 | 01.4 | 00.9 | 01.5 |
| | Spr. | 01.3[ac] | 01.5 | 01.9 | 01.5 | 01.0 | 01.5 | 01.3 | 01.5 | 01.1 |
| | Sum. | 01.8[b] | 01.6 | 01.9 | 01.3 | 02.0 | 01.9 | 01.3 | 02.8 | |
| | Fall | 01.5[c] | 02.7 | 01.8 | 01.0 | 00.7 | 02.7 | 01.0 | 01.5 | |
| *C. bovis* | Win. | 02.7[a] | 02.0 | 03.3 | 02.1 | 02.5 | 03.0 | 05.0 | 02.7 | 01.7 |
| | Spr. | 02.4[b] | 03.6 | 00.9 | 03.6 | 03.8 | 02.2 | 02.5 | 01.9 | 01.5 |
| | Sum. | 03.9[c] | 04.2 | 03.9 | 06.8 | 02.1 | 02.5 | 05.3 | 01.1 | |
| | Fall | 04.8[d] | 03.3 | 05.9 | 06.8 | 01.8 | 04.0 | 06.7 | 01.9 | |

[a,b,c,d]Overall seasonal values for each characteristic without common superscript differ significantly ($P < 0.05$).

Isolation of *Strep. agalactiae* was less likely in the spring as compared to summer (odds ratio = 0.77; $P < 0.001$) or fall (odds ratio = 0.70; $P < 0.001$) (Table 4). *Streptococcus agalactiae* were more likely to be isolated in the winters compared with spring (odds ratio = 1.7; $P < 0.001$), summer (odds ratio = 1.3; $P < 0.001$) or fall (odds ratio = 1.2; $P < 0.001$). *Streptococcus* spp. were more likely to be isolated in the winter compared with spring (odds ratio = 1.3; $P < 0.001$), summer (odds ratio = 1.4; $P < 0.001$) or fall (odds ratio = 1.3, $P < 0.001$) and in the spring compared with summer (odds ratio = 1.1; $P < 0.001$).

Season, year, and season × year significantly affected the proportion of *E. coli* ($P < 0.001$) and *C. bovis* ($P < 0.0002$) (Table 4). *Escherichia coli* was less likely to be isolated in the winter compared with spring (odds ratio = 0.83; $P < 0.001$), summer (odds ratio = 0.50; $P < 0.001$) or fall (odds ratio = 0.70; $P < 0.001$) and in the spring compared with summer (odds ratio = 0.60; $P < 0.001$) or fall (odds ratio = 0.85; $P = 0.002$). Isolation of *E. coli* was more likely in the summer compared with fall (odds ratio = 1.4; $P < 0.001$). Season and season × year significantly affected the proportion of *Klebsiella* spp. ($P < 0.001$) (Table 4). *Klebsiella* spp. were less likely to be isolated in winter compared with summer (odds ratio = 0.67; $P < 0.001$) or fall (0.78; $P = 0.014$) and in the spring compared with summer (odds ratio = 0.75; $P < 0.001$). *Corynebacterium bovis* were less likely in the winter compared with summer (odds ratio = 0.68; $P < 0.001$) or fall (odds ratio = 0.56; $P < 0.001$) and in the spring compared with summer (odds ratios = 0.60; $P < 0.001$) or fall (odds ratio = 0.49; $P < 0.001$) and in the summer compared with fall (odds ratio = 0.82; $P < 0.001$). Isolation of *C. bovis* was more likely in the winter compared with spring (odds ratio = 1.1; $P = 0.042$).

## DISCUSSION

Our study examined trends in isolation of major mastitis pathogens. Trends in the outcomes of the susceptibility tests performed on some of the samples have been previously reported (Makovec and Ruegg, 2003). We had a large dataset of milk samples submitted by veterinarians to a central laboratory. The use of data from a central laboratory eliminated potential bias that could be attributed to differences in laboratory procedures. Laboratory procedures remained consistent throughout the period of study. Results of all milk samples submitted to the diagnostic laboratory during our period of interest were included in this study. The origin of the milk samples included clinical and subclinical cases of mastitis and samples submitted for surveillance purposes and whole herd screening. The origin of the sample was not recorded for many records, but it is unlikely that there were large differences in the origin of samples submitted each year. Management information about the farms submitting cultures was not available. The lack of this information may have affected the significance of the logistic regression models because policies for milk culturing probably differed among farms. The analysis was conducted to identify trends in the outcomes of microbiological testing of milk samples, and there was no attempt to adjust the analysis for potential dependence between isolations of milk samples that were determined to contain multiple pathogens.

Significant goodness-of-fit values were identified for several pathogens indicating that factors other than year are influencing the isolation of bacterial pathogens. The nature of our data did not allow us to account for these factors because information about the farms that submitted the milk samples was not available. Factors influencing submission of milk samples for culture include milk price and individual farm policies. Our model identified changes in the proportion of selected pathogens isolated each year, but numerous other management factors would need to be considered to create a model that completely describes the data.

Our study was able to examine trends in isolation of mastitis pathogens over a 6.5-yr period. The prevalence of mastitis was compared in Finland between 1988 and 1995 by analyzing quarter samples from over 7000 cows (Myllys et al., 1998). Different trends were identified for various pathogens. The proportion of *Staph. aureus* isolated from milk samples decreased from 31.0 (1988) to 16.7% (1995). The authors reported similar results for *Strep. agalactiae*, which decreased from 4.7% (1988) of bacterial isolates to 0.55% (1995). The prevalence of mastitis pathogens in New York and Pennsylvania was evaluated from milk samples collected from 108,312 dairy cows (Wilson et al., 1997). *Staphylococcus aureus* and *Strep. agalactiae* were isolated from 9.1 and 10.1% of cows, respectively. The prevalence of isolation of *Staph. aureus* in our study was similar to previous reports but the prevalence of *Strep. agalactiae* was less than the prevalence previously reported in New York and Pennsylvania (Wilson et al., 1997). In that study, samples were obtained by trained personnel during herd visits performed to monitor mastitis. The greater prevalence of *Strep. agalactiae* found in that study may be attributed to the submission of a larger number of samples obtained from problem herds as compared to our study because 30% of herd visits were initiated in response to bulk milk SCC >750,000 cells/ml (Wilson et al., 1997). The proportion of *Staph. aureus* in our study decreased

from 17.7 (1994) to 9.7% (2001), and the proportion of *Strep. agalactiae* decreased from 8.1 (1994) to 3.0% (2001). These results were similar to the decreasing trends reported in Finland (Myllys et al., 1998).

Previous studies have recovered gram-negative bacteria from <2.0% of isolates (Wilson et al., 1997; Myllys et al., 1998). Our results were similar because a small proportion of gram-negative organisms were recovered from milk samples, and there was no significant relationship between isolation of *E. coli* and *Klebsiella* spp. and year. The immune system of the dairy cow is often capable of mounting an effective response to intramammary infections caused by gram-negative bacteria (Erskine, 2001). As a result, the natural duration of mastitis infections caused by gram-negative pathogens tends to be shorter than the duration of mastitis caused by gram-positive pathogens resulting a lower probability of recovery of these organisms (Erskine, 2001). The increased proportion of samples that resulted in no growth may reflect an increase in submission of samples obtained from cows experiencing mastitis caused by gram-negative pathogens.

The proportion of samples resulting in no growth was less than reported by others (51.5% in New York/Pennsylvania and 83.5% of samples in Finland) but increased dramatically between 1994 and 2001 (Wilson et al., 1997; Myllys et al., 1998). Some of the samples characterized as no growth may be attributed to *Mycoplasma* spp., which has specific growth requirements and was only tested on selected samples. Another possibility may be attributed to the decrease in *Strep. agalactiae*; *Strep. agalactiae* are easily cultured from milk samples, while other pathogens such as *Staph. aureus* are more difficult to culture due to intermittent shedding. As the prevalence of *Strep. agalactiae* has decreased, the probability of isolations may have also decreased. Samples were less likely to be characterized as no growth in the winter compared with all other seasons. Samples were more likely to be characterized as no growth in the spring compared with summer and fall. This could be a result of seasonal differences in pathogen type or severity of infections.

Similar to other surveys, *Staphylococcus* spp. were the most common bacteria isolated from milk samples. Isolation of gram-positive pathogens decreased significantly with year. The decreased prevalence of gram-positive pathogens may be attributed to successful implementation of control procedures or perhaps to the underlying changes in the demographics of herds submitting samples. In the period between 1994 and 2000, the number of dairy herds in Wisconsin declined from 29,000 to 21,000 (WI Dept. of Agriculture, 2001). It is possible that herds remaining in the dairy industry have been more successful in controlling contagious mastitis compared with herds that have exited the industry.

The proportion of contaminated samples was much higher in our study compared with the 3.0% and <1% rate of contaminated samples previously reported by Wilson et al. (1997) and Myllys et al. (1998), respectively. In both of the previous studies, trained technicians collected the samples. Information about collection procedures was not available for our data. In our study, it is likely that both veterinarians and farm personnel collected the samples that were submitted to the laboratory. The consistent decrease in the proportion of contaminated samples may indicate that persons submitting samples are becoming more aware of proper collection procedures.

Isolation of contagious mastitis pathogens was more likely in the winter compared with other seasons. Many cows in Wisconsin are housed in stall barns during the winter, which may increase transmission of contagious pathogens. It is also possible that farmers cultured more cows with subclinical infections during the winter (compared with other seasons) because they had more time to focus on mastitis control rather than performing fieldwork. Contagious mastitis pathogens are generally persistent, and infections acquired in the winter may persist into the spring. This may explain the increased prevalence of contagious mastitis pathogens in the winter and spring.

The odds of isolating *Streptococcus* spp. and *Staphylococcus* spp. varied by season. Seasonal trends in the isolation of environmental *Streptococcus* spp. have been inconsistent (Smith and Hogan, 1993; Bishop et al., 1980). In our study, isolation of *Streptococcus* spp. was more likely in the winter while isolation of *Staphylococcus* spp. was less likely. Similar to others, isolation of *E. coli* and *Klebsiella* spp. was more likely in the summer (Smith and Hogan, 1993; Bishop et al., 1980). Favorable conditions for the growth of gram-negative bacteria (hot and humid weather) probably result in increased exposure and increased infection with these pathogens.

## CONCLUSION

Significant differences in the proportion of bacteria isolated from milk samples by year and by season were identified. The proportion of samples characterized as no growth and contaminated comprised almost 50% of total submissions, and it is important that producers have realistic expectations when submitting milk samples. There was no association between year and isolation of coliform bacteria. The proportion of all other isolates significantly decreased by year, and the

contagious bacteria, *Staph. aureus* and *Strep. agalactiae*, demonstrated the most dramatic decreases. Environmental and contagious mastitis pathogens demonstrated characteristic differences by season.

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