PHYSIOLOGY AND MANAGEMENT

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

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ABSTRACT

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.

( **Key words:** *Streptococcus agalactiae*, prevalence, incidence, mastitis)

**Abbreviation key:** BTSCC = bulk tank SCC, GBS = group B streptococci, SPC = standard plate count.

INTRODUCTION

*Streptococcus agalactiae* is a highly contagious obligate parasite of the bovine mammary gland (20). Contagious mastitis pathogens such as *S. agalactiae* cause a low grade, persistent infection that generally does not have a high self-cure rate (11). Cattle that are unidentified as infected function as reservoirs of infection because they are not selected for treatment, segregation, or culling (31). Management programs for subclinical mastitis in general, and *S. agalactiae* in particular, are effective to control infections throughout the herd. The economics of such programs are generally very favorable when response is measured by changes in the incidence of clinical disease, the prevalence of herd infection, or by SCC, which is a crude measure of prevalence.

*Streptococcus agalactiae*, a Gram-positive streptococcus, grows in chains in milk and in liquid media. Cultures of milk samples that are found to be positive for CAMP factor and negative for esculin hydrolysis are commonly identified as *S. agalactiae* (33). Another feature of *S. agalactiae*, exploited for identification purposes, is its ability to produce pigmented colonies when grown anaerobically on media that contain starch (26).

Numerous studies have been conducted to determine herd prevalence of *S. agalactiae*. Census data from Mississippi (28) in 1982 (n = 998), Vermont (16) in 1985 (n = 2931), and Vermont (13) again in 1990 (n = 1971) indicated that prevalence rates for *S. agalactiae* were 44, 47, and 32%, respectively, based on the culture of milk from bulk tanks. In a random sample of southwestern Ontario herds (n = 250) in 1990, 42.4% of herds had milk that tested positive for *S. agalactiae* in at least one of four cultures of bulk tank milk (12). In a study of 50 herds in California (14), 44% were found to be infected based on cultures from bulk tank milk. In a stratified random sample of herds in Ohio in 1989 (2) (n = 49) and in 1992 (1) (n = 48) using milk samples from individual cows, 32 and 56% of herds had at least one positive culture, respectively. In contrast, in another study (30) in that same state, using cultures from the bulk tank milk from herds that sold milk to cooperatives that gave premiums for low SCC (n = 802), the prevalence of *S. agalactiae* was 5%. The
much lower percentage of infection in this study suggests that the problem was controlled at the herd level. Herd prevalence of infection with *S. agalactiae* in Prince Edward Island was not known previously. None of the previously reported studies included herd incidence rates (i.e., the frequency of new herd infections), which are essential for understanding the dynamics of herd infection.

The objectives of the present study were to determine the prevalence and incidence of *S. agalactiae* infection in dairy herds of Prince Edward Island, Canada; the effect of infection on milk quality; and the economic consequences to the dairy industry of the province. Prevalence and incidence data were determined from cultures of milk from the bulk tank of all herds that shipped milk in the province twice during the study period. Milk quality information was retrieved from the database of the Animal Productivity and Health Information Network (6) at the Atlantic Veterinary College, University of Prince Edward Island (Charlottetown, PE, Canada).

**MATERIALS AND METHODS**

**Study Population**

The dairy industry of Prince Edward Island consists of approximately 460 farms. Most of the dairy cows are registered or grade Holsteins. The Animal Productivity and Health Information Network at the Atlantic Veterinary College collects and collates information on health and production in the dairy industry of Prince Edward Island (6). From this database, the mean herd size of the 233 herds that participated in milk recording programs of the Atlantic Dairy Livestock Improvement Corporation in 1993 was determined to be approximately 36 lactating cows, and the mean milk production of this group was determined to be 23.1 L/d per cow. The mean bulk tank SCC (BTSCC) for all herds in the province in 1993 was 290 × 10³ cells/ml.

**Sampling Schedule and Handling**

For the first census (December 1992), two complete sets of bulk tank milk samples from all herds that shipped milk in the province were obtained between December 7, 1992 and January 4, 1993. For the second census (June 1994), two complete sets of bulk tank milk samples were obtained between June 13 and 20. The culture results of each of these samplings were used to establish the prevalence of infection. Results of bacteriological cultures on two media for each sample were interpreted in parallel. A herd was considered to be positive when *S. agalactiae* was isolated from either or both samples (19). The incidence of new herd infections was established using the census data from December 1992 as the base and cultures from June 1994 to identify new herd infections.

Bulk tank milk samples were collected and immediately refrigerated for delivery to the milk quality laboratory by milk transport personnel. For the December 1992 census, the majority of these samples were cultured within 24 h of arrival at the laboratory or 24 to 48 h after removal from the bulk tank. A few samples were older than 48 h at plating, but all were less than 96 h. For the June 1994 census, milk samples were only obtained during a brief period, and, as a result, samples for this census were frozen for storage prior to use. Most previous studies (5, 24, 27) have found no changes in either numbers of *S. agalactiae* bacteria per sample or classification of infection status when frozen samples were used instead of fresh samples. However, one study (31) indicated that, for samples from individual cows, the number of cows classified as infected increased when frozen samples were used. All samples were frozen within 24 h of arrival at the milk quality laboratory and were held at −20°C for a period not exceeding 10 d before being thawed at room temperature (20°C) and plated.

**Culture Methods**

The present study employed two media, a modified Edwards medium and group B streptococci (GBS) medium (Islam) (Oxoid Canada, Inc., Nepean, ON, Canada). Commercial modified Edwards medium base was further modified by the addition of ferric citrate (0.05 g/L), ovine blood (50 ml/L), and a crude staphylococcal β-hemolysin. The staphylococcal β-hemolysin was not commercially available and was prepared in the laboratory using the method described by Ward and Postle (32). The amount of the β-hemolysin extract added to the medium was determined by titrations with each new blood lot used for medium preparation. The pH was adjusted to 7.65 using 1 M NaOH. The pH of the solid agar had to be above 7.4 to allow maximum development of the CAMP reaction. The medium was stored at 4°C and used within 1 wk.

The second medium, GBS (Islam), was modified by the addition of sterile inactivated horse serum (50 ml/L of Oxoid® horse serum (Oxoid Canada, Inc.) and gentamicin (5 mg/L). The pH was adjusted to 7.6 with 1 M NaOH. The pH of the solid agar had to be 7.5 ± 0.1 to allow for good expression of pigment. A 150-μl aliquot of a well-mixed milk sample was dispensed by pipette onto the modified Edwards and modified GBS media. The inoculum was spread
TABLE 1. Five estimates of the sensitivity of individual protocols for bulk tank milk culture using modified Edwards and group B streptococci media.  

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<td>Positive herds, no.</td>
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<td>52</td>
<td>26</td>
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<td>Positive series, no.</td>
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<td>40</td>
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<td>Estimate of sensitivity</td>
<td>0.78</td>
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1Both media from Oxoid Canada, Inc. (Nepean, ON, Canada).
2Forty of 42 herds that had conflicting results between cultures 1 and 2 in December 1992 were recultured.

The sensitivity of the culture procedures used in this study has previously been reported to be 95% compared with results using cultures from individual cows (26). Although milk samples from individual cows were not cultured in this study, estimating the sensitivity of the culture procedure was possible using data for paired samples that had been generated during the December 1992 census and the June 1994 census and using a special culture of herds that had conflicting results in the December census. A herd was considered to be positive when *S. agalactiae* was cultured from one or both samples from the bulk tank milk. The specificity of the test was assumed to be 100% because of the obligate nature of the infection and because of the high specificity reported for the confirmatory latex agglutination test. Bartlett et al. (2) had previously made this assumption also.

Five separate estimates of sensitivity were made and are summarized in Table 1. Estimates ranged from 65 to 78%. If two independent tests are interpreted in parallel (i.e., a herd is considered to be positive when either test result is positive), the combined sensitivity of the test procedure is \( \alpha_1 + \alpha_2 - (\alpha_1 \alpha_2) \), where \( \alpha_1 \) and \( \alpha_2 \) are the sensitivities of the two tests. Based on this formula, the sensitivity of a testing program that consisted of cultures of two separate bulk tank samples would be 91%. However, this formula assumes that the two tests are independent. This assumption was tested by comparing the observed agreement between pairs of samples to that which would be expected because of chance. The expected agreement attributable to chance alone was calculated using the formula \( (\alpha_1 \times \alpha_2) + (1 - \alpha_1) \times (1 - \alpha_2) \). Because the observed agreement was actually less than that expected by chance, the two tests were assumed to be independent, and the testing program was estimated to have a sensitivity of 91%.

**RESULTS**

**Herd Prevalence of *S. agalactiae***

The apparent herd prevalence of *S. agalactiae* from the December 1992 data was 17.1%. Seventy-seven of 452 herds with culture results from each of the two samplings were positive on one or both cultures. True prevalence \( p \) can be calculated (25) from the apparent prevalence \( t \) and test characteristics [sensitivity \( (\alpha) \) and specificity \( (\beta) \)]. The formula is \( p = (t + \beta - 1)/(\alpha + \beta - 1) \). The estimated sensitivity of the testing procedure was 91%, and the specificity was assumed to be 100%. Therefore, the true herd prevalence in December 1992 was estimated to be 18.9%.
Prevalence was also calculated from the June 1994 data. Fifty-seven of the 434 herds that were tested had at least one positive culture, resulting in an apparent prevalence of 13.1% and an estimated true prevalence of 14.4%.

Herd Incidence of \textit{S. agalactiae}

Four hundred ten herds had two bulk tank milk cultures from both the December 1992 and June 1994 censuses. Three hundred forty-two of these herds tested negative in 1992 and were at risk of becoming infected during the 1.5 yr between December 1992 and June 1994. During this period (513 herd-years of risk), 18 herds became infected, and the incidence of new herd infections with \textit{S. agalactiae} was 0.0351 or 3.51 new herd infections per 100 herds per year.

Association of Infections with \textit{S. agalactiae} and BTSCC

The 5-mo mean BTSCC (computed using the BTSCC 2 mo prior to the culture, the BTSCC of the month of the culture, and the BTSCC 2 mo subsequent to the culture) of herds that tested positive for \textit{S. agalactiae} and herds that tested negative for \textit{S. agalactiae} were compared using the two-sample \textit{t} test procedure and the Mann-Whitney rank sum test. The log transformation of the BTSCC data was necessary prior to use of the \textit{t} test because the raw SCC were not normally distributed. Results shown in Table 2 indicate that BTSCC were higher in herds that tested positive for \textit{S. agalactiae} than in herds that tested negative for the bacteria.

Association with Milk Quality Penalties

On Prince Edward Island, penalties are assessed to producers using the following criteria for milk quality: BTSCC, standard plate count (SPC), and antibiotic residue testing. Persistent violations for either BTSCC or SPC result in the loss of ability to ship milk for fluid consumption. Penalties for BTSCC are imposed when counts exceed $500 \times 10^3$ or $750 \times 10^3$ cells/ml for 2 consecutive mo for fluid and industrial milk producers, respectively. Between October 1992 and March 1993, 35 BTSCC penalties were assigned to 16 of the 77 herds that were positive for \textit{S. agalactiae}, and 30 BTSCC penalties were assigned to 20 of the 375 herds that were negative for \textit{S. agalactiae}. Infected herds had more penalties (Mann-Whitney rank sum test, $P < 0.05$) and were 3.90 times as likely to be penalized (Taylor series 95% CI of 2.12 was less than the relative risk, which was less than 7.17) as were uninfected herds.

Penalties are imposed when SPC exceed $50 \times 10^3$ or $200 \times 10^3$ cfu/ml twice in a 2-wk period for fluid and industrial milk producers, respectively. Between October 1992 and March 1993, 15 penalties were assigned to 9 of the 77 herds that tested positive for \textit{S. agalactiae}, and 11 penalties were assigned to 8 of the 375 herds that tested negative for the bacteria. Infected herds were 5.48 times as likely to be penalized (Taylor series 95% CI of 2.18 was less than the relative risk, which was less than 13.75) as uninfected herds.

Seven of 32 herds that tested positive for \textit{S. agalactiae} had persistent penalties and lost the ability to ship milk for fluid consumption because of low standards. Similarly, 7 of 249 uninfected herds had persistent penalties and lost the ability to ship milk for fluid consumption. Infected herds were 7.78 times as likely to lose the ability to ship fluid milk (Taylor series 95% CI of 2.92 was less than the relative risk, which was less than 20.75) as uninfected herds.

Nine inhibitor violations (detection of antimicrobial residues) occurred in 8 dairy herds between November 1992 and April 1993. Two herds infected with \textit{S. agalactiae} (2.6%) had a total of three violations, and 6 herds that were not infected (1.6%) had
violations. The difference between these groups was not significant ($P = 0.55$ as determined by chi-square test), and there was no apparent association between inhibitor violations and infection status in this study.

**Estimate of Economic Impact of Infection**

Infections could contribute to three major sources of economic loss. Herds in census data that were infected with *S. agalactiae* had significantly higher BTSCC in the 5-mo period around sampling, which has previously been associated with reduced milk production (8). Second, herds with infection were more likely to incur milk quality penalties for both BTSCC and elevated bacterial counts than were herds without infection. Finally, infection with *S. agalactiae* was associated with the loss of ability to ship fluid milk for a mean period of 16 mo.

Each increase in BTSCC of $100 \times 10^3$ cells/ml above a threshold of $200 \times 10^3$ cells/ml has been associated with a decrease in mean herd production of approximately 2% (8). Using this value and the mean milk production and milk price of the dairy industry in Prince Edward Island [23.1 L/d per cow; $43.89/hl ($1 Canadian = $0.7455 US)] of industrial production and $47.62/hl of fluid production, the annual cost of elevated BTSCC for dairy farms that were detected positive for infection was $3866 more that the cost for herds without infection. The mean cost of BTSCC penalties on infected farms over uninfected farms was $550/yr. The estimated annual cost of penalties that were attributable to excessive bacterial counts in infected herds versus herds that cultured negative was $78 per infected herd.

Fourteen herds would have lost status as a producer of fluid milk because of elevated BTSCC in the 1992 to 1993 dairy year. The annual cost of lost status for the average dairy herd in Prince Edward Island was $9084. The estimated annual cost that was associated with the loss of fluid status across all infected dairy herds was $1699.

**DISCUSSION**

**Test Characteristics**

Sensitivity values calculated in this study were estimated from a history of infection that had been identified with the same diagnostic test. No calculation of the true sensitivity of the testing protocol could be generated without herd classification with an appropriate gold standard. Sensitivity estimates derived from single cultures ranged from 0.65 to 0.78. These values were somewhat less than the 95% (26) reported by the developers of the culture techniques used in the study, but far superior to the 20.5, 35.3, and 46.8% sensitivity values reported for other bulk tank culture methods (2, 12, 14). Pigment production is one of the main criteria for identifying *S. agalactiae* colonies on the GBS medium. In Prince Edward Island, the proportion of strains with the capability to produce pigment appeared to be lower (61.6%) than that observed in Alberta (91.5%), where 95% sensitivity was achieved. In a study in the northeastern US (21), only 15 of 40 (37.5%) strains were pigmented, and in a study in England, 3 of 13 (23%) strains from mastitic cows were pigmented (22). Differences in pigment production might have contributed to the lower sensitivity observed in this study.

The culture protocol (Edwards and GBS plate) was repeated twice over a 2-wk period at each census. The results of the two tests were interpreted in parallel, which improved sensitivity (19). When the results of the two diagnostic tests were independent, the estimated sensitivity of the combined protocol could be calculated based on the estimated sensitivity of a single test. The results of the second test protocol in the December 1992 census did not agree with the results of the first test more frequently than would be expected by chance. The two data files, therefore, were assumed to have been independent, and the sensitivity of the combined procedure was estimated at 91%. When used in this manner, the sensitivity of the test was far superior to the sensitivity of other bulk tank tests (2, 12, 14).

The specificity of the testing protocol has been assumed to be 100% because *S. agalactiae* is an obligate pathogen of the udder. The only plausible way to isolate it from the bulk tank is if it is shed by an infected cow. The samples used in this study were those taken by milk truck drivers at routine pickups. These samples have been normally used for regulatory purposes, and great care was taken to prevent cross-contamination. In this study, the samples were preselected based on their appearance on two selective media and then tested with a latex agglutination test that had a specificity of over 98%. The specificity testing program used in this study approached 100%.

**Herd Prevalence of *S. agalactiae***

The observed herd prevalence of *S. agalactiae* in the December 1992 census was 17.1%, and the estimated true prevalence based on this value and an estimate of the characteristics of the test was 18.9%. The observed and calculated values were 13.1 and
14.4%, respectively, for the follow-up census conducted in June 1994. The reduced prevalence of infection in the herd resulted primarily from the extension programs that were provided to infected herds during the interval between the two censuses. These prevalence values were lower than those recorded in other areas of North America where census or random sample bulk tank studies have been conducted (12, 13, 16, 28). However, because S. agalactiae can be successfully eradicated (20) and because herds can be maintained without infection under field conditions (30), levels of herd infection of 18.9 or 14.4% are substantial and indicate that S. agalactiae mastitis is still a major udder health problem for the dairy industry in Prince Edward Island.

**Herd Incidence of S. agalactiae**

Although herd prevalence studies of S. agalactiae infection are common, no studies that describe the incidence of new infections have been reported in the literature. Information regarding incidence rate is extremely valuable because it improves the understanding of disease dynamics. The calculated incidence of new herd infections with S. agalactiae of 3.5 herds per 100 herds per year indicates a considerable number of new herd infections. This value may be higher than in other dairy producing areas because in Prince Edward Island the mean herd size is only 36 cows, and many small producers do not find it economical to raise replacement heifers. As a result, there is considerable movement of cattle among farms within the province. Such movements could increase the transmission rate of S. agalactiae across herds.

**Association with BTSCC**

On average, herds infected with S. agalactiae had higher BTSCC than did uninfected herds. The single most important factor affecting SCC is the infection status of the mammary gland (7, 30). Streptococcus agalactiae has previously been associated with elevations in SCC at the cow and herd levels (10, 34). Others (9) have demonstrated reduced herd SCC after therapy to eradicate S. agalactiae. Between 1985 and 1990 in Vermont, state prevalence rate for herds infected with S. agalactiae was reduced from 47 to 32% of herds, and mean SCC declined 200 × 10^3/ml (10). The results of the present study reiterate that herd infection with S. agalactiae was associated with significant elevations in BTSCC.

**Association with Milk Quality Penalties**

Many milk marketing and processing organizations use high quality premiums or low quality penalties to encourage farmers to produce milk that meets higher standards for SCC and bacteria. Because the design and implementation of these schemes were not uniform, programs could not be compared. Among the criteria assessed on Prince Edward Island, BTSCC (10, 13, 34), SPC (16) and antibiotic residues (15) have previously been found to be associated with S. agalactiae infection. The SCC and bacteria standards of the program in Prince Edward Island were not stringent, but the risk of penalties for SCC and bacteria count were 3.90 and 5.48, respectively, compared with the risk of penalties for uninfected herds. With more rigorous standards, an even greater association between BTSCC penalties and infection status may occur.

Only 9 inhibitor violations occurred during the monitoring time period. Greer and Pearson (15) showed an increased risk of inhibitor violations in herds that were infected with S. agalactiae in Ireland. Although there was no apparent association between inhibitor violations and S. agalactiae infection status, the power of the study might have been insufficient to detect a difference, if one existed.

**Estimate of Economic Impact of Infection**

The annual cost associated with S. agalactiae to the dairy industry in Prince Edward Island is substantial. The negative economic effects of infection are even greater than this cost because the economic hardship is not spread evenly across herds. More than 80% of herds are uninfected. On the farm, S. agalactiae infection could cost individual producers $5000 to $15,000/yr. The greatest economic losses for an individual farm were associated with loss of premium for fluid milk as a result of chronic elevated BTSCC.

Herd that are infected with S. agalactiae might also have a higher prevalence of other chronic subclinical mammary pathogens, such as Staphylococcus aureus (10, 23). In addition, infection status could be correlated with poor premilking hygienic procedures (3), which augments the association between S. agalactiae and penalties for excessive bacterial counts. As a result, the associations with BTSCC and penalization because of BTSCC and bacteria count might be inflated. Nevertheless, S. agalactiae might be a serious economic burden to dairy producers with infected herds.

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