HERD PREVALENCE AND INCIDENCE OF *STREPTOCOCCUS AGALACTIAE* IN PRINCE EDWARD ISLAND AND EVALUATION OF THE EFFECTIVENESS OF AN ERADICATION EXTENSION PROGRAM

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ABSTRACT

The first two objectives of the study were to establish the herd prevalence and incidence of *Streptococcus agalactiae* mastitis in the Prince Edward Island (P.E.I.) dairy industry. The third and fourth goals were to quantify the effectiveness of an intensive "on farm" and a less intensive "mail out" extension protocol. An attempt was also made to identify the management procedures which affected herd level eradication of *S. agalactiae*.

The herd prevalence and incidence of mastitis caused by *S. agalactiae* were determined during December, 1992 and June, 1994. At each census, bulk tank milk samples from all dairy herds (n=452) in P.E.I. were tested on two separate occasions.

A 150 μl aliquot of bulk tank milk was plated on each of two media: Islam's GBS® and modified Edwards medium®. All suspect colonies were subcultured onto blood agar and identification confirmed with a latex agglutination test. These culture and identification techniques had a previously reported sensitivity of 95%. In the present study estimates of the sensitivity ranged from 65% to 78% on a single culture protocol. At each census herds were tested with this protocol twice and the results interpreted in parallel. The combined sensitivity was estimated to be 91%. *S. agalactiae* is an obligate pathogen of the bovine udder and the confirmatory latex agglutination test had previously reported specificities approaching 100%. The estimated specificity was assumed to be 100%.

The apparent prevalence of *S. agalactiae* in P.E.I. in December, 1992 and in June, 1994 was 17.7 and 13.1% respectively. Based on the test characteristics the estimated true prevalence was 18.9% in December, 1992 and 14.4% in June, 1994. Herd level infection with *S. agalactiae* was associated with milk quality penalties and elevated bulk tank somatic cell count, a surrogate measure of production loss. Evaluation of the previously negative herds (December, 1992) in June, 1994 yielded an estimate of the incidence of 3.51 new herd infections per 100 herds per year.

An intensive "on farm" and a less intensive "mail out" extension program to eliminate *S. agalactiae* from infected dairy herds were evaluated. *S. agalactiae* infected herds were randomly assigned to undergo the "on farm" education program (n=50) or act as positive controls (n=27). A randomly selected group of negative herds (n=50) was negative controls. Bulk tank samples were cultured from the study herds in December, 1993. The positive control herds were then informed of their status and supplied with the same educational materials as the "on farm" group by mail. Effectiveness of this campaign was assessed from data collected in the June, 1994 census. Herds in the "on farm" program were 4.62 times more likely to eradicate *S. agalactiae* than the positive control herds. Path analysis, using information generated from a survey of study herds, revealed that, in general, herds that eradicated *S. agalactiae* did so by following a particular pathway which included participation in the extension program, consultation with udder health professionals, and whole herd milk cultures. The effectiveness of the "mail out" extension effort was not statistically different than the "on farm" program. However, because of the small number of herds in each group, the study had limited power to discern a difference, if, in fact, there was one.

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It is often asked *why did you chose a career in veterinary medicine*? Some colleagues have told me that they choose the career out of a sincere love of animals, others have cited the broad scientific training and others had visions of a "James Herriot" like lifestyle. Each of these was a factor in my career choice, however, the most important was a sincere respect and admiration for farmers. My greatest admiration goes to dairy farmers. While others must *take* their end product from the animals, dairy cows *give* their milk. The most successful producers are able to stimulate cows to *give* more milk. Many thanks to the dairy farmers who have provided encouragement in my first few years of veterinary practice and throughout this project.

Dr. Ian Dohoo, my graduate supervisor, has shown great patience as I struggled through this project as a part time student. Ian often worked his schedule around mine, having meetings on weekends or at night when my timetable permitted. Ian is not only a great teacher and researcher he has the friendly open spirit that makes him a great human being. I first met Dr. Ken Leslie as an undergraduate at the University of Guelph. Ken is one of the few great educators who teaches by making his career an example of excellence to his students. Although not directly connected to the project, he gave me encouragement when few people believed in the value of my research.

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Chapter 1

Literature Review

1.1 INTRODUCTION

Mastitis is a major source of economic loss to the dairy industry.[1] A lower incidence of mastitis and improved milk quality are major factors in determining farm profitability.[2] Even since a sustained extension and education effort began in the early 1970's mastitis remains the single most influential health factor affecting milk production, with both clinical and subclinical mastitis having a major effect on milk yield.[3] In addition, infections with mastitis pathogens, which go uncured, represent a reservoir of the disease organism in the herd.[4] Dairy producers and processors share the economic detriment because of decreased production and milk quality losses.[1] It is anticipated that over the next decade consumer concern with food safety and wholesomeness will continue to escalate.[2] These issues will play an important role in success or failure of maintaining or expanding markets by the dairy industry.[2]

The cost of mastitis was reviewed by Blosser in 1979 and estimates ranged from $35 to $294 per cow per year.[5] Based on responses to a questionnaire sent to dairy personnel in each state, the annual cost of mastitis in the United States was estimated at 1.3 billion dollars in 1976.[5] This represents eleven percent of total farm receipts for milk sales. Other studies have estimated a ten percent annual milk loss due to mastitis caused by the major contagious pathogens, *Streptococcus agalactiae* and *Staphylococcus aureus*.[6]

Jensen commented that one in ten cows is fed, managed and milked for nothing as a result of mastitis.[6]

Prior to 1945, most of the research into mastitis was in the areas of pathology and diagnosis. Very little information on control, therapy and prevention was available. However, as early as 1933 Minett was able to demonstrate that a herd could be assembled and maintained free of *S. agalactiae*.[7] In 1945 penicillin became available for use in veterinary medicine. Access to this antibiotic represented the first breakthrough in mastitis treatment. The eradication of *Streptococcus agalactiae* was now a practical possibility. By 1955, it was clear that antibiotic therapy alone was not sufficient to control mastitis. As exposure of the udder to mastitis pathogens plays the most important role in establishing new infections, a system of udder hygiene was needed to decrease teat-end challenge.[8] It was not until the National Institute for Research in Dairying (NIRD) program was formulated in the 1970's that a comprehensive udder health scheme was established.[8] The most immediate and obvious benefit to the dairy producer, of such a program, was reduced cases of mastitis and decreased amounts of milk discarded due to antimicrobial treatment.[9]

Veterinarians play an important role in mastitis management. They are increasingly relied upon for information related to herd management of animal health as well as individual animal diseases. In California, dairy herd managers ranked mastitis as the most important disease on their farms.[10] These farmers consulted veterinarians more frequently than any other professional group about this problem. It is important to note, however, that 16 of 42 producers would seek advice from someone other than a

veterinarian, such as a processors field representative, county extension agent, or feed company representative, as their primary source of mastitis information.[10] It is evident that if veterinarians are to remain in a leadership position relative to the provision of udder health services to dairy producers, they must continue to upgrade their knowledge and skills.[11]

1.1.1 Overview of *Streptococcus agalactiae*

Streptococcus agalactiae was a major cause of mastitis in the pre-antibiotic era. It remains a significant cause of chronic mastitis in many herds, in spite of the fact that it can be readily eliminated.[12] Procedures for the diagnosis and treatment of intramammary infections due to the bacterium are well established.[13] Since it can only survive for long periods within the mammary gland and is susceptible to penicillin therapy, eradication within a closed herd is possible.[14] Herds can be maintained free of infection with *S. agalactiae* under field conditions.[15]

S. agalactiae has the ability to adhere to the mammary tissue of cows and the specific microenvironment of the bovine udder is necessary for the growth of the bacteria.[16] The virulence of various strains is related to differences in their ability to adhere to the mammary epithelium.[12] In addition, the interaction of the bacterium with the major milk protein, casein, may enhance its ability to parasitize the bovine mammary gland.[16]

S. agalactiae also causes neonatal septicemia in humans. Although some surface

antigens seem specific to the bovine,[17] overall there is considerable homology between strains isolated from septicemic infants and mastitic cows.[18] Human infection is generally acquired from other human sources, although there may be some risk associated with direct exposure to infected animals or their products. *S. agalactiae* is now recognized to be part of the normal bacterial flora in the human throat, genitourinary tract, and rectum.[19]

1.1.2 Epidemiology

Streptococcus agalactiae is a highly contagious obligate parasite of the bovine mammary gland.[14] Contagious mastitis pathogens like *S. agalactiae* cause a low grade persistent type of infection and generally do not have high self cure rates.[20] Unidentified infected cattle function as reservoirs of infection because they are not selected for treatment, segregation or culling.[21] For an obligate intramammary pathogen like *S. agalactiae*, the bovine udder is recognized as the only reasonable source of the organism in the milk. Consequently, isolates in the bulk tank can usually be assumed to have come from the udder.[22,23]

When a herd is infected with *S. agalactiae* traditionally there has been a high within herd prevalence.[23] In 1982, Sears found an average of 39.5% of cows infected within positive herds in Mississippi.[24] Oliver and Mitchell found an intraherd prevalence of 44.7% among infected herds in Massachusetts between 1976 and 1982.[25] More recent studies in Ontario (1990)[26] and Ohio (1992)[27] showed a trend toward lower within herd prevalences. Individual cow infection levels in positive herds averaged 7.9% and 10%,

respectively. In the Ohio study (mean prevalence 10%), the authors noted that infection status had a marked positive skew. Most herds had a very low percentage of quarters infected and a few herds had a high percentage of quarters infected.

Within herd mastitis prevalence was compared between *S. agalactiae* negative herds and positive herds in the Massachusetts study. In the positive herds, major pathogens were isolated from 58.5% of the 1105 cattle tested and 37.0% of the quarters. *S. agalactiae* was the most common isolate accounting for 69% of the total isolates. In negative herds 26.3% of cows and 10.2% of the quarters were infected. Other streptococci were the most common group of organisms.[25]

Herd level infection with *S. agalactiae* has been associated with failure to use post milking teat dip and selective or nonuse of dry cow therapy.[15,26,28] The use of a common wash rag or sponge has also been found to be a risk factor.[28] Inadequate treatment of clinical cases of mastitis was observed more frequently in herds that were infected.[15] In California, larger herd size and non-participation in the DHIA program were associated with the disease.[29] In contrast, Bartlett *et al* (1992)[27] found that there was no association between post milking teat dip and dry cow therapy and *S. agalactiae* infection status of herds. The researchers speculated that this was because the practices were widely adopted by both infected and noninfected herds. It is possible that the widespread use of these procedures has lead to a decrease in within herd prevalence, from the high levels noted in the 1970's and early 80's, but not to complete eradication.

1.1.3 Herd prevalence studies

Prevalence studies have been conducted in a number of areas. Some studies are based on the culture of milk samples from individual cows, while others are based on the culture of bulk tank milk samples. All figures quoted below are of herd level prevalence. *S. agalactiae* was either isolated from the bulk tank milk or from the culture of at least one individual cow sample in the herd. Some of the prevalence data cited are based on census of the entire study population, while others are based on a sample of the population. Not all of these samples were random. A series of pie charts depicting the results of these studies are located in Figures I and II. Details on the methods used in the various studies can be found in the text that follows.

In a census of herds in Mississippi (n=998), in which each herd had its bulk tank milk cultured three times, 435 herds or 44% were positive for *Streptococcus agalactiae*.[24] In a census of the 2,931 herds shipping milk in Vermont in 1985, *S. agalactiae* was isolated on a single bulk tank milk culture from 47% of herds.[30] In 1990, the study was repeated on the 1,971 herds still in production and the herd prevalence was found to be 32%.[31]

In a random sample of southwestern Ontario herds in 1990, 42.4% of herds were found to be positive on at least one of four bulk tank milk cultures.[26]

Figure I

Herd level infection with *Streptococcus agalactiae* based on bulk tank culture

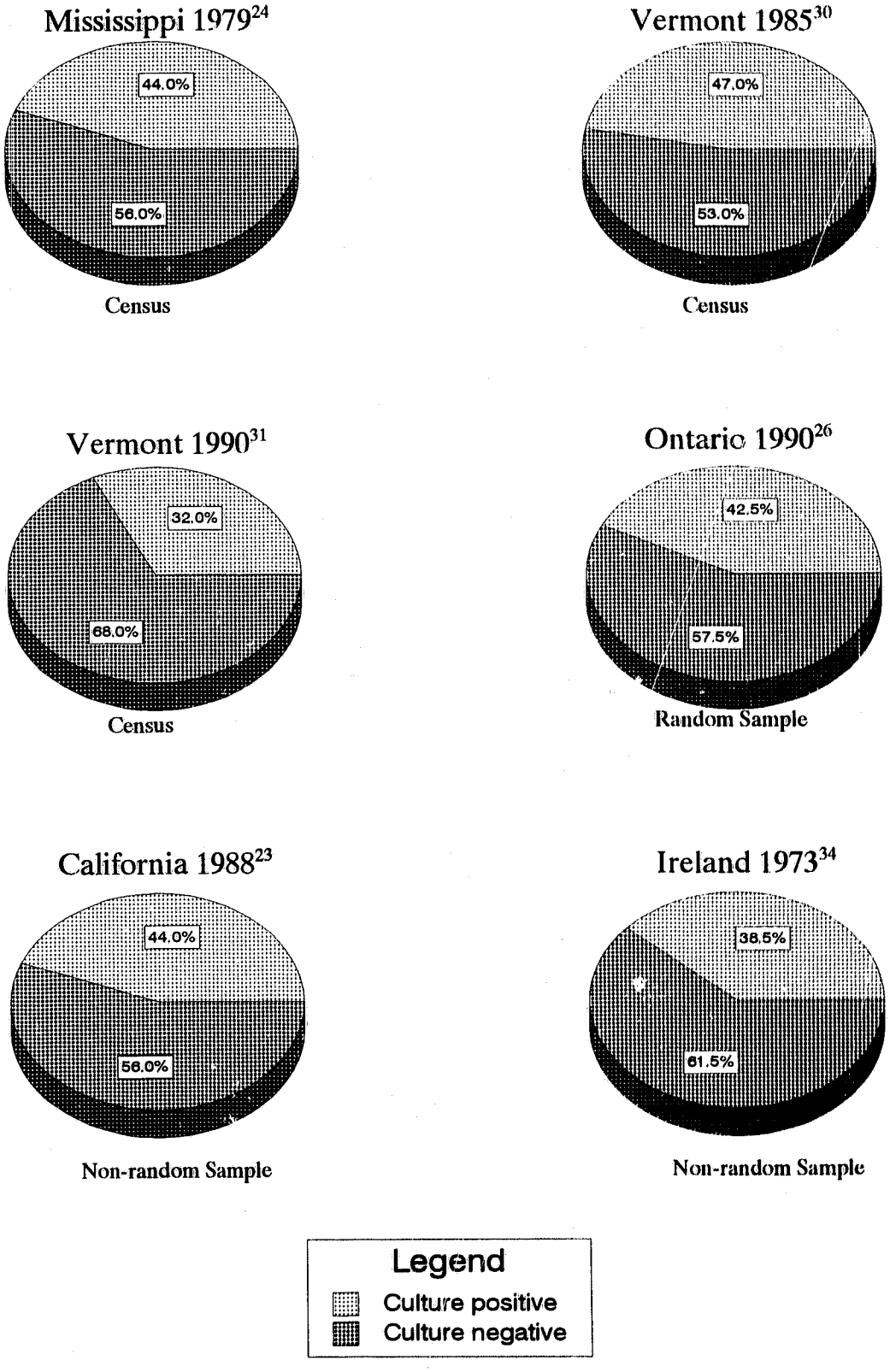
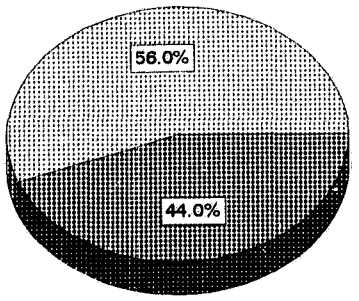


Figure II

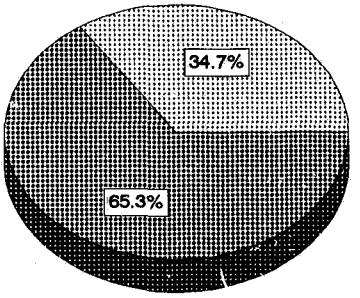
Herd level infection with *Streptococcus agalactiae* based on individual cow cultures

Ohio 1992[27]



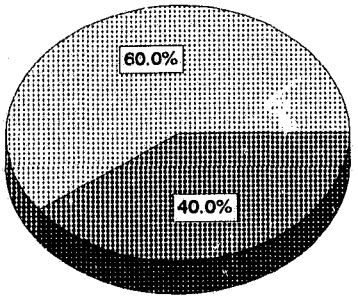
Stratified Random Sample

Ohio 1989[22]



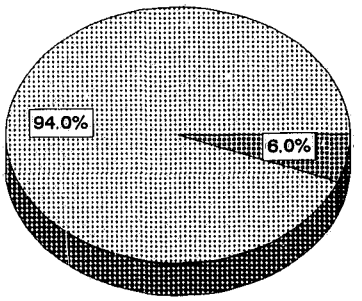
Stratified Random Sample

Pennsylvania 1982[32]



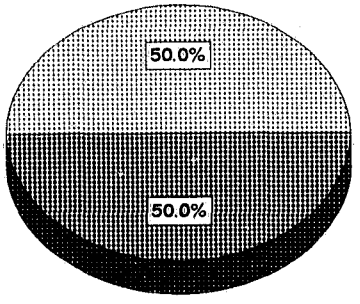
Non-random Sample

California 1988[33]



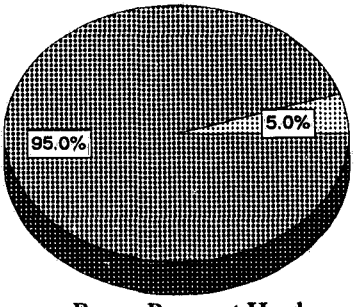
Non-random Sample

New York 1989[28]

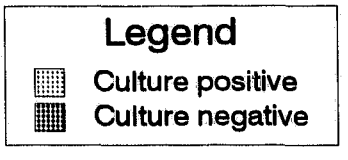


Non-random Sample

Ohio 1991[15]



Bonus Payment Herds



In a stratified random sample of herds in Ohio in 1992, using cultures of individual cow samples, 56% of herds had at least one positive culture.[27] In an earlier study (1988/89), in which quarter samples were cultured from a random sample, stratified based on herd size and geographic location of herds in the same state, 34.7% of the herds were found to be positive using the same criteria.[22]

In Pennsylvania in 1982, a study was conducted on 29 herds.[32] The selection criteria were not specified. Most of the herds had quarter milk samples taken three times over an eighteen month study period. Forty-eight of 80 samplings or 60% had at least one cow infected with *S. agalactiae*.

In California in 1988[33], 47 of 50 herds in a non-randomly selected group had at least one cow infected with *S. agalactiae* (herd prevalence 94%). Nineteen of the herds were chosen because of herd level mastitis problems. The remaining thirty-one were a convenience sample of other herds in the local area. *S. agalactiae* was the most prevalent bacteria from individual cow cultures. Of the 23,138 cows tested, 7.81% were positive. In a separate report using the same herds the prevalence based on a single bulk tank milk sample was 44%.[23]

In a review of 19,000 herd surveys conducted by the New York State Quality Milk Promotion Service (QMPS) more than half had at least one cow infected with *S. agalactiae*.[28] Of the herds using the QMPS, 60% are forced into the program because they have an average SCC in excess of 1,500,000.

In an Irish study, 38.5% of herds tested in a monthly bulk tank culture program were positive during the year long study.[34]

In an Ohio study of herds selling milk to a Cooperative giving premiums for low SCC, the herd prevalence of *S. agalactiae* was 5% based on 802 bulk tank cultures.[15]

It is evident from these figures that *Streptococcus agalactiae* remains an important problem in the North American dairy industry. These values represent serious economic losses to the industry. It is encouraging to note that in the Ohio study of herds shipping milk to a dairy processor paying quality premiums, the level of infection seems drastically reduced over other studies in the same state. This suggests that the problem can be controlled at the field level.

1.2 MICROBIOLOGY, CULTURE AND IDENTIFICATION

If eradication programs for *Streptococcus agalactiae* are to be effective, methods for identification of the pathogen both at the herd and individual cow level need to be inexpensive, accurate and non-labour intensive. Researchers continue to work on the advancement of diagnostic tests both through refinements of the traditional bacteriological techniques and in the area of diagnostic immunology.

1.2.1 Microbiology of *Streptococcus agalactiae*

Streptococcus agalactiae or group B streptococci, as it is more frequently referred to in the medical literature, is a Gram positive cocci often noted growing in chains in milk and in liquid media. Pasteur, Koch and Neisser, the first proponents of the germ theory

of disease, recognized the role of streptococci in disease. With the introduction of solid media for laboratory culture in the late 19th century, the first species of streptococci were isolated.[35] Nocard and Mollereau reported the first investigation of the cause of bovine mastitis. In 1889, they isolated "*Streptococcus nocardi*", later renamed *Streptococcus agalactiae*, from the milk of an infected quarter. Prior to 1940 and the widespread use of penicillin, researchers stated that 90% of all bovine mastitis was caused by streptococci.[36]

Several researchers have tried to exploit various unique characteristics of this bacterium in order to facilitate identification. [37,38,39,40,41,42,43,44,45] The combination of CAMP factor, β haemolysis, and negative esculin hydrolysis has been the most common criteria used for *S. agalactiae* identification. The most versatile medium for the diagnosis of udder infections is blood-esculin agar.[46]

Group B streptococci produce a zone of complete haemolysis in blood agar known as β haemolysis. In addition, they also produce a diffusible extracellular product known as CAMP factor (named for the discoverers of the phenomenon Chrisie, Atkins and Munch-Peterson). In the presence of staphylococcal β haemolysin this factor causes a rapid and complete haemolysis of sheep erythrocytes.[43] Traditionally, the CAMP reaction has been read by streaking a β haemolytic staphylococcus perpendicular to streaks of suspect streptococci. Ward and Postle, as early as 1968, showed that β haemolysin can be extracted from staphylococci. This toxin can be incorporated into media to allow the direct observation of the CAMP reaction on primary plates.[40] In the same year, working independently, Jasper and Dellinger noted that the CAMP reaction observed using crude

β haemolysin extract, which they streaked on the plates surface, was identical to that observed using streaks of living β-staphylococci.[41] Others have used drops of the β haemolysin to observe the CAMP reaction.[42] β haemolysin is not commercially available. Details on its production are presented in the Materials and Methods section of Chapter 2.

Unlike most other species of streptococci, *S. agalactiae* does not hydrolyse esculin.[35] The addition of ferric citrate to media, as an iron source, augments the dark colour production by esculin splitting bacteria, facilitating differentiation of these bacteria from group B streptococci.[35]

Ward *et al* (1970) attempted to optimize the recovery of *S. agalactiae* from milk cultures using a selective media.[44] To a trypticase soy, crystal violet, thallium acetate agar base they added 5% washed sheep erythrocytes (to observe the haemolytic phenomenon), esculin, ferric citrate (for esculin hydrolysis) and staphylococcal β haemolysin (for direct observation of the CAMP reaction). The use of this TKT/FC media was able to identify group B streptococci infected bulk tank milk samples twice as frequently as nonselective methods.[44] Recently, the sensitivity of this method has been questioned. [22,26]

Another feature of group B streptococci, that has been exploited for identification purposes, is its ability to produce pigmented colonies when grown anaerobically on starch containing media. The production of pigmented colonies by streptococci was first described by Orla Jensen in 1919.[38] Lancefield noted that when a strain of group B streptococcus, which she had been investigating, lost its haemolytic properties it also lost its pigment production capabilities.[37] A close linkage has been suggested between the

genes associated with pigment production and haemolysis.[45] Some strains seemed to be able to produce much more pigment than others. Pigment production is very much dependent on anaerobic conditions, inclusion of starch in the media (0.1%), and the pH of the media (>7.3).[38] The pigment has the characteristics of a carotenoid localized in the membranous portion of the cell wall.[45] Production of the pigment appears to be more consistent among human than bovine strains.[19,47]

Fresh or frozen milk samples have been successfully used in the culture of both pooled bulk tank and individual cow samples for *S. agalactiae*.[21,48,49,50] There has been either no difference, or a slight increase, in recovery rates in previously frozen samples. The viability of *S. agalactiae* in milk was determined by quantifying the number of colony forming units before and after freezing. Storage at −20 °C for four weeks did not effect the number of colonies recovered.[51]

1.2.2 Culture from bulk tank samples

Bacteria in bulk tank milk can originate from one of two sources. Bacteria can be present within the cow's udder or may result from environmental contamination as milk is removed from the cow, handled and processed.[52] Presence of *S. agalactiae* in bulk milk is due exclusively to the shedding of bacteria from infected quarters.[53] Milk quality laboratories traditionally have been more concerned with milk contamination after removal from the udder. It has been suggested that they should identify mastitis pathogens in bulk tank milk as well as check on milk quality.[52] This would require the

use of more selective media: the standard plate count is not able to identify specific mastitis pathogens. Comprehensive reports from routine bulk tank analysis could then be made available.[52] An inexpensive screening test for *S. agalactiae* within dairy herds would enable other agencies, such as those now providing somatic cell count services, to offer testing procedures.[22] Such services would make eradication programs more attractive. If such undertakings are to succeed, culture tests for the detection of this organism must approach 100% accuracy.[44]

Oz *et al* (1985) proposed a standard method of bulk tank culture.[9] Milk taken at three different times would be preserved by freezing. The samples would then be thawed and processed to break up bacterial clumps. A large inoculum of the processed sample would then be plated onto several selective media. Ward *et al* (1970) had previously proposed a selective media for *S. agalactiae* isolation.[44] They also suggested an eradication protocol and certification criteria based on this culture procedure. Johnson (1986)[54] and Pankey (1987)[46] suggested bulk tank milk sampling should be part of a regular herd health program. If *S. agalactiae* or *S. aureus* were isolated from the bulk tank milk, all cows in the herd should be tested to determine which are infected.[46,54]

Based on the obligate nature of the disease, Bartlett (1991) stated that the assumption of 100% specificity on bulk tank milk samples is reasonably valid.[22] *S. agalactiae* is usually shed in high numbers from infected glands,[49,55] with a cyclic shedding pattern being typical.[4] The number of *S. agalactiae* in bulk tank milk is a function of the number of infected quarters shedding the organism. In a study of 7 herds, *S. agalactiae* did not multiply within the milking system or in the tank, except at

temperatures greater than 27°C.[23]

Although the specificity of bulk tank milk culture can be assumed to be very high, the sensitivity has been variable between studies. Not all researchers have used the same methods. As a result, it is difficult to compare test characteristics among the various protocols. Variations in the sensitivity of bulk tank milk culture are probably a function of differences in the protocols, variation in the intraherd prevalence and variations in the rate of bacterial shedding related to the stage of infection.[23]

In an Ohio study of 49 herds, stratified on the basis of herd size and geographic location, bulk tank milk samples and milk filter culture samples were compared to individual quarter milk samples[22]. The method used was to streak an 0.01 ml aliquot on a quadrant of sheep blood esculin agar, and TKT media. The prevalence of *S. agalactiae*, based on at least one cow in the herd culture positive, was 34.7%. The sensitivity of bulk tank milk culture was 35.3% (6/17). The specificity of the test was 96.9% (31/32). The positive predictive value and negative predictive value were 85.7% and 73.8%, respectively. In the same study the use of the milk filter was found to have a sensitivity of 23.5% (5/17).

In a large California study[23], in which composite cow and bulk tank milk samples were taken from 23,138 cows in 50 herds, one loopful (0.01 ml) of milk from the composite or bulk tank milk samples was streaked on a half or a full blood agar plate respectively. A streak of β haemolysin was placed on the media so the CAMP reaction could be read directly. The sensitivity of bulk tank milk cultures using individual cow sampling as the gold standard was 50%. Correlations obtained between the number of

colonies and the intraherd prevalence was at .714 Spearman ($p<0.0005$). Forty-six percent of the observed variation in the intraherd prevalence could be explained on the basis of the number of colonies isolated per ml of bulk tank milk. If 7% of the cows in the herd were infected, greater than 4,000 colonies of *S. agalactiae* were isolated per ml. In positive herds, in which *S. agalactiae* was not isolated from the bulk tank, on average 3.9% of the cows were infected. In herds in which it was isolated from the bulk tank on average 17.97% of cows were infected. In this group the intraherd prevalences ranged from 0.56 to 44.94%.[23]

In an Ontario study, the use of TKT/FC media, with a 0.01 ml inoculum volume on bulk tank milk samples, yielded a sensitivity of only 20.5% when compared to a gold standard of individual cow cultures.[26] In this survey, the average cow prevalence within infected herds was 7%. The intraherd prevalence is consistent with the 10% prevalence found in positive herds recently in Ohio,[27] but much lower than the 40% levels encountered in the 70's and early 80's.[24,25]

The widespread adoption of post-milking teat dip and dry cow therapy may have led to a drop in the intraherd prevalence, but not to total eradication. Smith and Ward speculated this as early as 1975.[56] In response to the low intraherd prevalence and low sensitivity of bulk tank milk culture observed in their Ontario study, Godkin and Leslie suggested that, in the future, the sensitivity of bulk tank milk culture for *S. agalactiae* could be improved by using more selective media and a larger volume of inoculum.[26]

Schoonderwoerd *et al* (1993) employed two selective media in a technique for the isolation of *S. agalactiae.* They used a starch medium, modified to make it more

selective and with anaerobic conditions so that pigment production could be observed, and a CAMP/esculin medium. When this technique was employed on bulk tank milk samples a large inoculum was used and the results of the cultures were interpreted in parallel.[39] Interpreting diagnostic tests in parallel reduces the false negative classification rate.[57] The sensitivity of this protocol was reported to be 95% and the specificity 100%.[39] Details on the technique can be found in Chapter 2.

1.2.3 Culture from individual cow samples

Pankey and coworkers[46] suggested a mastitis control monitoring program based on sampling of cows at first milking, when clinical mastitis cases occurred and at dry off. They submitted that changes in the prevalence of mastitis pathogens during the lactational and dry period would be revealed by this method. Others have stated that whole herd culture gives a reliable picture of the level of contagious mastitis pathogens.[20] Each of these protocols assumes that the culture of individual cows is accurate. It is difficult to define the accuracy of individual cow samples as they are often the gold standard against which other culture methods are measured. The published studies measure the agreement between tests or measure the effect, if any, of various enrichment procedures.

Jensen and Knudsen (1989) questioned the ability of individual quarter cultures to predict subclinical mastitis.[58] Although *S. agalactiae* was not included in their study, the other species of bacteria examined all had very high negative predictive values. When a test was negative the cow was truly negative. Where the authors found fault was in the

positive predictive value. A proportion of cows, which were culture positive, did not have an inflammatory response as measured by SCC. In Jensen and Knudsen's definition, these animals did not have mastitis. They blamed the discrepancy between the number of culture positive cows and inflammatory responses on streak canal infections.[58] If an eradication program for *S. agalactiae* is being considered, streak canal infections could serve as a reservoir of infection and their treatment would be beneficial.

Because no gold standard is available, Jasper *et al* (1974) attempted to establish the ability of bacterial culture to identify *S. agalactiae* infected quarters by measuring the level of agreement between duplicate samples.[59] One quadrant of a TKT/FC plate was inoculated using 0.01 ml of milk. One hundred and seventy-three samples were eventually identified as positive. One hundred and seventy-two were identified on the initial sample.

A study, involving 167 cows on 4 farms with a prevalence of *S. agalactiae* between 25% and 65% of cows, was carried out to determine the effects of several different sampling and cultural protocols on the number of cows diagnosed as positive.[13] Quarter and composite samples containing 0.01 ml of milk were streaked on a half plates of trypticase soy blood agar with 0.1% added esculin. Alternatively, 0.05 ml of a composite sample was inoculated on a whole plate. No differences were noted for recovery rates from milk taken before, immediately after or five hours after milking. The use of quarter samples did not increase the number of cows identified as positive over composite samples. The use of a larger inoculum volume with composite samples did not effect the disease classification. The sensitivity and specificity of a single culture based

on infection history ranged between 95 and 100%.

Dinesmore *et al* (1992) evaluated the effects of augmented culture techniques on the recovery of bacteria from clinical mastitis cases.[49] Results from cultures on blood agar incubated for 48 hours at 37°C were compared to those in which pre-culture freezing, pre-culture incubation and increased inoculum sizes were used. Other species of bacteria responded by increasing growth and recovery rates. However, for *S. agalactiae* there was no effect. The authors speculated that this was because when present *S. agalactiae* is usually shed in high numbers.[49] Thurmond *et al* (1989) found a positive effect of pre-enrichment in a brain heart infusion (BHI) broth on the recovery rate of *S. agalactiae* over plating of 0.01 ml on blood agar.[60]

Generally, freezing has had no effect on the recovery of *S. agalactiae* from the milk of infected cows.[13,49,50] However, in one report freezing increased the number of isolations.[21]

In the absence of a gold standard, based upon a different biological principle, it is difficult to assess the accuracy of individual cow cultures. The level of bacterial shedding, the inability of augmenting procedures to enhance recovery in most instances, and the fact that the test is highly repeatable all indicate that sensitivity and specificity of the test are high. The unique biological features of the bacterium, being CAMP factor positive and esculin hydrolysis negative, substantiate an estimate of specificity which approaches 100%. Estimates of sensitivity of culture of milk samples from individual cows are more difficult. Based on repeatability, both within and across culture protocols, a sensitivity greater than 90% can be assumed.

1.2.4 Other tests for *Streptococcus agalactiae* mastitis

There is a need for rapid, accurate screening tests for the identification of *S. agalactiae* both from bulk tank milk and individual cow milk samples. Preliminary cultures or pre-incubation of milk samples must occur before the current tests can be used. The most commonly used of these tests is latex agglutination. Particles of latex that are coated with immunoglobulin agglutinate when they come in contact with a sufficient quantity of Lancefield group-B carbohydrate antigen.

The accuracy of latex agglutination as a confirmatory test on bacterial colonies obtained by preliminary milk culture is well established. When used on isolates from bulk tank milk samples the sensitivity and specificity of latex agglutination were 97.6 and 98.2% respectively.[61] In other studies, latex agglutination was 100% sensitive [62,63,64] and had a specificity between 98 and 100%.[63,64] A major advantage of latex agglutination as a confirmatory test is the rapidity in which test results are obtained.[61]

When used on the whey fraction of milk, which was incubated for 18 hours but not cultured to obtain individual colonies, 14 of 16 infected cows were detected using latex agglutination.[65] Only one sample from pooled bulk tank milk was used. It was found to be positive. The authors speculated that the test may have a use in bulk tank samples because routine bacteriology plates may often be overgrown with contaminants.

The Phadebact slide coagglutination method is similar to latex agglutination but the immunoglobulin is coated onto staphylococcal bacteria instead of latex beads. Once again preliminary incubation must be used to produce the necessary 5-8 colonies. In one

study all 66 group B streptococci were correctly identified by this method.[66]

Both ELISA and indirect fluorescent antibody tests have also been developed to aid in the identification of *S. agalactiae*. Each of these tests also require the samples be plated for 24 hours before use. Even with this restriction they reduce diagnostic time by 24 hours.[67]

A plethora of surrogate mastitis tests have been investigated. These include SCC, adenosine triphosphate, N-acetyl-β-D-glucosaminidase (NAGase), bovine serum albumin, antitrypsin and conductivity. An assessment of the value of these tests in predicting mastitis is beyond the scope of this thesis and appears elsewhere.[68]

1.3 MILK QUALITY

The profitability of the dairy industry is driven by both the quantity and quality of the milk produced. Although milk has often been described in the popular media as "nature's most perfect food", it cannot escape consumer scrutiny of quality and wholesomeness. As a result, many milk marketing and processing organizations have begun to penalize poor quality and give financial rewards for superior quality milk. In the first ten years of a bonus payment scheme based on bulk tank somatic cell count (BTSCC) and bacteriological criteria, producers in a western US cooperative received $5,000,000 in bonus payments.[69] The data from that cooperative show a steady increase in the quality of milk during that time period suggesting that farmers do respond to bonus payment schemes.[69]

1.3.1 Somatic Cell Count

Development of the technology for rapid measurement of somatic cell counts (SCC) has contributed greatly to the advancement of mastitis control programs. Cell counting provided tangible evidence of the existence of subclinical mastitis to producers.[8] Somatic cell counts can be used to assess milk quality and udder health or to monitor mastitis control. In addition, they may offer an important tool in the epidemiological investigation of the mastitis complex.[70]

Summaries of international, national and provincial somatic cell count data are presented in Tables I and II and Figure III. Within the international community Canada is average with respect to milk quality as measured by BTSCC.[71] Prince Edward Island is slightly below the national average in percent herds with BTSCC less than 500,000.[72]

High BTSCC have been shown to be correlated with poor milk quality, reduced quantity and quality of processed milk products and shorter shelf life[2] and are used by the dairy industry as a measure of raw milk quality.[2,73] In a review, Reneau and Packard (1991) noted that a Wisconsin dairy cooperative found with decreasing BTSCC there was a parallel decrease in antibiotic residue violations.[2] They also noted that there is a high correlation (.77 to .90) between an average of multiple BTSCC and prevalence of subclinical mastitis. Greer and Pearson (1973) in Northern Ireland noted a similar relationship between antibiotic residue violations, elevated cell counts and *S. agalactiae* infection status.[34] In an Ohio study of 1032 farms, the highest average SCC was found for farms with *S. agalactiae* in their bulk tank milk.[74]

Table I

Summary of results of the 1987 International Dairy Federation questionnaire on herd milk cell counts[71]

	Number of dairy herds ($\times 10^3$)	Average number of cows/herd	Herd arithmetic mean cell count ($\times 10^3$)	Cow arithmetic mean cell count ($\times 10^3$)
Canada	30.7	43	337	NA
Australia	15.9	NA	NA	349
Belgium	31.2	30	410	415
Great Britain	44.6	65	388	NA
Japan	72.5	20	NA	310
Netherlands	52.8	39.8	NA	327
Norway	30.4	11.8	NA	221
Sweden	30.9	19	NA	271

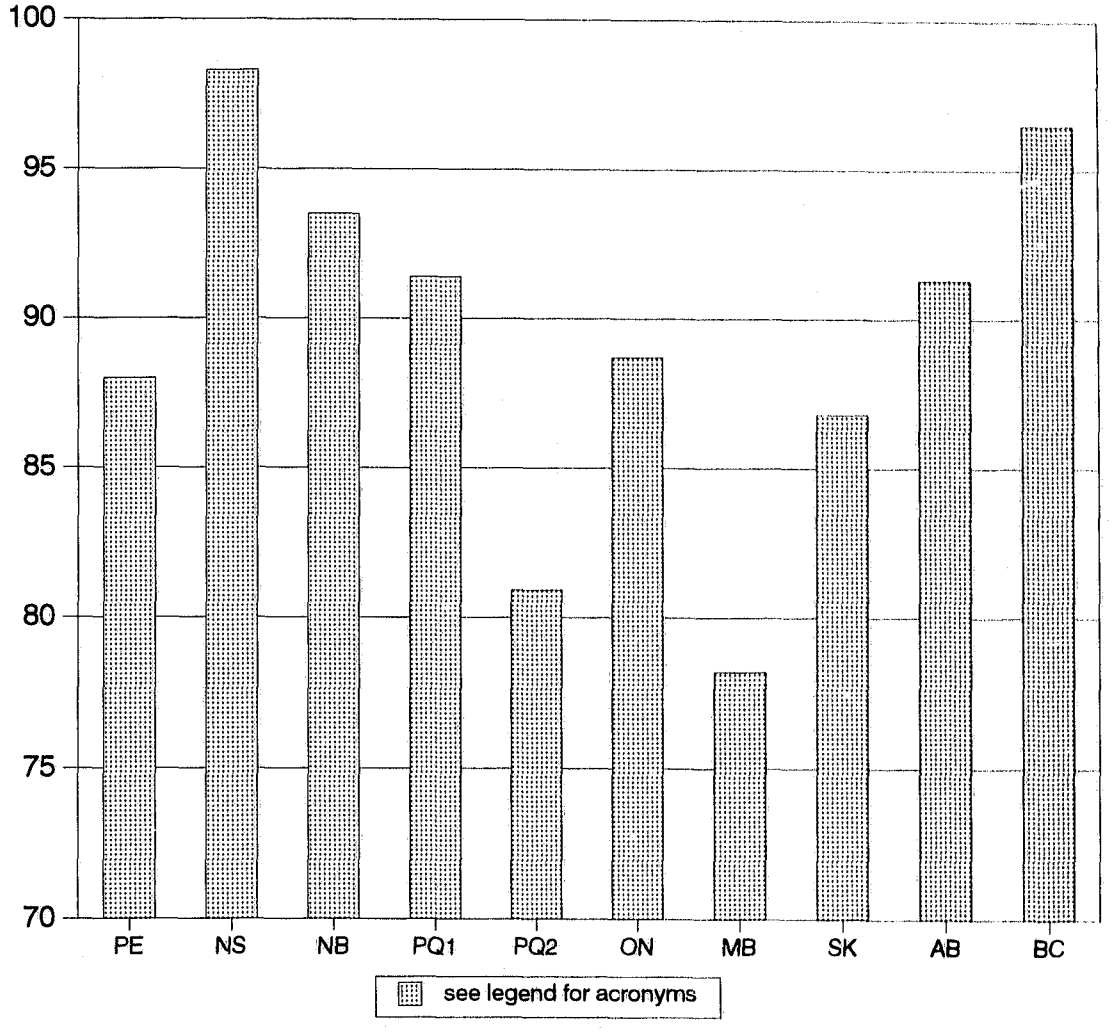
Table II

Summary of results of the International Dairy Federation questionnaire on the percent distribution of herds according to their annual mean cell count[71]

	SCC x 10^3							
	<100	>100 <199	>200 <299	>300 <399	>400 <499	>500 >699	>700 >999	>1,000
Canada	6.0	26.0	23.0	15.0	10.0	10.0	5.0	5.0
Australia	2.0	21.0	31.0	22.0	12.0	9.0	3.0	1.0
Belgium	0.7	11.8	24.1	21.6	14.8	16.0	8.5	2.5
Great Britain	1.0	15.0	30.0	22.0	12.0	12.0	6.0	2.0
Netherlands	3.0	25.3	27.2	18.1	10.8	10.0	4.1	1.5
Norway	18.6	34.2	21.5	11.2	5.9	5.0	2.3	1.2

Figure III

Summary of percent of herd with less than 500 X 10^3 bulk tank somatic cell count by province* in Canada[72]



* Data for Newfoundland unavailable

PE = Prince Edward Island, NS = Nova Scotia, NB = New Brunswick,
PQ1 = Quebec Grade 1 dairies, PQ2 = Quebec Grade 2 dairies, ON = Ontario,
MB = Manitoba, SK = Saskatchewan, AB = Alberta, BC = British Columbia

The single most important factor affecting SCC in milk is the infection status of the mammary gland.[3,15,73,75] Fifty-nine percent of the variation in BTSCC was due to the prevalence of intramammary infection, based on the comparison of test day individual cow cultures with the BTSCC from the same day. The resulting regression equation predicted that 6% of cows would be infected with a BTSCC of 200,000, 16% at 500,000, 32% at 1,000,000 and 48% at 1,500,000.[32] In another study, using individual cow SCC to predict infection status, 43% of the change in BTSCC was related to changes in prevalence of subclinical infection. As herd size increases the correlation between BTSCC and infection status increases.[76] In general, bulk tank counts under 250,000 indicate good udder health, and over 500,000 indicates a problem with subclinical mastitis.[15,73]

Other factors besides infection status that can have an impact on SCC include the age of the cow, the stage of lactation, and minor factors including season, stress diurnal variation and day to day variation. Compared to infection status, however, all of these factors are minor.[3,73,77]

With the development of automated somatic cell counting equipment the measurement of individual cow SCC has gained wide popularity. Individual cow somatic cell counts can be used for several purposes. These values can be interpreted at the herd level. Such an interpretation would allow the measure of milk quality, estimation of the prevalence of mastitis, analysis of patterns of mastitis in the herd and comparison of mastitis prevalence across a large number of herds. A BTSCC is easier to obtain and gives a better indication of milk quality than does averages of individual SCC. Estimates

of prevalence of mastitis can use either raw somatic cell counts or linear scores. Linear scores are the result of conversion of raw SCC on a natural logarithmic scale. Patterns of mastitis, within a herd or across herds, can be determined by analysing central tendencies or by calculating frequency distributions within certain threshold and signalment categories. The optimal number of intervals in a frequency interval is dependent on herd size.[70]

Individual cow SCC can also be interpreted at the individual cow level. Thus, SCC could be used to identify cows for culture and sensitivity, early dry off and dry cow therapy, culling and alterations in the milking order. Some producers and veterinary practitioners continue to use SCC to identify cows for treatment during lactation without the use of milk culture. This is not likely economically beneficial as many cows with resolved infections and uninfected cows may be treated and cows with infections with poor response rates (*S. aureus*) will not be identified.[73]

The predictive value of SCC is dramatically affected by the prevalence of infection.[78,79] The ability of SCC to predict infection status was examined in 12 herds with 719 cows in New York.[78] At a threshold of 400,000 cells, in a herd with a prevalence of infection of 7%, the positive predictive value (PPV) was only .26, while the PPV rose to .78 at a prevalence of 43%.

S. agalactiae produces high SCC in individual cows. These cattle have a significant influence on the BTSCC. In a group of herds with BTSCC greater than 700,000, the geometric mean SCC for *S. agalactiae* infected cows was 2,238,700.[80] In another study, the arithmetic mean SCC for *S. agalactiae* infected cows was 900,000.[81]

In an Australian study, 80% of cows with greater than 500,000 SCC in herds with a BTSCC greater than 800,000 had *S. agalactiae*.[82] In a study of twelve herds, blitz therapy for *S. agalactiae* dropped the average herd SCC from 918,000 to 439,000 in 30 days and, with the implementation of post milking teat dip (PMTD) and dry cow therapy (DCT), to 268,000 in one year. In the same study 94.8% of cows with *S. agalactiae* had a linear score SCC greater than 4 and 69.4% of cows had a linear score SCC of greater than 6. The midpoint cell count of linear score 4 would be 200 x 10^3 and the midpoint cell count of a linear score 6 would be 400 x 10^3.[83]

In Vermont, a drop in the herd prevalence of *S. agalactiae* from 47 to 32% corresponded with a drop in the state average BTSCC from 539 x 10^3 to 337 x 10^3.[31]

In a randomly selected group of herds with an average SCC greater than 700 x 10^3 cells/ml all herds had at least one cow infected with *S. agalactiae*. Twenty-six percent of quarters were infected with this pathogen. In herds with less than 150 x 10^3 average SCC, the percentage of quarters infected with *S. agalactiae* was 0.1%.[80]

Somatic cell counts are increasingly used as a measure of milk quality and to estimate the economic losses associated with mastitis. Infection with *S. agalactiae* is associated with elevations in SCC, both at the cow and herd level.

1.3.2 Standard Plate Count

The total bacterial count can be substantially increased by the presence of *S. agalactiae* mastitis in a dairy herd.[30,55,84] Bulk tank milk samples from infected herds

frequently contain bacteria counts in the range of 20,000 to 100,000 colony forming units (cfu).[53] A cow in the early stages of infection with *S. agalactiae* can shed up to 100 x 10^6 bacteria per ml.[55] The standard plate count (SPC) dropped from 99,000 to 2,000 after the implementation of a modified blitz therapy regimen and hygiene practices to control *S. agalactiae* on a British dairy farm.[85]

1.3.3 Milk Products

Milk from mastitic cows, even after dilution with noninfected milk, may be unsuitable for the production of certain dairy products.[86] High cell count milk has lower levels of fats, nonfat solids and lactose and higher levels of sodium, chloride and free fatty acids than low cell count milk.[36] In the manufacturing of cheese high SCC lowered yield due to lower casein and fat levels.[5,9,86,87] High SCC in milk also increases the time required to make cheese.[88] The cheese produced has a higher moisture content than that produced from low SCC milk[88] and is often downgraded due to quality.[86]

Milk from cows with subclinical mastitis decreases the quality of other manufactured products.[86] These changes in milk composition result in reduced nutritional value of milk, increased processing problems and "off flavours".[9] The shelf life of fluid milk products is also decreased due to the augmentation of the growth of spoilage bacteria.[9,86]

Milk with high SCC has increased proteolytic activity.[89] This activity, which is especially high in milk from quarters infected with *S. agalactiae*,[84] accelerates the growth

of some starter organisms for cultured milk products, but may also favour the growth of some psychrotrophic Gram negative spoilage bacteria.[89] Psychrotrophs rely on protein degradation products, free amino acids and peptides, as they are unable to utilize lactose for their carbon sources.

Regardless of their origin, bacteria in the milk supply are ultimately responsible for reduced shelf life of fluid milk and damage to manufactured milk products.[52]

Streptococcus agalactiae infection in dairy cattle plays an important role in reducing the production of quality milk and milk products. This effect is manifest through its impact on SCC and bacteria count and the detrimental effect of the bacterium on the quantity and quality of processed milk products.

1.4 CONTROL, PREVENTION AND THERAPY

Since the early 1970's, when veterinarians began to analyze herd level health problems, the emphasis has shifted from pathology and therapeutics in individual mastitis cases to development of methods for the prevention, control and herd level therapy of mastitis.

1.4.1 Milking machine function

The degree to which the milking machine is actually responsible for new infections is unknown and has been the source of some controversy.[90] There is no consistent field

evidence that milking machine malfunction is a major cause of herd subclinical mastitis problems. When the milking machine is not properly maintained there are often other deficiencies in the herd management program associated with a high prevalence of subclinical infection.[1] A study in Denmark indicated that only 6.6% of infections in pipeline milked herds were accounted for by the machine.[91] Grindal (1988), in Britain has suggested that 25-50% of new infections are related to improper machine function.[92]

The milking machine can contribute to mastitis in various ways. It can act as a fomite to transfer contagious bacteria between cows or from the environment to the cow. Tracer bacteria placed in the machine liners have been shown to persist for at least the next six cows.[93] Mastitis causing bacteria can be inoculated from the environment, or from infected quarters to mastitis free quarters, by slippage of the liner on the teat during milking[94], or by allowing large amounts of air to enter the system rapidly at the time of cluster removal.[92,95] During these events rapid changes in internal vacuum levels cause droplets of bacteria laden milk to be propelled backwards through the streak canal.[90] The use of shields fitted in the short milking tubes, or a multivalve claw, can significantly reduce the incidence of new infections.[92,95]

The other major influence that machine milking may have on mastitis incidence is through trauma to the teat-end. Trauma can reduce the teats resistance to infection by damaging or removing the keratin lining.[92] Inadequate pulsation undermines the integrity of the keratin lining and causes teat-end damage.[96] Pulsation failure may consist of mechanical failure of the pulsator, shortness of the liner barrel or too short a rest phase in the pulsation cycle.[97]

1.4.2 Management and Udder hygiene

Pre-milking udder hygiene and good cow husbandry are essential parts of a quality milk program. Although protocols for routine udder hygiene have been reviewed more extensively elsewhere[98], a brief examination of the more relevant techniques is appropriate for this discussion.

The original comprehensive udder health program was developed by the National Institute for Research in Dairying (NIRD) in the 1960's. The program was based on a simplified system of hygiene measures. The basic tenets included stock management, teat dipping, dry cow therapy, therapy of clinical cases, culling, monitoring and machine maintenance.[99] Most of the measures in the NIRD program were not new. The idea of teat dipping, for example, had been put forward as early as 1916.[100] It was, however, the first time that a comprehensive program had been advanced to producers via an extension effort.

Twenty-five years after the advancement of the NIRD program researchers in Texas and Ontario investigated the relationships of various hygiene practices to the level of subclinical mastitis as measured by SCC. In each study, teat dip use had the most dramatic effect on SCC levels. Other practices associated with lower SCC include proper washing and drying of udders, regular milking system analysis, and regular veterinary attention. The use of a common rag or sponge to dry udders was found to increase SCC in both studies.[101,102,103] In each of these studies, and in a third in Ohio, teat dip was shown to be the most cost effective control procedure.[104]

Conventional teat dips appear to operate by killing potential mastitis pathogens on the teats and thereby preventing colonization of lesions and of the teat orifice.[98] Teat disinfection after milking can reduce new infection rates by contagious pathogens by 50% or more.[105]

The percentage of dairy producers using post milking teat dip was significantly higher in herds with low SCC than in high SCC herds.[80,82,106] In addition, herds with *S. agalactiae* have been shown to use teat dip less frequently than uninfected herds.[15,26,28] A wide variety of teat dips have shown some efficacy in preventing new infections with *S. agalactiae*. In challenge studies and field trials, various teat dips have prevented 80% (n=20);[107] 67.5% (n=121);[108] 68.1% (n=121);[109] 46.2% (0.5% iodine) and 70.7% (1.0% iodine) (n=955 quarters);[110] 61.5% (n=131);[111] 52.5% (n=66);[112] and 51.75 (n=133).[113]

In an Ohio study, cow husbandry and hygiene components found to be risk factors for *S. agalactiae* herd level infections included cleanliness of the cows, cleanliness of the exercise area, herd size and the use of a common wash rag.[27] Failure to use post milking teat dip was not statistically associated with infection status, probably because almost all herds in the study were using it.

Some authors have stated that if applied in a conscientious program post milking teat dip in combination with dry cow therapy will eliminate *S. agalactiae* from herds.[8,14] Others have disagreed.[56] What does seem certain is failure to use these two procedures to follow up a blitz therapy eradication program can lead to considerable frustration and expense on the part of dairy producers due to reemergence of infection within the herd.[1,114]

1.4.3 Therapy

The therapeutic and preventative effectiveness of antimicrobial drugs for bovine mastitis is dependent upon the etiological agent, proper use of the drug under consideration, dairy husbandry, sanitation procedures and the phase of the disease.[115] When selecting drugs for the treatment of subclinical mastitis, selection should be based on cost, safety, residue potential, distribution properties and sensitivity data.[116]

1.4.3.1 Lactating cow therapy

S. agalactiae is generally sensitive to intramammary therapy using a variety of commercially available preparations. Systemic therapy has also been reported to be effective but offers no clear medical or economic benefits over intramammary therapy. *S. agalactiae* eradication programs are practical and advantageous because the organism is an obligate parasite of the bovine udder and infected cows serve as the reservoir for noninfected herdmates.[116] Although novel methods for the treatment of intramammary infection (IMI) have been tried,[117,118] the basis of treatment for most IMI remains antibiotic therapy. Treatment of *S. agalactiae* mastitis with intramammary infusion products will result in a high percentage of infections eliminated in a cost effective manner, with few residue concerns provided milk withholding times are observed.[119] In herds with high prevalence of *S. agalactiae* the use of these products in cows, with both clinical and subclinical mastitis, is justified because it stops bacterial shedding. It should

be noted, however, that the treatment of clinical cases is not effective in reducing the herd prevalence unless it is part of a total control program.[120]

A considerable number of studies have looked at the *in vitro* activity of various antimicrobials against *S. agalactiae*. In a study, which included 39 strains of *S. agalactiae* from Florida, Louisiana, New York, Vermont, Washington and West Virginia, none were found to be resistant to penicillin.[121] Ninety five percent of *S. agalactiae* strains were susceptible to lincomycin and erythromycin. Seventy-five percent were susceptible to tetracyclines but susceptibility to streptomycin and spectinomycin was much lower.[121] Macdonald *et al* (1976) reported that in a study of a large number of streptococcal species all *S. agalactiae* (n=7) tested were sensitive to penicillin and its derivatives, and to tetracyclines and erythromycin.[122] In a separate report, none of the 14 *S. agalactiae* isolates tested in one study showed resistance to penicillin or it derivatives, or to erythromycin or tetracyclines.[123]

Sensitivity testing, *in vitro*, is an attempt to predict the likely activity of the material *in vivo*,[122] but the *in vivo* behaviour of the product may be different.[123] As a result, it is important to examine some reports of activity within the mammary gland. Craven (1987) reviewed the literature and found a mean cure rate for *S. agalactiae* of 85%.[124] Others have noted that both lactational and dry cow therapy results in greater than 90% cure rates.[116] Huber (1977) reported that 98% of *S. agalactiae* infected cows responded to treatment with penicillin or its derivatives.[115] He also reported that the current sensitivity to penicillin is about the same as it was when penicillin was first introduced.[115] In an English study, 98% of the cows infected with *S. agalactiae*

eliminated the bacteria after therapy with cloxacillin.[114] In a dairy herd in Scotland, 22 cows in a herd of 83 were found to be shedding *S. agalactiae* on composite cultures. All infected cows were treated with cloxacillin for three treatments and none were found to be culture positive one week later.[125]

In a large Pennsylvania study[83] of farms with long standing udder health problems, quarter milk samples were used to identify infected cows. All animals shedding the bacteria were treated with a penicillin/novobiocin intramammary infusion product. Each producer was to follow up the therapy protocol with a rigorous program of post milking teat dip and treatment of all quarters at dry off. Using this strategy, the producers attained a cure rate of 92.6% for quarters and 88.3% for cows in 30 days. The quarter infection rate dropped from 23% to 3.4% in 30 days and to 1.6% in one year and the cow infection rate dropped from 46% to 9.3% in 30 days and to 4.2% in one year.[83]

In a California study[126], 220 cows were identified by composite milk culture as infected and were stratified by lactation number. One group was treated with a commercially available intramammary infusion product containing 100,000 IU of penicillin and 150 mg novobiocin; the other group was given 1.2×10^6 IU of procaine penicillin G in 10 ml of sterile saline. When the infected cows were cultured again, 21 to 25 days after treatment, 90% were no longer shedding the bacteria. The cure rate in the commercial product was higher at 94%, than the 87% recorded for the home made penicillin suspension. The previous SCC, as estimated by the California mastitis test was the next most important factor affecting the treatment outcome with high SCC cows less likely to have the infection eliminated. Heifers had improved recovery rates. Herd of

origin was also associated with the outcome of therapy.[126]

There are occasions when it may be beneficial to try to reduce the prevalence of subclinical mastitis in a dairy herd more rapidly than can be achieved with dry cow therapy and post milking teat dip.[1] In particular, lactational therapy of all infected animals in herds with *S. agalactiae* may be economical.[1,4,83,85,127,128,129] The term "blitz therapy" can refer to treatment of all cows in the herd at one time, but more commonly refers to the immediate treatment of all cows found to be culture positive.[116,130] An outline of an eradication program, adapted from Kirk and Mellenberger,[130] employing a blitz therapy protocol is included in Appendix A.

1.4.3.2 Dry cow therapy

In field experiments conducted by the National Institute for Research in Dairying use of post milking teat dip was seen to reduce the new infection rate, but had little effect on existing infections.[8] The management procedure, that can most easily alter the percent of quarters infected in a herd without destroying saleable milk, is dry cow therapy.[1] Dry cow treatment generally enhances the cure rate of existing infections and should be used in herds with contagious mastitis pathogens.[131] It should be noted that mammary secretions during the dry period support the growth of mastitis associated streptococci better than milk obtained during lactation.[132] Only 36 to 42% of cows were able to spontaneously eliminate the infections due to streptococci during the dry period. The use

of dry cow therapy was associated with a cure rate of 95% during the same period.[133] The combined use of post milking teat dip and dry cow therapy is the fundamental means of controlling contagious mastitis.[1]

The application of dry cow therapy to all cows in the herd was found to be associated with decreased SCC. Although the financial returns to investment in dry-cow therapy vary greatly among farms, in general, the benefits of dry cow therapy exceed the costs.[134]

A Pennsylvania study of 16 high and 16 low SCC herds found that 81.3% of herds with low SCC were treating all cows at drying off compared to 56.3% of herds with high SCC.[80] In a larger study in the same state, 70.8% of the low SCC herds used post milking teat dip and total dry cow therapy, while only 36.2% of the high SCC group used these practices.[106]

In an Ohio study of herds shipping milk to a cooperative giving premiums for low SCC milk, managers in herds with *S. agalactiae* were less likely to dry cow treat.[15] In a study of 1,032 farms in the same state, herds with *S. agalactiae* were also less likely to use dry cow therapy.[74] The use of selective dry cow therapy, as opposed to treatment of all cows at dry off, was associated with a higher herd prevalence of *S. agalactiae* in two different studies.[26,28]

Erskine (1992) noted that only herds using post milking teat dip and dry cow therapy after blitz therapy programs for *S. agalactiae* maintained the reduced SCC levels achieved by treatment protocol.[1]

Although it has been stated that dry cow therapy and post milking teat dip will

eradicate *S. agalactiae* from herds[8,14,53], and there has been widespread adoption of these two practices,[22,26,27,32] there is still a high herd prevalence of the infection.[22,24,26,27,30,31] It appears that at the field level these procedures are more useful for reduction of infection levels than the complete elimination of the infection from dairy herds.[56]

1.4.4 National Programs

Some countries have undertaken national or regional programs aimed at the eradication of *Streptococcus agalactiae*. These programs have legislative authority to implement control measures and have been successful in dramatically lowering the prevalence of the disease.

Israel has a system of regional laboratories with regulatory authority to implement a *S. agalactiae* eradication program. At one regional laboratory, a decrease in herd prevalence of *S. agalactiae* from 28% of herds to less than 2% was noted in the first 5 years of the scheme. There were approximately 625 herds under the jurisdiction of the laboratory and they ranged in size from 20 to 500 cows. Each of the dairies had their bulk tank milk cultured monthly and in infected herds, milk from all the lactating cows was examined individually. In addition, an assessment was made of management, sanitation and milking practices. All cows found to be positive were treated with 600,000 IU of procaine penicillin G intramammary twice at 24 hour intervals. Cows were resampled either weekly or every other week depending on the size of the farm. Animals that were still positive after the first treatment were either culled or treated again.

Previously infected herds were declared to be free of the disease after a rigorous culturing protocol which lasted 18 months. Less than 3% of infected cows needed to be culled because they were refractory to treatment.[135]

In Denmark herd milk supplies are cultured annually for the presence of *S. agalactiae*. All cows on farms found to be positive on bulk tank culture are sampled individually.[136] Herd prevalence of the disease decreased from 15% to 2% between the 1950's and 1970's after implementation of this program.[137]

Control and prevention programs either at the farm, regional or national level, using both management tools and therapeutic regimens, have been effective in reducing *Streptococcus agalactiae* levels.

1.5 ECONOMICS

A large body of evidence has been generated documenting the economic impact of subclinical mastitis in general and *Streptococcus agalactiae* in particular. This impact has been measured both through the direct effect of infection and through surrogate measures such as SCC.

1.5.1 General: Subclinical Mastitis

In a literature review, Blosser (1979) found estimates of the cost of mastitis to range from $35 to $294 per cow per year or approximately 1.3 billion dollars in the

United States in 1976.[5] Reneau (1991), also in a literature review, noted that 70-80% of the loss due to mastitis was due to the subclinical form and 20-30% was due to clinical mastitis.[2] Janzen (1970) found that milk yield losses due to mastitis were 2.8 to 83.9% of potential production with the majority in the 10 to 20% range.[6] In one study, infection with mastitis reduced the milk output of a quarter by 30% on average. The net reduction was 20% after allowing for the compensating affect of adjacent quarters.[138] In 1969 King reported that quarters infected with *S. agalactiae* produced 18% less milk, 8.6% less fat and 3.1% less solids-nonfat than did noninfected quarters.[4]

In an Ohio study to determine the economic impact of disease on dairy farms, mastitis was found to be the most significant disorder[139]. The estimate of $45 per cow per year for the cost of mastitis was largely based on the level of clinical disease as reported by producers. If SCC data were included, it was anticipated that the value would have been much higher.[139] In another study, the 365 day rolling herd production in herds with greater than 700,000 average SCC, was 5,900 kg whereas yield was 8,134 kg in herds with less than 150,000 average SCC.[80]

The major costs associated with mastitis include lost production, lost milk premiums, culling, discarded milk, treatment costs and veterinary fees, and added labour requirements.[4,140] When all these factors were accounted for, the mean economic loss associated with mastitis was $266.68/cow/year in four herds, two with high SCC and two with low SCC.[140]

It is critical in any udder health management program to set appropriate goals for both SCC and clinical mastitis levels. The return on investment of a mastitis control

program based on milking hygiene, teat dipping, therapy of all quarters at dry off, treatment of clinical cases and culling of chronic cases has been as high as 600%.[141] Other researchers have shown negative marginal values of implementation of udder health management programs.[140]

1.5.2 Effect of elevated somatic cell count

Leslie *et al* (1983) reviewed the literature and found that elevated SCC indicated production losses associated with subclinical mastitis.[73] Herds with high SCC usually have chronic infections with contagious pathogens while low SCC herds tend to have environmental infections characterized by more acute toxic effects. The economic impact of chronic subclinical mastitis is greater because more cows and quarters tend to be infected. The value of elevated SCC as a protection against intramammary infection has been questioned. A healthy cow is able to respond to mammary infection with adequate cells regardless of her initial cell count.[142] The estimates of production losses associated with elevated SCC in individual cows vary. They are generally in excess of 20% of potential production.[73] The effect of a BTSCC of 500,000 above a baseline of 200,000 has been reported to be a 5% production loss. The effect of a 1,500,000 BTSCC over the same threshold was estimated to be a 25% production loss.[32]

Dairy Herd Improvement Association (DHIA) linear scores are natural logarithmic transformations of raw SCC values. The midpoint of linear score 1 is 25 x 10^3 cells. Each doubling of SCC increases the linear score by 1 level. Linear score 2 has a

midpoint of 50 x 10^3; linear score 3 corresponds to a midpoint SCC of 100 x 10^3 and so on. Linear scores on individual cows are a better indicator of the economic loss associated with mastitis than is the BTSCC.[143] Because of the curvilinear relationship that exists between SCC and milk loss, greater production losses are associated with given increases of SCC in low SCC cows.[144]

In a large study of 10,705 cows from 770 herds, an increase in linear score SCC of one level was associated with an decrease in milk production over predicted production levels between first and second lactations of 205 kg. The effect between second and third lactations was slightly larger at 221 kg. The effect of SCC in a previous lactation, regardless of level, on milk production in a subsequent lactation was much less than the effect of SCC in the current lactation.[15] This indicates that if infection can be cured and SCC return to normal then the milk production will also be restored. Fetrow (1988) noted a 190 kg decrease in milk production per lactation for each increase in linear score of 1 level.[145] Reneau (1986) reviewed the literature and found a daily milk loss of between 0.7 and 1.44 kg of milk per day associated with increasing log increments of SCC.[3]

The prevalence of high SCC was greater when either *S. agalactiae* or coagulase positive staphylococci were isolated from bulk tank milk. When analyzed in a regression model, including a wide variety of management factors, the presence of *S. agalactiae* in the bulk tank milk culture was the single most important source of variation.[48] In one study, quarters in which subclinical *S. agalactiae* infection was diagnosed had an average SCC of 900,000.[81] In another investigation, the geometric mean SCC for *S. agalactiae* infected cows was 2,238,700.[80]

It is important to note that while cows with subclinical mastitis infections have an elevated SCC, it is not economical to treat lactating cows based on SCC.[3,146] The cost of treatment was not returned when lactating cows were treated based on a SCC elevated over a 400,000 cells/ml threshold. The poor response may have been due to infections that are refractory to treatment (*S. aureus*) or because a lot of negative cows were identified as infected (false positives). The net loss per cow treated with this program was $19.65.[146]

1.5.3 Clinical mastitis management

Most investigations have found a reduction in the levels of clinical mastitis with improved udder hygiene and reduced SCC. The costs of clinical mastitis was estimated at $107 per case or $38 per cow per year in an Ohio study.[69] The incidence of clinical mastitis dropped from approximately 140 cases per 100 cows per year to 50 cases per 100 cows per year in the 15 years following publication of the National Institute for Research in Dairying (NIRD) program in the UK in the early 1970s. The prevalence of subclinical mastitis has fallen from 50% of cows to 32% of cows and the national average BTSCC has gone from 570,000 to 352,000 during that same period.[99,147]

In England, after the implementation of an udder health monitoring scheme in 1979, the incidence of clinical mastitis dropped from 51 to 32 cases per 100 cows per year over a five year period. The herd mean SCC decreased from 346,000 to 243,000 during that same time.[148] Dohoo *et al* (1984) found an increased risk of developing a

clinical episode of mastitis in cows that had elevated SCC.[75]

In contrast, in a study of clinical mastitis levels on farms with high or low SCC, the low SCC group had 4.2 clinical episodes per 100 cows per month whereas the high SCC herds had 2.9 clinical episodes per 100 cows per month. Over 40% of the clinical cases in the high SCC group were attributable to *S. agalactiae*. In herds with a low SCC nearly 50% of mastitis cases occurred in the first month, whereas the episodes of clinical mastitis in the other herds were spread evenly throughout the lactation.[149]

1.5.4 *Streptococcus agalactiae* eradication programs

Sears (1982) estimated that the loss to the dairy industry in Mississippi due to infection with *S. agalactiae* to be 3.6 million dollars.[24] Sandholm *et al* (1990) reported that in countries where antibiotics have been widely used, *S. agalactiae* has been eradicated, but they did not cite references.[150] National programs in Denmark and Israel have drastically decreased the herd prevalence in these countries.[135,137]

S. agalactiae is the only subclinical mastitis pathogen that can be treated economically during lactation.[127] *S. agalactiae* was eliminated from herds that were blitz treated with antibiotics and followed up with good sanitation procedures more quickly than blitz treated herds that did not have follow up sanitation.[56] Cows that are nonresponsive to the first treatment and are not identified for further treatment or culling can serve as reservoirs of infection. In herds where teat dipping and other hygiene practices are not adequately performed the bacteria can quickly spread to the noninfected

cows.[127]

The literature contains several references to eradication programs and their cost effectiveness.[4,83,85,128,129] In twelve herds, where the yearly mean herd SCC was greater than 700,000, Erskine and Eberhart (1990) found more than 25% of cows had *S. agalactiae*.[83] All cows were quarter sampled. Infected cows were treated with a penicillin/novobiocin intramammary infusion. Each herd also started on a program of post milking teat dip and blanket dry cow therapy. Cure rates were 92.5% for quarters and 88.3% for cows in the first 30 day period. This program yielded a net benefit of 512 kg of milk and 14 kg of fat per cow in the first year. Costs included in the analysis were treatment costs, cost of withholding milk, and the cost of collecting and processing the samples. The benefits were based on increased milk production over a control group with similar starting SCC levels. The cost benefit ratio was calculated at 2.28:1 for the entire program. Comparing total blitz therapy, treatment over a certain SCC threshold or culture and treatment of positive animals, the last had the best cost benefit ratio.[83]

In a study of an epidemic of *S. agalactiae* mastitis in a large California herd (n=627) composite samples were taken from all lactating cows. Ninety nine infected cows were treated with penicillin or a penicillin/novobiocin combination. Three weeks after the blitz treatment 97 of the 99 cows were found to be free of the infection. Expected lactation curves of the infected cows without treatment were compared to curves after treatment and to a series of control cow's lactation curves. The control cows were matched for production, stage of lactation and gestation and lactation number to each case animal. The evaluation revealed no significant difference between the milk production

of infected cows post treatment and cows that had never been infected. The level of production returned to normal after treatment during the same lactation. The economic benefits of the mastitis treatment program were also calculated. The simulation model predicted depressed lactation yields of approximately 25, 16, and 8% over the 305 day lactation period for cows becoming infected at 14, 63, or 126 days in milk respectively. As a result, the benefits of treatment differ with the stage of lactation. Treatment of infected cows early in lactation (< 60 days) yielded a net benefit of $396 and therapy of cows in mid-lactation (61-120 days) yielded a net benefit of $237. Treatment of cows with *Streptococcus agalactiae* in late lactation was associated with a net loss of $55 per cow. In this group, the increase in production was not maintained long enough to offset the costs associated with treatment. The overall benefit to cost ratio for the blitz therapy program was 2.25 to 1.[4]

S. agalactiae is the most common contagious pathogen causing herd level infection in St. Croix, U.S. Virgin Islands. In a random survey of cows 96.5% were infected with a pathogen including 26% which had *S. agalactiae*. None of the producers were teat dipping or using complete dry cow therapy and most were using a common wash rag to clean cows. The authors estimated that implementing a control program could return $317 to $999 per cow per year.[129]

On *S. agalactiae* infected farms where blitz treatment of all infected cows is not possible, treatment protocols have been modified. A modified blitz therapy program was used as part of a herd program to eliminate *S. agalactiae* from a seasonal herd in England. A basic udder health management program was instituted and the herd was

divided in two based on a SCC threshold of 500,000. Those cows in the high SCC category were treated with 300 mg of erythromycin intramammary. When lactating cow numbers ebbed to their lowest point, all animals were treated with the same product. Cows were treated at dry off with 500 mg of cloxacillin and 250 mg of ampicillin. When the herd was in full production again all cows were cultured. None of the animals present in the herd during the treatment protocol were still positive. The program was found to have a benefit cost ratio of 1.41:1.[128] In another modification of the blitz therapy protocol all cows with clinical mastitis or a CMT in any quarter greater than two were medicated. This therapy was combined with improvements in milking equipment and sanitation. In a case report using this method, the herd SCC dropped from 1,600,000 to 250,000 and SPC went from 99,000 to 2,000.[85]

Management programs for subclinical mastitis in general and *Streptococcus agalactiae* in particular are effective in controlling herd infection. The economics of such programs are generally very favourable when response is measured by changes in the prevalence of herd infection, incidence of clinical disease or SCC.

1.6 EXTENSION AND EDUCATION

The programs for control of subclinical mastitis have been developed and shown to be cost effective. There is a need to advance this information to the dairy producer and professionals in the industry.

1.6.1 Extension to Producers

Over the last 25 years, implementation of the National Institute for Research in Dairying (NIRD) program (which was later adopted by the National Mastitis Council (NMC) in the U.S.) at the farm level has been slow. Farmers have not been convinced of the enormous impact of subclinical mastitis and there have, in the past, been no economic incentives to produce higher quality milk.[2] Motivation of marginal clients is very challenging in mastitis control programs.[54] There must be reasonable and achievable goals for SCC levels so that they can be used for the evaluation of a herd's mastitis status and for motivating changes.[145] In general, programs fail because dairymen do not implement known control procedures, implementation is inadequate or there is a milking equipment malfunction.[54]

In 1987, a random group of producers in Pennsylvania who were enrolled in the SCC option of DHIA and had an average SCC greater than 700 x 10^3 were surveyed. None of the farmers would seek professional help for a herd average SCC less than 500,000 and 37.5% would not seek help for a herd level SCC of less than 1,000,000.[80] The standard for SCC under the U.S. Pasteurized Milk Ordinance for interstate milk transport dropped from 1,000,000 to 750,000 July 1, 1993.[1] Lack of understanding of mastitis or motivation to deal with it on behalf of farmers may be a critical factor in herds having problems with subclinical mastitis.[80] Unfortunately, reports on udder health and milk quality are usually not presented to the dairy producer in an integrated and organized manner to the dairyman. In addition, comprehensible interpretation of the results by

professionals in the field is often lacking.[52]

In a Texas study involving 138 producers on the DHIA SCC option, only 30% used all of the recommendations of the NMC mastitis control plan.[151] In a similar study in Ontario, 31.6% of farmers had adopted all of the NMC recommendations.[102] In a survey of milking practices and udder health in California, only 30% of the milkers used single service paper towel to dry udders and only 17% of dairies teat dipped all four teats adequately.[152]

Despite the poor implementation of these udder health management strategies, there is evidence that they can be beneficial. In a study of dairy farmers participating in a "Mastering Mastitis Program" extension scheme in Australia, the control practices reduced mastitis and increased production. For herds not involved in an udder health scheme previously, the gross increase in income was $70/cow/year.[153] As noted in section 1.5.3, the incidence of clinical mastitis and prevalence of subclinical mastitis has dropped since the implementation of the NIRD program in the UK.[99,147]

In a study of the effectiveness of education programs in the implementation of mastitis control procedures, those producers in a more intense education group used drycow therapy and post milking teat dip more frequently than controls. Prevalence of infection declined more rapidly in the herds taking part in the intense education program compared to a less intense program or controls.[154]

Many producers have inconsistent and fragmented views of the interrelationships of milking management and mastitis.[152] Although only 30% of producers were using all of the recommendations of the NMC, when surveyed on the important points of an udder

health program they listed the same procedures as NMC members.[101] This has lead some researchers to conclude that many farmers will only adopt a new technique to try to solve problems not to prevent them.[102] There is a continuing need to educate producers about the importance of subclinical mastitis.

1.6.2 Extension to Professionals

There have been very few studies to quantify the udder health knowledge of professionals in the field. In a Texas survey, the mastitis knowledge of extension personnel was found to be inconsistent or lacking.[151] Study of herd level disease and production dynamics has been identified as an important educational need in the veterinary community.[10] Despite this fact, a recent survey of veterinarians in Ohio shows some disappointing results.[155]

A random sample of 50 Ohio veterinarians in large or mixed animal practice was interviewed. While 96% reported using bacteriological culture of milk samples in the previous year, only 44% used microbiology to establish trends of herd infection. Eighty-eight percent reported using culture to determine the cause of failure to respond to treatment. Thirty-five percent of practitioners did not distinguish between *S. agalactiae* and other streptococci, although the epidemiology and control strategies for each are very different.[155] Only 54% of practitioners expressed a familiarity with linear scores and 86% preferred raw SCC. Veterinarians were more likely to use SCC for setting a milking order and to select cows for culture than were producers. However, 72% of veterinarians

cited the use of SCC to select cows for lactational treatment[155], despite the fact that research does not support this practice.[146]

1.6.3 *Streptococcus agalactiae* extension programs

The NMC and other organizations have produced extension materials to inform producers and professionals in the industry of the merits of mastitis control programs. Education on the epidemiology and eradication of *Streptococcus agalactiae* has been highlighted in these publications.[130,156] A description of one such program, adapted from Kirk and Mellenberger,[130] can be found in Appendix A.

There is no information in the veterinary journals which attempts to quantify the effectiveness of *S. agalactiae* extension efforts. Evaluation of the impact of education programs is limited to surveys of the uptake of general control measures, which have been the backbone of mastitis control for decades.[102,151,154] No studies have followed up dissemination of information on *S. agalactiae* mastitis to see the short and medium term effect on management procedures.

1.7 OVERALL OBJECTIVES OF THE STUDY

The objectives of this study were fourfold. The first objective was to establish the herd prevalence of *Streptococcus agalactiae* in the Prince Edward Island dairy industry. Prevalence studies documented in section 1.1.2 of this thesis have shown *S. agalactiae* to be an important cause of mastitis in other areas of North America. The second objective was to show the incidence of herd level infection in a group of previously negative herds over an 18 month period. Although prevalence studies are common, no studies showing the incidence of new herd infections have been published. Incidence data is valuable in understanding the dynamics of *S. agalactiae* infection in the population. The third and forth goals were to quantify the effectiveness of an intensive "on farm" education effort and a less intensive "mail out" extension protocol. Evaluation of the efficacy of such a targeted program for mastitis control and eradication has not been published in the veterinary journals. In addition to the above stated major goals, an attempt was made to identify the management procedures which affected herd level eradication of *S. agalactiae*.

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Chapter 2

Herd prevalence and incidence of *Streptococcus agalactiae* in the Prince Edward Island dairy industry

2.1 INTRODUCTION

An extensive review of the epidemiology and microbiology of *Streptococcus agalactiae* and an assessment of its effect on milk quality is presented in Chapter 1 of this thesis. An outline of control and therapy programs including their economic consequences is also provided.

Herd prevalence studies have been conducted in numerous jurisdictions. In census data from Mississippi[1] in 1982 (n=998), Vermont[2] in 1985 (n= 2931) and Vermont[3] again in 1990 (n=1971), herd prevalence rates for *S. agalactiae* were 44%, 47% and 32% respectively based on bulk tank milk culture. In a random sample of southwestern Ontario herds (n=250) in 1990, 42.4% of herds were found to be positive on at least one of four bulk tank milk cultures.[4] In an Irish study, 38.5% of herds tested (n=379) in a monthly bulk tank culture program were positive during the year long study.[5]

In a stratified random sample of herds in Ohio in 1989[6] (n=49) and 1992[7] (n=48), using individual cow milk samples, 32% and 56% of herds had at least one positive culture. In a Pennsylvania study[8] (n=29) in 1982, which also used individual cow culture, 60% of herds had at least one cow infected with *S. agalactiae*.

In a non-randomly selected group of California dairy herds in 1988, 47 of 50 herds

(94%) had at least one cow infected with *S. agalactiae*[9]. Prevalence in the same group of herds using bulk tank milk culture was 44%.[10]

In a review of 19,000 herd surveys conducted by the New York State Quality Milk Promotion Service (QMPS), more than half had at least one cow infected with *S. agalactiae*[11]. Many of these herds had been referred to the service because of chronic udder health problems. By contrast, in an Ohio study (n=802) of herds selling milk to a Cooperative giving premiums for low SCC, the herd prevalence of *S. agalactiae* was 5% based on bulk tank cultures.[12] The much lower levels of infection in this Ohio study suggest that the problem can be controlled at the field level.

S. agalactiae remains an important and prevalent cause of subclinical mastitis in the North American dairy industry. The herd prevalence of infection in Prince Edward Island (P.E.I.) was not previously known. The major objectives of the present study were to determine the prevalence and incidence of *S. agalactiae* infection in P.E.I. dairy herds. Milk quality and economic criteria were also examined to determine associations with infection status.

2.2 MATERIALS AND METHODS

2.2.1 Study population

The Prince Edward Island (P.E.I.) dairy industry consists of approximately 460 farms. Most of the milk cows are registered or grade Holsteins. The Animal Productivity

and Health Information Network (APHIN) at the Atlantic Veterinary College collects and collates information on health and production in the P.E.I. dairy industry.[13] From this database, it was determined that the average herd size of the 233 herds on milk recording programs with the Atlantic Dairy Livestock Improvement Corporation (ADLIC) was approximately 36 lactating cows in 1993 and the average daily milk production of this group was 23.1 litres. The average BTSCC for all herds in the province in 1993 was 290 x 10^3

2.2.2 Sampling schedule

For the census, which will be referred to as the *December 1992 Census*, two complete sets of bulk tank milk samples from all herds shipping milk in the province were obtained. The first sampling took place between December 7th and 14th and the second sampling occurred between December 28, 1992 and January 4, 1993. For the *June 1994 Census* two complete sets of bulk tank milk samples were obtained between June 13th and 20th. The culture results of each of these samplings were used to establish the prevalence of infection. The results of the two tests were interpreted in parallel, that is, a herd was considered positive if one or both tests were positive.[14] The incidence of new herd infections was established using the census data from December, 1992 as the baseline and the June, 1994 cultures to identify new infections.

2.2.3 Sample handling

Milk samples routinely collected by the truck drivers who haul milk to the dairies were used in this study. A sample of approximately 20 ml was taken at each milk pick up (typically every second day). The samples were immediately refrigerated and transported on ice to the provincial regulatory laboratory within 24 hours. In the laboratory the samples are used for assessment of milk quality, including periodic analysis of SCC, bacteria count and antibiotic residues. While milk samples were taken at each pick up, not all were needed to meet the requirements of the regulatory testing programs. The samples used in the present study were excess to the requirements of the regulatory program and were not used for any other purpose. For the *December 1992 Census* fresh milk samples were used. The majority of these samples were cultured within 24 hours of arrival at the laboratory or 24 to 48 hours after removal from the bulk tank. A small number of samples were greater than 48 hours old at plating but all were less than 96 hours.

In June, 1994 milk samples, which were not required to meet regulatory requirements of the milk quality laboratory, were only obtainable in a small time period. Availability of laboratory facilities dictated that not all samples could be cultured within these time constraints. As a result, samples for the *June 1994 Census* were frozen for storage prior to use. Most studies in the literature have found no changes, in either numbers of *S. agalactiae* bacteria per sample or classification of infection status, when frozen rather than fresh samples were used.[15,16,17] However, one study indicated, on

individual cow samples, an increase in cows classified as infected when frozen samples were used.[18] All samples were frozen within 24 hours of arrival at the regulatory laboratory, and were held at -20 °C for a period not exceeding 10 days before being thawed at room temperature and plated.

2.2.4 Media preparation

Various unique microbiological features of *S. agalactiae* have been exploited to identify the bacterium in the laboratory. The two most common attributes used are the production of the CAMP factor, a diffusible product which causes lysis of red blood cells in the presence of staphylococcal β haemolysin, and the inability of *S. agalactiae* to hydrolyse esculin.[19] More recent research has focused on the production of an orange pigment by *S. agalactiae* when grown on a selective media anaerobically.[20] The present study employed two media which utilized all of these characteristics.

All media were prepared from commercial agar bases by the media preparation section of Central Laboratory Services at the Atlantic Veterinary College. The media were modified as per the recommendations of Schoonderwoerd *et al* (1993).[20]

2.2.4.1 β haemolysin

Crude staphylococcal β haemolysin is incorporated into Edward's media to allow visualization of the CAMP reaction characteristic of *S. agalactiae* on primary isolation

plates. β haemolysin is not commercially available. The procedure for extraction of the toxin from *Staphylococcus aureus* has been described previously by Jasper and Dellinger (1968)[21] and Ward and Postle (1969).[22]

A fresh culture of *Staphylococcus aureus*, American Type Culture Collection (ATCC) number 25923, was grown on citrated 5% ovine blood agar. A 24 hour growth was inoculated into 500 ml of brain heart infusion (BHI) broth at a pH of 7.4. and incubated aerobically at 35°C for four days with constant agitation. It was then centrifuged at 15,300 x g for 20 minutes at 4 °C. The cell free supernatant was sterilized using a 0.22μ Nalgene® disposable filter unit. The resulting sterile crude β haemolysin solution was stored in screw top bottles in a light excluding chamber at 4°C.

Potential variability in the concentration of haemolysin, due to the crude nature of the extraction procedures, and differences in the susceptibility of ovine erythrocytes to lysis between blood lots, required that the quantity of haemolysin for inclusion in the Edward's media be titrated for each new blood lot. The concentration of β haemolysin required was determined by two methods. The first titration procedure, hot-cold tube titration, set a range of levels to be further evaluated in a within media titration using known cultures of CAMP positive and negative bacteria.

In order for the first titration to be conducted, it was necessary to prepare a 2% suspension of washed ovine erythrocytes from the blood lot to be used in the modified Edward's media. Defibrinated sheep blood was washed three times by alternating suspension in 10 ml of normal saline and centrifugation. After the final wash, 0.2 ml of packed red cells was added to 10 ml of normal saline to provide the necessary 2%

suspension for the hot-cold tube titration procedure.

Twelve serial dilutions of β haemolysin ranging from 1 in 16 to 1 in 32,000 and negative and positive controls containing brain heart infusion (BHI) and undiluted β haemolysin were tested in 1% ovine erythrocytes during the hot-cold tube titration. These mixtures were incubated for 30 minutes at 37°C and then moved to a refrigerator where they were kept at 4°C for 4 hours before reading. The resulting haemolysis was recorded as complete, incomplete or negative. Complete haemolysis was recorded when red tinged supernatant was found and no build up of red cells was located at the bottom of the test tube. Tubes were classified as incompletely hemolyzed when there was a red tinged supernatant and a small accumulation of red cells at the bottom of the test tube. Negative tubes had a clear supernatant with a large collection of erythrocytes at the tube base. Four successive dilutions were chosen to be used for the media titration. The last tube showing complete haemolysis and the subsequent three dilutions were selected. This allowed inclusion of levels demonstrating complete, incomplete or no haemolysis in each media titration.

The agar used for the media titration was the modified Edward's described below. Four plates of each dilution of β haemolysin were prepared. Four bacterial strains were inoculated at each haemolysin level; *S. agalactiae* (ATCC 13813) a non β haemolytic, non pigment producing strain with very weak CAMP (β enhancement) reaction; a Prince Edward Island (P.E.I.) field strain of *S. agalactiae* that was both β haemolytic and pigment producing with strong CAMP reaction; a CAMP positive Alberta field strain of *Streptococcus uberis* that was non pigmented and not β haemolytic; and a P.E.I. field

strain of *Staphylococcus aureus*. Those culture plates, on which the clearest β enhancement (CAMP reaction) could be demonstrated, were considered to contain the most useful dilution of the crude β haemolysin.

2.2.4.2 Modified Edwards® media

Commercial modified Edwards® media base by Oxoid® was further modified by addition of ferric citrate (0.05 g/L), ovine blood (50 ml/L) and staphylococcal β haemolysin (the amount determined by the previously described titrations). The pH was adjusted to 7.65 using 1 M NaOH. The pH of the solid agar must be above 7.4 to allow maximum development of the CAMP reaction. The media was stored at 4°C and used within one week.

2.2.4.3 Modified Islam's GBS® media

Commercial group B strep media, Islam's GBS® by Oxoid was modified by the addition of sterile inactivated horse serum (50 ml/L Oxiod Horse Serum®) and gentamicin (5 mg/L). The pH was adjusted to 7.6 by using 1 M NaOH. The pH of the solid agar must be 7.5 ± 0.1 to allow for good expression of pigment producing capabilities. The agar plates were stored at 4°C.

2.2.5 Inoculation and incubation

Bulk tank milk samples were allowed to reach room temperature and were shaken to break up bacterial clumps. A 150 µl aliquot of the well mixed milk sample was dispensed by pipette onto the modified Edwards® and modified GBS® media. Each plate was individually identified with the producer's shipping number. The inoculum was spread evenly over the entire plate using a sterile glass spreading rod. The milk was allowed to air dry on the media before inversion for incubation.

The Edwards® media was incubated aerobically at 35°C for 48 hours. Colonies were classified as negative, suspicious or highly suspicious. The criteria for classification of isolates on Edwards® media is outlined in Appendix B.

The GBS® media was incubated anaerobically at 35°C for 48 hours. Colonies were classified as negative, suspicious or highly suspicious. The criteria for classification of isolates on GBS® media is outlined in Appendix C.

2.2.6 Subculturing and identification

All plates, with bacterial colonies that were either suspicious or highly suspicious on GBS® or Edwards® agar, were subcultured onto quadrants of 5% ovine blood agar. The blood agar plates were incubated aerobically for 24 hours. Identification was confirmed using the Streptex® latex agglutination test kit by Wellcome®. Latex agglutination, as a confirmatory test on bacterial colonies, is very accurate. Sensitivity

and specificity of latex agglutination was 97.6 and 98.2% for *S. agalactiae*, respectively, in a study of bacterial isolates from bulk tank milk.[23] Other studies have reported sensitivity and specificity levels of 98 to 100% for *S. agalactiae*.[24,25,26]

2.2.7 Data processing and analysis

Data were stored as collected in dBASE III PLUS®[27] (Ashton-Tate) and analyzed using the Statistix® statistical software program[28] version 4.0 (Analytical Software, St. Paul, Minnesota) and Epi Info[29] version 5.01a (Centres for Disease Control, Atlanta, Georgia and World Health Organization, Geneva, Switerland).

2.3 RESULTS

2.3.1 Test characteristics (sensitivity, specificity, predictive value)

The sensitivity of the culture procedures used had previously been reported to be 95% when compared to a gold standard of individual cow culture.[20] Although individual cow cultures were not carried out in this study, it was possible to estimate the sensitivity of the culture procedure using data generated during the *December 1992 Census* and *June 1994 Census* and a special culture of herds, with conflicting results, in December, 1992, which was conducted in January, 1993.(see Appendix D)

A total of 77 herds were found to be infected with *S. agalactiae* on the original

series of two bulk tank cultures in December. A herd was considered to be positive if *S. agalactiae* was cultured from one or both bulk tank milk cultures. The specificity of the test was assumed to be 100% due to the obligate nature of infection and the reported specificity of the confirmatory latex agglutination test. This supposition has been made by other researchers.[6] Sixty of the 77 herds were identified on the original culture giving an estimate of the sensitivity of 78%. Fifty-two of the 77 positive herds were identified on the second culture yielding a sensitivity estimate of 68%.

Out of the 452 herds, having bulk tank milk cultures at each sampling in the *December 1992 Census*, a total of 42 herds had conflicting results between culture one and two (they were positive on one of the cultures but not both). Forty of these 42 herds with conflicting results had a third bulk tank milk sample cultured in January of 1993. On this culture 26 of these 40 herds were identified as *S. agalactiae* positive. The estimate of sensitivity yielded by this method is the most conservative at 65%. These 40 herds may have been more difficult to diagnose as positive because some colony characteristics differed between this group and the 35 herds (70 cultures) that were positive on both of the December, 1992 cultures. The differences between these two groups of herds, with respect to the visible CAMP reaction and pigmentation, is outlined in Table III.

Table III Pigment production and visible β enhancement (CAMP) of 112 milk culture isolates from 77 herds identified as infected with *S. agalactiae* in the December, 1992 milk culture series.

	number of isolates	pigmented (%)	β enhancement (CAMP) zone (%)	both pigmented and β enhancement (%)
culture results agreed[a]	70	57 (81.4%)	48 (68.6%)	48 (68.6%)
culture results disagreed[b]	42	12 (28.6%)	16 (38.1%)	7 (16.7%)

[a]35 herds had *S.agalactiae* isolated on both the first and second culture protocols (70 cultures)
[b]42 herds had *S. agalactiae* isolated on one culture protocol but not the other

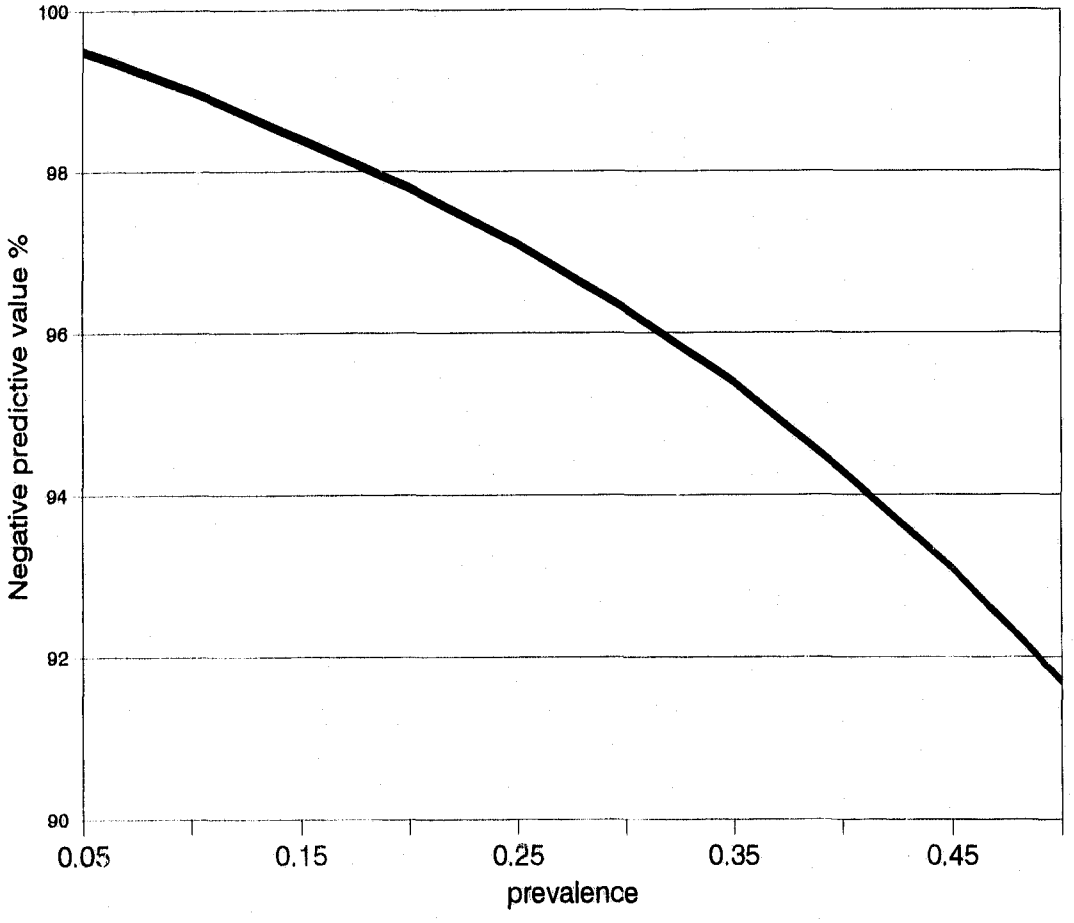
Similar estimates of the sensitivity of the bulk tank culture protocol were generated from the *June 1994 census*. A total of 57 herds were classified as infected at that time based on parallel interpretation of the results of two samplings. Thirty-eight and 42 herds were identified at each respective culture. These values yield sensitivity estimates of 67% and 74%, respectively.(see Appendix D)

Based on the above estimates, it was reasonable to assume the sensitivity of a single culture protocol (one Edwards® and one GBS® culture) was approximately 70%. Since each bulk tank was checked twice over a three week period, the combined estimate of the sensitivity of the test procedure, when the results are interpreted in parallel, was approximately 91%.(see Appendix D) This value assumes that the tests are independent and an evaluation of this assumption is presented in Appendix D.

The predictive value of a testing protocol is dictated by the sensitivity and specificity of the test and the prevalence of infection in the population being tested. The specificity of the testing procedure in this study was assumed to be 100%. The positive predictive value, the proportion of positive herds that are truly infected, is therefore, also 100%. The negative predictive value, the portion of negative herds that are true negatives, can be calculated from the sensitivity, specificity and prevalence of the disease in the population. The negative predictive value for the test, when the prevalence of diseases is less than 20%, is greater than 97.8%. (see Figure IV)

Figure IV

Negative predictive value of parallel interpretation[a] of 2 bulk tank milk culture protocols each using GBS® and modified Edwards® media for identification of *S. agalactiae*



[a] Estimated sensitivity of parallel interpretation was equal to 0.91

2.3.2 Herd prevalence of *Streptococcus agalactiae*

The calculated herd prevalence of *Streptococcus agalactiae* from the *December 1992 Census* 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. If the results from the two samplings are interpreted in parallel, and this interpretation has an estimated sensitivity of 91% (see section 2.3.1), a more accurate estimate of the true herd prevalence at that time would be 18.9%.(see Table IV)

Herd prevalence can also be calculated from the *June 1994 Census* data. A total of 434 herds had culture results available from each of the two samplings. Fifty-seven of these herds were found to be infected on at least one of these cultures. The calculated herd prevalence from this data was 13.1%. If the assumption of 91% sensitivity on the two cultures interpreted in parallel is valid, a more accurate estimate of the herd prevalence would be 14.4%.(see Table IV)

Table IV Herd Prevalence of *Streptococcus agalactiae* in Prince Edward Island in December 1992, and June 1994.

	number of herds[a]	number of positive herds[b]	apparent prevalence	estimated true prevalence[c]
December 1992 census	452	77	17.1%	18.9%
June 1994 census	434	57	13.1%	14.4%

[a]Herds with culture results on both GBS® and Edwards® media at each sampling (2) during the census
[b]Herds with *S. agalactiae* isolated on one or both bulk tank milk cultures
[c]Formula for estimation of the true prevalence of disease based on the apparent prevalence and the test characteristics[30] (derivation of the sensitivity and specificity values for the culture protocol is outlined in section 2.2.1 and Appendix F)

$$p = \frac{t + \beta - 1}{\alpha + \beta - 1}$$

Where,

p = estimate of true prevalence
t = apparent prevalence
β = estimate of specificity
α = estimate of sensitivity

2.3.3 Herd incidence of *Streptococcus agalactiae*

There were 410 herds that had 2 bulk tank milk cultures from both the *December 1992 Census* and *June 1994 Census*. Those herds with culture results on the *June 1994 Census*, and which were found to be infected using the original culture protocol (*December 1992 Census*) were excluded. Removing these 68 herds left a population of 342 herds at risk of becoming infected. The time frame of the incidence study was December, 1992 to June, 1994 or 1.5 years. During this period of 513 herd years of risk, 18 herds became infected. The incidence of new herd infections with *S. agalactiae* was 0.0351, or 3.51 new herd infections per 100 herds per year.

2.3.4 Association of *S. agalactiae* infection and bulk tank SCC

A comparison of the five month average of bulk tank somatic cell count (BTSCC) between the *S. agalactiae* positive and *S. agalactiae* negative herds was made using the two sample t-test procedure and the Mann-Whitney rank sum test in Statistix®. The five month average BTSCC was computed using the 2 months prior to the culture, the month of the culture and the two months subsequent to the culture. Log transformation of the BTSCC data was necessary prior to use of the t-test because the raw cell counts were not normally distributed. Four hundred and twenty-three herds had both complete culture and BTSCC data. Descriptive statistics and the association between infection status and BTSCC are outlined in Table V. There was a higher BTSCC in *S. agalactiae* positive

Table V Descriptive statistics and association between *S. agalactiae* infection in December, 1992 and average BTSCC in October, November, and December, 1992 and January and February, 1993

	number of herds[a]	mean BTSCC ($x 10^3$)	median BTSCC ($x10^3$)	geometric mean BTSCC ($x 10^3$)
S. agalactiae negative	348	273	242[b]	237[c]
S. agalactiae positive	75	462	388[b]	402[c]

[a]Only herds with both complete culture results from the *December 1992 Census* and BTSCC values for all five months were included

[b]difference significant using the Mann-Whitney rank sum test ($p < 0.001$)

[c]difference significant using a two sample t-test ($p < 0.001$)

herds compared to culture negative herds.

Conducting a census of the milk shipping population is both expensive and time consuming. It may be desirable to sample a subsection of the population to decrease costs. While this was not done in the present study, it is of interest to determine what proportion of the herds identified as infected would have been included in various subsamples of the population based on BTSCC history. Table VI illustrates the proportion of infected herds that would be included in the subsample at various five month average BTSCC thresholds. Figure V illustrates the percentage of infected and culture negative herds in various five month average BTSCC categories. The population base for each sample was the 423 herds that had complete BTSCC data from October, 1992 to February, 1993.

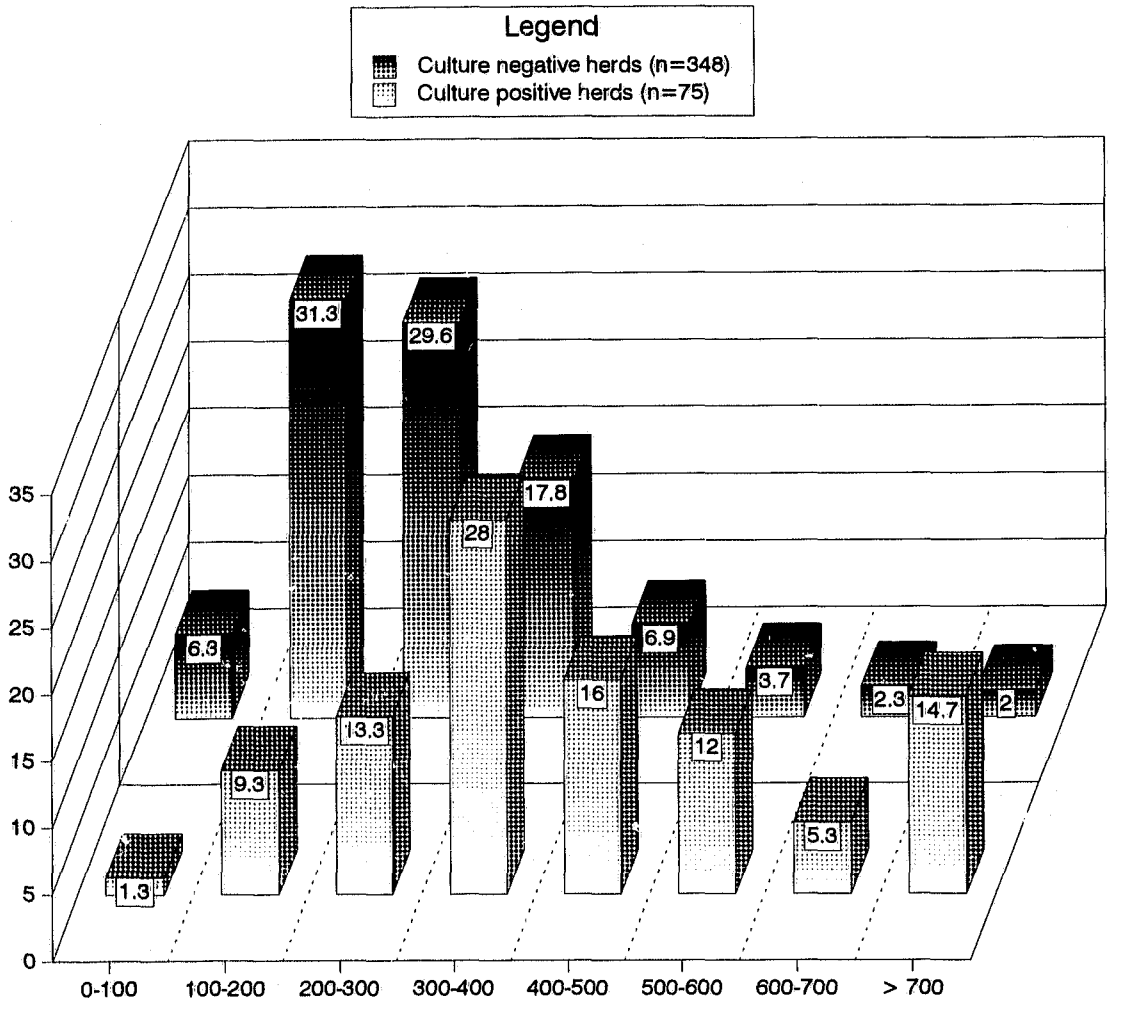
Table VI Proportion of infected herds[a] included within various subsamples of the P.E.I. dairy industry based on average BTSCC from October, 1992 to February, 1993

threshold value	number of herds cultured	number of infected herds cultured	percent of infected herds included
census[b]	423	75	100%
BTSCC greater than median (263×10^3)	211	61	81%
BTSCC greater than 300×10^3	171	57	76%
BTSCC greater than 350×10^3	129	46	61%

[a]Infection status based on December, 1992 census results. Seventy five of the 77 herds identified as infected had complete BTSCC values from October, 1992 to February, 1993

[b]Only herds with BTSCC data in each of the five test months were included in this analysis (n=423)

Figure V Percentage of *S. agalactiae* infected and culture negative herds within various categories based on the five month average BTSCC between October 1992, and February 1993



Average bulk tank somatic cell count

2.3.5 Association with milk quality penalties

The provincial milk regulatory laboratory uses multiple criteria to assess milk quality. In Prince Edward Island, penalties are assessed to producers based on the following measures of milk quality; BTSCC, standard plate counts and antibiotic residue testing.

Penalties for BTSCC are imposed based on two scales. Industrial milk producers, those shipping milk for processing only, are penalized 2¢ per litre for one month if their BTSCC rises above a threshold of 750 x 10^3 cells per ml for two consecutive months. Fluid milk producers, who have a portion of their milk used for fluid consumption, are penalized 2¢ per litre plus the loss of their fluid producers premium for that month if their BTSCC is in excess of 500 x 10^3 cells per ml for two consecutive months. If a fluid milk producer has BTSCC in excess of this limit for four months in the same dairy year (August 1 to July 31) that producer loses their fluid producer status for the remainder of the dairy year and the following year.

The association between the incidence of BTSCC penalties over the months October 1992 to March 1993 (five two month penalty periods) and infection status in December of 1992 was analyzed. The chi-square statistic was used to assess whether infected herds were more likely to receive one or more penalties, whereas the Mann-Whitney rank sum test was employed to determine if penalties were more frequent in the infected herds. Results of the analysis are outlined in Table VII. The risk of being penalized for BTSCC violations is 3.90 times greater in the infected herds than the culture

Table VII Association between total number of BTSCC penalties and number of herds penalized between October, 1992 and March, 1993[a] and the *S. agalactiae* infection status in December, 1992.

	Number of herds	number of BTSCC penalties	penalties per 100 herds	number of herds penalized
S. agalactiae positive	77	35	45[b]	16[c]
S. agalactiae negative	375	30	8[b]	20[c]

[a]Five 2 month penalty periods

[b]Positive herds receive more penalties as analyzed by the Mann-Whitney rank sum test ($p = 0.03$)

[c]Positive herds are more likely to be penalized as analyzed by the chi-square test ($p < 0.001$)

negative herds (Taylor series 95% confidence interval 2.12<RR<7.17).

Bacteria counts on milk shipped from P.E.I. dairy herds are also monitored using the standard plate count (SPC). Industrial milk shippers are penalized 2¢ per litre if the SPC is elevated above a threshold of 200 x 10^3 colony forming units (cfu) per ml twice in a two week pay period. Fluid milk producers are penalized 2¢ per litre and lose their fluid premium for a two week pay period if the SPC on milk that they ship rises above 50 x 10^3 cfu per ml twice during a pay period. If a fluid producer has 4 such violations in a dairy year (August 1 to July 31) that producer loses fluid status for the remainder of the dairy year plus the following dairy year. Twenty-six bacteria penalties were imposed on 17 farms in the period October 1, 1992 to February 28, 1993. Nine of the penalized farms (15 penalties) were from the infected group (12%) and 8 penalized farms were from the culture negative group (2%). Employing the chi-square statistic *S. agalactiae* infection is associated with SPC violations. ($p < 0.001$) The relative risk of penalty for the infected group was 5.48 times higher than the culture negative group (Taylor series 95% confidence limits 2.1?<RR<13.75).

Nine inhibitor violations (antimicrobial residues detected) occurred in 8 dairy herds in the six month period November, 1992 to April, 1993. Two *S. agalactiae* infected herds (2.6%) had violations (one herd had two violations) and 6 culture negative herds (1.6%) tested positive for residues. The difference between these groups was not significant ($\chi^2 = 0.37$, $p = 0.5454$). There was no apparent association between inhibitor violations and *S. agalactiae* infection status in P.E.I.

The program in which fluid producers lose their fluid status due to excess BTSCC

penalties was not instituted until the 1992-1993 dairy year was partially over. Despite this, calculations were based on an entire year to more closely estimate the cost of such penalties. Fourteen dairy herds that shipped milk at a premium price because a portion of that milk was used as fluid product would have lost fluid status in P.E.I. in the August 1, 1992 to July 31, 1993 dairy year. The average length of loss of fluid status would have been 16 months. The average herd losing status reaches 4 penalties at month eight and would lose fluid status for the remainder of that dairy year and for the following year. Seven of these producers were from the *S. agalactiae* infected group (n=32) and 7 were from the culture negative herds (n=249). A chi-square analysis of this data revealed that *S. agalactiae* infection was significantly associated with loss of fluid status ($p < 0.001$). The risk of losing fluid premium status was 7.78 times higher in the infected herds (Taylor series 95% confidence interval $2.92<RR<20.75$).

2.3.6 Estimate of economic impact of infection

There are three major sources of economic loss to which *S. agalactiae* infection may contribute. Herds in this census that were infected with *S. agalactiae* had significantly higher BTSCC in the 5 month period around sampling and this would have been associated with a lower level of milk production. Secondly, herds with the infection were more likely to incur milk quality penalties for both BTSCC and elevated bacteria counts than were culture negative herds. Finally, infection with the bacteria was associated with the loss of fluid status for a period averaging 16 months.

Each increase in BTSCC of 100 x 10^3 above a threshold of 200 x 10^3 has been associated with a decrease in herd average production of approximately 2%.[8] The individual losses in herd production based on this threshold in the *S. agalactiae* infected herds and culture negative herds average 5.5% and 2.0% respectively. The decrease in production attributable to infection status is 3.4%. For the average P.E.I. herd shipping 23.1 L of milk per day from 30.1 cows in a 30 day month this represents a loss of 716 L of production. The industrial milk price in P.E.I. in December, 1992 was $43.89 per hectolitre. The fluid milk price was $62.04. Approximately 20% of a qualifying dairies production was used as fluid product and secured the higher price. The average monthly value of lost milk production, calculated from production losses and milk prices, was $322.16. The yearly cost of elevated BTSCC on infected farms over culture negative herds was $3,866. In December 1992, the estimated herd prevalence of *S. agalactiae* was 18.9%. The cost of elevated BTSCC, associated with *S. agalactiae* to the P.E.I. dairy industry, was approximately $336,110. (see Appendix E)

Sixty-five penalties for elevated BTSCC were assessed to 36 herds over five two month penalty periods between October, 1992 and March, 1993. The average cost of a 2¢ per litre penalty to industrial milk producers was $417 per month. The average additional cost of losing fluid premium for one month for qualifying producers was $757. The cost of the 23 penalties in the infected industrial shippers in the five penalty periods was $7,506 more than the anticipated cost of penalties based on the 13 penalties assessed to culture negative industrial herds during the same time frame. The yearly total cost of BTSCC penalties associated with *S. agalactiae* in this group was $16,513. The cost of

the 12 penalties in the infected fluid producers over the five penalty periods was $11,740 more than the anticipated cost of penalties based on the 17 penalties assessed to culture negative fluid herds over the same time frame. The yearly total cost of BTSCC penalties associated with *S. agalactiae* in this group was $25,828. The average cost of penalties on infected farms over noninfected farms was $550 per year. The cost of these penalties to the P.E.I. dairy sector was approximately $47,817 (see Appendix E).

There were a total of 26 penalties for excess bacteria counts in the five month period between October, 1992 and February, 1993. The value in excess of the anticipated cost based on penalties in the culture negative herds was $2,502 over the test period. The estimated annual excess cost of penalties in the infected group was $6,005 or $78 per infected herd (see Appendix E).

Fourteen herds would have lost fluid status due to elevated BTSCC in the 1992-1993 dairy year if the program had operated for the whole year. The annual cost of losing fluid premium in the average P.E.I. dairy herd is $9,084. The estimated annual cost of losing fluid status associated with *S.agalactiae* in P.E.I. in 1992 was $54,504.(see Appendix E)

The total annual cost associated with *S. agalactiae* to the P.E.I. dairy industry identified in this study was approximately $445,000.

2.4 DISCUSSION

2.4.1 Test characteristics (sensitivity, specificity, predictive value)

Sensitivity values calculated in this study were estimates based on a history of infection identified with the same diagnostic test. No calculation of the true sensitivity of the testing protocol can be generated without herd classification with an appropriate gold standard. Single culture protocol sensitivity estimates ranged from .65 to .78. These values were somewhat less than the 95%[20] reported by the developers of the culture techniques used in the study, but superior to the 20.5%, 35% and 50% sensitivity values reported for other bulk tank culture methods.[4,6,10] In the Alberta study, where 95% sensitivity was achieved, 91.5% of the bulk tank samples from which *S. agalactiae* was isolated contained pigmented strains. In the present study, only 61.6% of colonies isolated were pigmented. Strains of human *S. agalactiae* appear to produce pigmented colonies more consistently than do bovine strains. In a study in the U.S. northeast, 290/297 (97%) human strains of *S. agalactiae* produced pigmented colonies whereas only 15/40 (37.5%) bovine strains were pigmented.[31] In an English study, 93/99 (94%) human strains of *S. agalactiae* were pigment producers compared to 3/13 (23%) of bovine strains.[32] Pigment production is one of the main criteria for identifying *S. agalactiae* colonies on the GBS® media. The proportion of bovine strains with pigment producing capabilities in P.E.I. appears to be lower than that observed in Alberta, but closer to levels observed in other areas. Differences in pigment production may have contributed to the

lower sensitivity observed in this study.

In the present study, only 57.1% of colonies eventually identified as *S. agalactiae* showed visible hemolytic zones (CAMP reaction) surrounding individual colonies. Failure to observe β enhancement zones may have been due to several factors. Darkening of the media on some cultures by large numbers of esculin splitting bacteria may have interfered with observation of the CAMP phenomenon. On occasion, the whole plate would appear hemolyzed making it impossible to observe individual haemolytic zones. Although almost all *S. agalactiae* isolates are CAMP positive, some strains may show stronger β enhancement than others. Schoonderwoerd *et al* noted that with decreasing pigment level on GBS® media the clarity of β enhancement was obscured and β haemolysis on blood agar was weak or not present.[20] Others have noted that when strains loss their pigment producing capability, they also loss their β haemolytic properties on blood agar.[33] In the Alberta study, some non-pigmented strains had good CAMP reactions, however, only 4 of 47 cultures included in that study were totally devoid of pigment.[20] In this study, 55/69 (80%) of pigmented strains had visible CAMP reactions on the Edwards® media, whereas only 9/43 (21%) of nonpigmented stains had observable β enhancement. Variation in the strength of β enhancement produced by the Alberta strains, in which pigment production was more commonly observed, and the P.E.I. strains may have lessened the sensitivity of the culture protocol.

The culture protocol (1 Edwards® and 1 GBS® plate) was repeated twice over a two week period at each census. The results of the two tests were interpreted in parallel. When the results of diagnostic tests are interpreted in parallel, the sensitivity is

improved.[14] If the results of the two diagnostic tests are independent, the estimated sensitivity of the combined protocol can be calculated based on the estimated sensitivity of the single test. (see Appendix D) 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 expected due to chance. They can, therefore, be assumed to be independent and the sensitivity of the combined procedure can be calculated at 91%. When used in this manner, the tests sensitivity is far superior to levels calculated by other researchers.[4,6,23]

The specificity of the testing protocol has been assumed to be 100%. *S. agalactiae* is an obligate pathogen of the udder. The only plausible way to isolate it from the bulk tank is if it is being shed by an infected cow. The samples used in this study were those taken by the milk haulers at routine pick up. These samples are normally used for regulatory purposes and great care is taken to prevent cross contamination. The only potential source of error in the specificity is the confirmatory latex agglutination test. Others have previously reported specificity of this test to be 98% to 100%,[23,24,25,26] when used on *S. agalactiae*. In this study, the samples were preselected based on their appearance on two selective media. This should augment the predictive value of a positive test. It is reasonable to assume that the specificity and positive predictive values of the test approached 100%.

The negative predictive value of the test, if the true prevalence of disease is less than 20%, is greater than 97.8%. This value is a function of prevalence, sensitivity and specificity. Defence of the derivation of each of these numbers has been presented previously in this discussion and no further explanation is warranted.

2.4.2 Herd prevalence of *Streptococcus agalactiae*

The observed herd prevalence 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%. These prevalence values are lower than those recorded in other areas of North America, where census or random sample bulk tank studies have been conducted.[1,2,3,4] However, they are substantially higher than those anticipated by veterinary practitioners and personnel in the diagnostic microbiology laboratory at the Atlantic Veterinary College at the University of Prince Edward Island.

The observed and calculated values for the follow up census conducted in June, 1994 were 13.1% and 14.4% respectively. The reduction in the herd prevalence of infection resulted primarily from the extension programs provided to infected herds, in the period of time between the two censuses. *S. agalactiae* can be successfully eradicated[34] and herds can be maintained infection free under field conditions.[12] Levels of herd infection of 18.9% or 14.4% are substantial and indicate that *S. agalactiae* mastitis is a major udder health problem for the P.E.I. dairy industry.

2.4.3 Herd incidence of *Streptococcus agalactiae*

While prevalence studies of herd level infection with *S. agalactiae* are common, no studies describing the incidence of new infections have been reported in the literature. Incidence rate information is extremely valuable. It improves the understanding of disease

dynamics beyond that provided by prevalence data. The calculated incidence of herd infection with *S. agalactiae* of 3.5 new herd infections per 100 herds per year indicates a considerable level of new infections. This value may be higher than in other areas because of the make up of the P.E.I. dairy industry. The average herd size in the province is only 36 cows. Many small producers do not find it economical to raise replacement animals. As a result, there is considerable movement of cattle from farm to farm within the province. Such movements would increase the transmission rate of *S. agalactiae* among herds.

2.4.4 Association with bulk tank SCC

Descriptive statistics indicate a trend toward higher BTSCC in the infected group compared to the culture negative herds. Because the raw BTSCC data were not normally distributed, the raw cell counts were log transformed for analysis. Plotting the transformed values on a histogram showed a near normal distribution. The resulting t-test indicated that the geometric mean BTSCC of the infected herds was significantly higher than that of the culture negative herds. Non-parametric tests were also used to analyze the non-normally distributed data. The Mann-Whitney rank sum test also showed that the BTSCC in the infected group was higher than that in the culture negative group.

The single most important factor affecting SCC is the infection status of the mammary gland.[12,35,36,37] *S. agalactiae* has previously been associated with elevations in

SCC at the cow and herd level.[38,38,40] Others have demonstrated a reduction in herd SCC after therapy to eradicate *S. agalactiae*.[41] In Vermont, a reduction in the state prevalence between 1985 and 1990 from 47% to 32% of herds coincided with a 200 $x10^3$ drop in average SCC.[38] The results of the present study reiterate that herd level infection with *S. agalactiae* was associated with significant elevations in BTSCC.

Although not employed in the present study, sampling the population based on a BTSCC threshold could substantially reduce the cost associated with conducting the culture procedures. Depending on the desired sensitivity and the available financial resources a threshold value could be identified which would optimize the balance between sensitivity and cost. Using the median of a five month average BTSCC would have included 81% of the infected herds. The doubling of the cost associated with the culture of the remaining 50% of herds to identify the other infected herds (19%) may not be prudent.

2.4.5 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 which meets better standards for SCC and bacteria levels. There is no uniformity in the design and implementation of these schemes, so it is impossible to compare programs. Among the criteria assessed in P.E.I. BTSCC,[3,38,38,40] standard plate counts,[2,42] and antibiotic residues[5,43] have previously been found to be associated with *S. agalactiae* infection. The SCC and bacteria standards

of the P.E.I. program are not stringent. Both the chi-square of BTSCC penalty versus non-penalty herds and the rank sum test examining the total number of BTSCC penalties in the infected and culture negative herds were significant. The risk of penalty was 3.90 times higher in the infected herds. If standards were more rigorous, an even greater association between BTSCC penalties and infection status may occur.

Only nine inhibitor violators occurred during the monitoring time period. Although there was no apparent association between inhibitor violations and *S. agalactiae* infection status, there may not have been sufficient power in the study to detect a difference, if there was one.

2.4.6 Estimate of economic impact of infection

The total annual cost associated with *S. agalactiae* to the P.E.I. dairy industry, identified in this study, was approximately $445,000. While this figure is substantial, the effect is more dramatic when you consider that this economic hardship is not spread evenly across herds. More than 80% of herds are uninfected. At the farm level it is conceivable that *S. agalactiae* infection could cost individual producers from $5,000 to $15,000 per year. The greatest economic losses at the farm level were associated with loss of fluid premium as a result of chronic elevated BTSCC.

Herds that are infected with *S. agalactiae* may have a higher prevalence of other chronic subclinical mammary pathogens such as *Staphylococcus aureus*[38,44]. In addition, there may be a correlation between infection status and poor premilking hygienic

procedures,[11] which is augmenting the association between *S. agalactiae* and penalties for excessive bacteria counts. As a result, the associations with BTSCC and penalization due to BTSCC and bacteria count, may be inflated. Even with this caveat, it appears that, at the individual farm level, *S. agalactiae* may be a serious economic burden to P.E.I. dairy producers with infected herds.

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Chapter 3

Evaluation of an extension effort aimed at eradication of *Streptococcus agalactiae* from Prince Edward Island dairy farms

3.1 INTRODUCTION

Extension efforts to decrease the prevalence of subclinical mastitis and improve milk quality appear to be widespread throughout the developed world. These programs are often funded through national or regional governments or through milk marketing organizations. Most studies of the effectiveness of mastitis education programs have concentrated on the adoption of hygiene and therapy protocols, put forward by such organizations as the National Mastitis Council.[1,2,3,4] One study examined the effectiveness of education programs to change general mastitis control practices[5]. However, no research has examined response to targeted extension programs, exclusively directed at eradication of *Streptococcus agalactiae*. The efficacy and cost effectiveness of herd level programs for the prevention, control and eradication of *S. agalactiae* has been well established. A review of such programs was presented in Chapter 1 of this thesis. Despite the fact that these programs have been developed, *S. agalactiae* remains a prevalent cause of chronic subclinical mastitis and elevated SCC (see Chapter 2).

The major objective of this study was to analyze the response of dairy producers with *S. agalactiae* infected herds to a targeted "on farm" extension program. The study also examined the management practices on each farm. In addition, the pathway by which most farms achieved eradication is presented. Thirdly, response to a "mail out"

extension program was compared to the "on farm" program.

3.2 MATERIALS AND METHODS

3.2.1 Study population

The Prince Edward Island (P.E.I.) dairy industry consists of approximately 460 farms. Most of the milk cows are registered or grade Holsteins. The Animal Productivity and Health Information Network (APHIN) at the Atlantic Veterinary College collects and collates information on health and production in the P.E.I. dairy industry.[6] From this database, it was determined that the average herd size of the 233 herds on milk recording programs with the Atlantic Dairy Livestock Improvement Corporation (ADLIC) was approximately 36 lactating cows in 1993, and the average daily milk production of this group was 23.1 litres. The average BTSCC for all herds in the province in 1993 was 290 x 10^3.

3.2.2 Study design

Duplicate samples of bulk tank milk from all herds shipping milk in December of 1992 were used to establish the initial infection status of each herd. Seventy-seven of the 452 herds, which had culture results from each of the two samplings, were found to be infected with *S. agalactiae*. Dairy farm numbers identifying these 77 herds were placed

in a hat and 50 were chosen to take part in an "on farm" extension program in the spring and summer of 1993 and will be referred to as "program herds". The remaining 27 herds were "positive control" herds. Their infection status was not reported to them until after the effectiveness of the "on farm" extension effort was established by bulk tank culture in December of 1993. In April of 1994, all herds found to be infected in the December 1993 culture were informed of their status by mail. The positive control herds were provided with the same educational materials distributed during the "on farm" extension effort. Evaluation of this "mail out" extension effort was performed using culture results from bulk tank samples collected from these herds in June, 1994. A third group of herds ("negative control" herds, n=50) were chosen by random number generation using the Minitab® statistical software program[7] from the 375 herds that were culture negative in December, 1992. These 50 herds were recultured in December, 1993 and again in June, 1994 (as part of a complete census). A schematic of the study design is presented in Table VIII.

3.2.3 "On farm" extension effort

Forty-six of the 50 herds, randomly chosen from the 77 *S. agalactiae* infected dairies identified in the *December 1992 census*, were visited between April and June of 1993. Appointments were made by telephone. Of the four herds not included in the "on farm" visits, three producers were no longer shipping milk and one refused to take part in the educational sessions. This last dairyman asked that all educational materials be

Table VIII Study design[a] for the evaluation of "on farm" and "mail out" extension programs for the eradication of *Streptococcus agalactiae* from Prince Edward Island dairy farms

December, 1992	April-June, 1993	December, 1993	April, 1994	June, 1994
bulk tank milk culture (all herds)	farm visits for extension effort	bulk tank milk culture (127 herds)	"mail out" extension effort	bulk tank milk culture (all herds)
77 positive *S. agalactiae*	50[b] randomly selected herds	50[b] herds		50[a] herds
		27[c] herds	27[c] herds	27[c] herds
375 negative *S. agalactiae*		50[d] randomly selected herds		375 herds

[a] Actual numbers may differ due to herds leaving the industry or changing infection status

[b] *S. agalactiae* positive herds enrolled in "on farm" extension effort

[c] *S. agalactiae* positive herds not participating in "on farm" extension effort, later involved in "mail out" extension program

[d] *S. agalactiae* negative herds selected randomly from all negative herds

directed to his local veterinarian. Since he had been informed of his status and was given access to the educational material through his veterinarian, this producer was included as an extension program herd for analysis.

Individual meetings were arranged in the home of each farmer. Each session lasted 30 to 45 minutes. A detailed explanation of the eradication program outlined in Appendix A was given. The economics of *S. agalactiae* infection was also discussed. The desirability of input by local veterinary practitioners, and the provincial udder health technologist, was emphasized along with the necessity of long term measures to control mastitis after blitz therapy . Producers were encouraged to ask questions about the program. Each dairyman was asked for permission to release information on their infection status to their veterinarian and the provincial udder health technician. Forty-one producers complied with this request. At the end of each meeting, copies of the educational material (Appendix A) and a checklist of culture and therapy procedures, tailored to their particular situation, was provided for future reference.

3.2.4 Reculture of study herds

Infection status of program herds, and of positive and negative control herds, was reassessed by bulk tank culture in December of 1993. Forty-six of the 47 herds chosen to be participants in the extension program outlined above had milk samples available for culture. Twenty-five of the 27 positive controls and 48 of the 50 negative controls had samples cultured in December, 1993.

In Chapter 2, the estimated sensitivity of the microbiological procedures was 91%. The obligate nature of the infection and the specificity of the confirmatory latex agglutination test have previously been the basis for an assumption that the specificity was 100%.[8]

3.2.5 "Mail out" extension effort

Following the December, 1993 bulk tank culture, positive control herds (herds that were infected with *S. agalactiae* that were not previously involved in the "on farm" extension scheme) were targeted for a "mail out" extension program. Of the 27 positive control herds, two were no longer shipping milk and four had eliminated the infection without benefit of the education program. As a result, 21 herds were available for the evaluation of the "mail out" extension effort. Each of these farms received a copy of the eradication protocol (Appendix A) and a covering letter explaining how their infection status had been assessed. Producers were encouraged to seek advice on control procedures from local veterinary practitioners and the provincial udder health technician.

3.2.6 Reculture of study population

In June of 1994, duplicate bulk tank milk samples from all herds in the province (n=434) were cultured. Culture techniques were the same as used in the previous census and subsample studies, except that bulk tank milk samples utilized for this culture had

been frozen, at -20°C, for a period not exceeding 10 days. Most studies in the literature have found no changes in either numbers of *S. agalactiae* bacteria per sample, or classification of infection status, when frozen rather than fresh samples were used.[9,10,11] However, one study, conducted using individual cow samples, found an increase in the number of cows classified as infected when frozen samples were used.[12]

3.2.7 Survey of management practices in study herds

A telephone survey, in which information about selected udder health management practices was obtained, was carried out in April of 1994. Attempts were made to reach all 71 of the 77 original positive herds still shipping milk and the 48 randomly selected negative herds from the December, 1993 culture. A copy of the survey questionnaire is provided in Appendix F.

A separate survey of the veterinarians and udder health personnel servicing these herds was undertaken. Due to the high response rate on the survey of dairy producers (109 of 119 surveys completed) and the similarities between the survey questions, the material contained in the survey of service professionals was found to be redundant.

3.2.8 Data processing and analysis

Data were stored as collected in dBASE III PLUS® (Ashton-Tate)[13] and analyzed using the Statistix® statistical software program[14] version 4.0 (Analytical Software, St.

Paul, Minnesota) and Epi Info[15] version 5.01a (Centres for Disease Control, Atlanta, Georgia and World Health Organization, Geneva, Switerland).

3.3 RESULTS

3.3.1 Results of "on farm" extension effort and management survey

The change in herd status of the three groups involved in the evaluation of the effectiveness of the more intensive "on farm" extension effort are summarized in Table IX. Forty-one percent of the program farms and 16% of the positive control farms had eliminated the infection between the two tests. This difference is statistically significant ($\chi^2 = 4.74$, $p = 0.03$). The relative risk (RR) of cure in the extension group was 2.58 compared with spontaneous elimination from positive control herds (Taylor series 95% confidence interval $0.99<RR<6.75$)(see Appendix G).

Due to the imperfect nature of the diagnostic test there is inherent bias in the chi-square statistic and the relative risk. The misclassification was assumed to be nondifferential, that is, the rate of misclassification was equal in the positive control herds and the program herds, and was of disease status only and not exposure to the extension program. Nondifferential misclassification always biases the outcome towards the null.[16] Adjusting the calculated relative risk to account for the misclassification bias more closely estimates the strength of this association. The adjusted relative risk was 4.62 (Taylor series 95% confidence limits $2.25<RR<9.51$)(see Appendix G).

Table IX. Changes in *S. agalactiae* infection status in dairy herds undergoing an "on farm" extension effort, positive control farms and negative control farms

	number cultured December 1992	number cultured December 1993	*S. agalactiae* positive December 1993	proportion eliminating infection
program[a] herds	47	46	27	41%
positive[b] controls	27	25	21	16%
negative[c] controls	50	48	1	N/A

[a] Herds that were *S. agalactiae* positive in December of 1992 and were offered the "on farm" extension program

[b] Herds that were *S. agalactiae* positive in December of 1992 but were not participants in the "on farm" extension program

[c] Herds that were culture negative for *S. agalactiae* in December of 1992 which acted as negative controls

A survey of management practices on the 119 farms with culture data in December, 1993 was conducted. Of 119 potential respondents 109 surveys (92%) were completed. A summary of the results from the survey is presented in Appendix H. Associations between survey responses and the provision of the extension program and herd level eradication of *S. agalactiae* are outlined in Table X.

Herds that received the "on farm" extension program reported seeking professional help for elevated BTSCC or *S. agalactiae* and having whole herd cultures more frequently than the positive control herds. There was no statistical association between being in the extension program and teat dip use, dry cow therapy or maintenance of milking equipment.

Herds that eradicated *S. agalactiae* between the *December 1992 Census* and December, 1993 were more likely to always use teat dip, to have consulted a professional for help and to have conducted a whole herd culture. There was no statistical association between use of complete dry cow therapy versus selective or no dry cow therapy, or milking equipment maintenance in the last 6 months, and eradication of the bacteria from the herd.

Table X Associations between management practices, provision of the "on farm' extension program and eradication of *S. agalactiae*

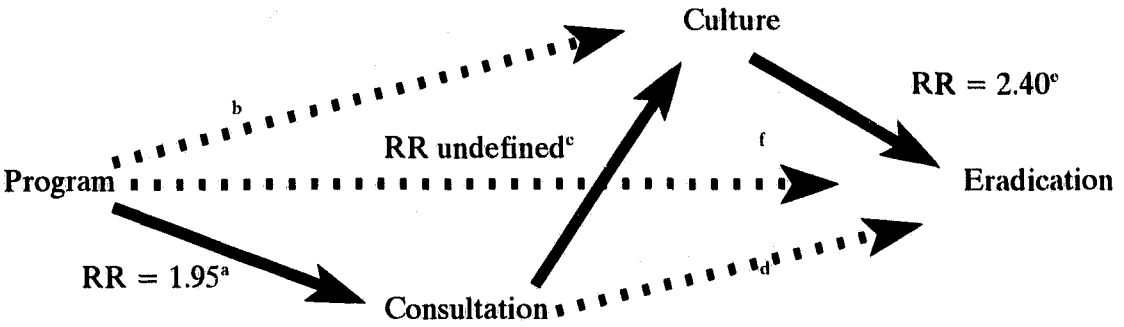
			extension group		eradication	
		number of herds	program	positive control	yes	no
teat dip	always	50	79%[a]	75%[a]	96%[a]	55%[b]
	sometimes or never	14			RR = 1.75	
dry cow therapy	all cows	30	42%[a]	57%[a]	52%[a]	44%[a]
	selective or none	34				
equipment last serviced	less than 6 months	25	37%[a]	43%[a]	48%[a]	34%[a]
	more than 6 months	39				
consulted professional	yes	30	56%[a]	29%[b]	74%[a]	32%[b]
	no	34	RR = 1.95		RR = 2.31	
milk cultures	all cows	13	28%[a]	5%[b]	48%[a]	5%[b]
	individual or no cows	51	RR = 5.6		RR = 9.6	

[a b] The difference between values in the same category with different superscripts is statistically significant ($p < 0.05$)

Stratified analyses of the program group, selected management practices and eradication revealed that there was little or no direct effect of the extension program. The benefits of the extension program were indirect through increased frequency of consultations with professionals and increased whole herd microbiological culture. Herds that were in the extension program were 1.95 times more likely to consult an udder health professional. Consulting a professional increased the probability of a whole herd culture being performed. No herds that did not consult a professional had a whole herd culture performed. Herds, which conducted a whole herd culture were 2.40, times more likely to eradicate *S. agalactiae* compared to herds that did not do a whole herd culture. Other pathways were not significant. See Figure VI and Appendix I for details.

Figure VI

Proposed path diagram of the effect of the extension program on *Streptococcus agalactiae* eradication. Solid lines indicate significant associations ($p < .05$).



[a] Herds that were involved in the extension program were 1.95 times more likely to consult a professional for help due to elevated SCC or subclinical mastitis than positive control herds (see Appendix I, subsection A)

[b] The risk of culture in both the extension program and control herds was 0 if they did not consult (see Appendix I, subsection B)

[c] The relative risk of culture in the consulting and non-consulting herds was undefined but there was clearly a strong association between consulting and carrying out a whole herd culture since 13 of the 30 herds (43%) that had professional consultation carried out a whole herd culture while none of the herds that did not consult carried out a culture (see Appendix I, subsection C)

[d] There was no significant association between consulting and eradication among herds that did not do a whole herd culture (see Appendix I, subsection E)

[e] The risk of eradication in herds that performed whole herd culture was 2.40 times higher than herds not doing a whole herd culture. (see Appendix I, subsection F for stratified analysis)

[f] There was no significant direct effect of extension program on eradication (see Appendix I, subsection G)

3.3.2 Results of "mail out" extension effort

The changes in herd status of the positive control herds during the entire study period are summarized in Table XI. These herds were found to be infected with *S. agalactiae* in December, 1992 but were not part of the "on farm" extension program in the spring and summer of 1993. The 21 herds in this group that were found to be infected at the December, 1993 culture were informed of their status by mail in April of 1994. At that time, they received the same educational materials that the "on farm" extension group had been given. Twenty of these herds were available for follow up culture in June, 1994.

We assumed that dairy herds that would implement eradication programs would likely do so within the first two months after notification. Five of the 20 dairy herds (25%), that were included in the "mail out" extension effort, and had culture results from the *June 1994 Census*, eliminated the infection. An estimate of the number of herds expected to have spontaneous elimination of the bacteria during the period of December, 1993 to June, 1994, was made based on the frequency of spontaneous eliminations in the same group of herds in the period December, 1992 to December, 1993. This estimate was 1.6 herds (Appendix J). The proportion responding to the mail out extension program was compared to the proportional response to the "on farm" extension program using a z statistic. The numbers responding to the "on farm" program was also adjusted to reflect the expected spontaneous eliminations in this group (Appendix J). Eighteen percent of herds eradicated *S. agalactiae* as a result of the "mail out" extension program and 30%

due to the "on farm" program. These proportions are not statistically different ($z = .93$, $p > .05$).

The standard error of the proportion of herds eradicating due to the "mail out" extension program (18.5%) was 9.0%. Although there appears to be a substantial difference between the response to the "mail out" education program (18.5%) and the "on farm" extension effort (30.1%), this difference was not statistically significant.

Table XI Changes in the *S. agalactiae* infection status of 27 Prince Edward Island dairy herds which were found to be infected in December, 1992, and were informed of their infection status and given educational materials by mail in April, 1994.

S. agalactiae infection status	Date of culture			reduction in herd level infection December, 1993 to June, 1994
	December 1992	December 1993	June 1994	
positive	27	21	15	(5/20) 25%[b]
negative	0	4	9	
missing[a]	0	2	3	

[a] Herds did not have milk samples available during the month of the culture because they had left the industry.

[b] 20 of the 21 positive herds in December, 1993 had cultures in June, 1994 and 15 remained infected.

3.4 DISCUSSION

3.4.1 "On farm" extension effort

Systematic inaccuracies in classification of the exposure or outcome variables in a study may occur when measurement of exposure is faulty, or when the sensitivity or specificity of the diagnostic procedure used to classify the outcome are less than 100%.[16] Exposure in the present study (provision of the extension program) was assigned by the investigators and therefore, was unlikely to be recorded erroneously. However, some herds would have been misclassified on the outcome variable (*S. agalactiae* infection). When misclassification occurs equally in the program herds and the positive control herds, that is, when the sensitivity and specificity of the diagnostic procedures in each group are equal, such misclassification is said to be nondifferential. Since herds in the present study were assigned to exposure (extension group) by a formal random procedure, the sensitivity and specificity of the diagnostic procedures should be equal in each group and misclassification of infection status would be nondifferential. Nondifferential misclassification will always bias towards the null. It will always spuriously diminish the apparent effect of the exposure on the outcome variable.

There was a statistical association between exposure to the extension program and herd level eradication of *S. agalactiae*. The strength of this association was measured by calculating the relative risk, because the study was a field trial of an extension service. Relative risk estimates were calculated in Epi Info[15] using the Mantel-Haenszel technique.

Confidence intervals for the relative risk in Epi Info are computed by the Greenland-Robins method,[17] which, for a single stratum table, is equivalent to the Taylor series 95% confidence interval. Although the χ^2 for the association between program and eradication was < 0.03 the more conservative Taylor series 95% confidence interval just included 1 (0.99-6.75). Adjustment of the relative risk to account for misclassification indicates, as expected, a stronger association between the extension program and eradication than before adjustment, and the Taylor series 95% confidence interval no longer included 1. Given the fairly large relative risk and the fact that field trail data provide stronger evidence of causal associations than do observational studies, we can conclude that there was a causal association between provision of the "on farm" extension program and eradication of *S. agalactiae*.

Until recently, veterinary practitioners have traditionally focused their efforts on therapy of individual animals.[18] Given the economics of herd level infection with *S. agalactiae* outlined in Chapter 2, and the apparent effectiveness of educational programs to initiate herd eradication, veterinarians and other agricultural professionals should concentrate on such areas. Goodger and Rupanner (1982) found that veterinarians were relied upon most frequently to deal with mastitis problems at the herd level.[19] The survey of program and control herds indicates that in P.E.I. 82% of dairy producers seeking advice on subclinical mastitis consult their veterinarian. It is incumbent on veterinarians to develop and initiate udder health management and education programs.

Herds that were infected with *S. agalactiae* in December of 1992, and which remained infected until December 1993, were less likely to teat dip all cows all the time

than herds which eradicated the bacteria in that time period. Dargent-Molina *et al* (1988) had previously reported that herds that did not teat dip were 7.1 times more likely to be infected with *S. agalactiae* than herds that did teat dip.[20] Erskine and Eberhart (1990) found that, in herds that did not teat dip and dry cow treat all cows after initial blitz therapy within one year, herd average SCC had returned to previous levels.[21] Herds that conducted whole herd cultures appear to teat dip more (92%) than the herds which did not conduct whole herd culture (76%). Producers that teat dip may be more willing to embark on other management schemes to fight subclinical mastitis. Because teat dipping was commonly practised among herds that remained infected, as well as those that eradicated, it was likely not associated directly with eradication.

Dargent-Molina *et al* (1988) compared dry cow therapy use in herds with high levels of *S. agalactiae* infection with *S. agalactiae* negative herds.[20] While total dry cow therapy had a sparing effect on infection there was no difference in the odds ratios for selective and no dry cow therapy. Godkin and Leslie found that selective dry cow therapy was a risk factor for *S. agalactiae* infection.[22] Based on these studies herds were divided into those using dry cow therapy on all cows and those using dry cow therapy on none or a selected group of cows. Herd level eradication of *S. agalactiae* was not associated with the level of dry cow therapy in this study.

Reports, which estimate the percentage of new infections caused by improper milking machine function, range from 6.6%[23] to 50%.[24] Despite this fact, machine maintenance interval was not identified as a risk factor for herd level infection with *S. agalactiae* in two previous studies.[22,25] There was no association between program group

and the probability of having milking equipment serviced in the last six months in this study. There was also no association between equipment service in the last six months and the probability of eradicating *S. agalactiae*.

Infected herds consulted professionals in the field more frequently (47%) than did culture negative herds (15%) for advice on subclinical mastitis. This may have been partially due to the fact that *S. agalactiae* infected herds had significantly higher BTSCC, and as a result received a relatively higher number of milk quality penalties than culture negative herds. Part of the increase in use of professionals appears to be in response to the extension program, since 56% of the program herds and 28% of the positive controls consulted with professionals. Consultation was positively associated with eradication although the effect was an indirect one mediated through whole herd culture.

Whole herd culture to identify infected cows is the initial step of most blitz therapy programs for the elimination of *S. agalactiae*. Forty-eight percent of the herds eradicating the bacteria reported doing whole herd culture in the 12 months prior to the survey, whereas, only 5% of the herds which remained culture positive had conducted a whole herds culture. While use of whole herd culture was strongly associated with eradication (relative risk = 3.3), a substantial number (12 herds) managed to eradicate the bacteria without whole herd culture. Some herds that eradicated *S. agalactiae* reported culling all cows with elevated SCC, or individual cow culture and therapy based on history of elevated SCC. Some of the reduction observed may have been the result of continued use of good milking hygiene, including postmilking teat dip and dry cow therapy. Eleven of 13 herds (85%) conducting whole herd culture appear to have

eradicated the bacteria. This suggests that herds that embarked on eradication programs were generally successful. At the individual cow level, cure rates for *S. agalactiae* have ranged from 88% to 100%[21,26,27] after a single therapeutic regimen with penicillin or it's derivatives. The eradication protocol put forward in the extension materials called for reculture and retreatment or culling if therapy was unsuccessful. The two herds that did not eradicate after whole herd culture may not have followed the recommended procedures, or may represent true failures of the eradication program. Stratified analysis revealed that the relative risk of eradication was the same for herds which used whole herd culture, regardless of whether or not they were in the extension program. The extension program appears to have caused more producers to have whole herd cultures and resulted in eradication through this pathway.

3.4.2 Results of "mail out" extension effort

Proportional reduction in herd prevalence of *S. agalactiae* attributable to the "mail out" extension effort was not statistically different from the reduction attributable to the "on farm" extension program. Although there was a trend towards greater response in the herds that received on site visits (30% versus 18%), there was not sufficient power in the study to decern a difference, if, in fact, there was one.

The length of time required to eliminate the bacteria from most farms, based on the extension materials provided was between 1 and 4 weeks. It seems reasonable to assume the most producers that would initiate eradication programs would do so within

the first two months after notification. As a result, although there was a substantial difference between the amount of time allowed for eradication in the "on farm" extension study compared to the "mail out" study, it likely did not bias the results. If the results were biased they would favour the "on farm" extension program.

Since there was no concurrent control group for the evaluation of the "mail-out" program, estimation of the efficacy of the program was adjusted based on the number of "spontaneous eliminations" in the study herds in the previous year. It was possible that heightened awareness of *S. agalactiae* mastitis, within the relatively small agricultural and veterinary communities in P.E.I., may have lead some producers to make management changes, which contributed to the number of eradications in this group. If such a scenario were true, the number of "spontaneous" eliminations in both the "on farm" and "mail out" groups would be higher than would be expected in other locations.

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Chapter 4

Conclusions

4.1 INTRODUCTION

Four major objectives were addressed in this study. The first of these objectives was to establish the herd prevalence of *Streptococcus agalactiae* in Prince Edward Island. *S. agalactiae* has previously been shown to be an important cause of chronic subclinical mastitis in other parts of North America. Although prevalence data at the herd level has frequently been published, no studies of the incidence of new herd infections have been published. Calculation of the incidence of new herd infections in a previously negative group of herds was the second major focus of the study. Herds found to be infected on the initial prevalence investigation were subdivided to enable fulfilment of the final two objectives, evaluation of an intensive "on farm" extension program and a less intensive "mail out" extension effort. No such evaluation of the efficacy of such a targeted education program for *S. agalactiae* mastitis control and eradication has been published in the veterinary journals.

4.2 HERD PREVALENCE AND ECONOMIC EFFECT OF *S. agalactiae* INFECTION IN P.E.I.

Prevalence of herd level infection with *S. agalactiae* in North American dairy herds has previously been reported to range from 5 to 94% (Ohio[1] (1993) 5%; Vermont[2]

(1990) 32%; Ohio[3] (1989) 32%; Ontario[4] (1990) 42%; Mississippi[5] (1982) 44%; California[6] (1988) 44%; Vermont[7] (1985) 47%; Ohio[8] (1992) 56%; California[9] (1988) 94%). The estimated true herd prevalence of infection in Prince Edward Island in December of 1992 was 18.9%. After an extension program, which included a directed "on farm" or "mail out" education component, was put forward the estimated true herd prevalence of infection was 14.4% in June of 1994. While these estimates of herd level infection are somewhat lower than those observed in other areas, they indicate that *S. agalactiae* is a major cause of chronic subclinical mastitis in the P.E.I. dairy industry. Herds that were infected had higher BTSCC, received more financial penalties, due to excessive BTSCC and total bacteria count, and were at greater risk of losing fluid milk shipper status and the corresponding milk premium payment. The estimated annual cost to the P.E.I. dairy industry, associated with infection with *S. agalactiae*, was $445,000. This figure represents substantial monetary loss to the P.E.I. dairy industry. Individual herd losses are more devastating when you consider that this cost is spread over a small proportion of the industry.

4.3 INCIDENCE OF HERD LEVEL INFECTION WITH *S. agalactiae* IN P.E.I.

To establish the incidence of new herd level infections with *S. agalactiae* culture negative herds from the December, 1992 census were retested in June of 1994. Eighteen herds that were previously negative were culture positive at that time. The calculated incidence was 3.51 new infections per 100 herds per year. *S. agalactiae* is an obligate

pathogen of the bovine mammary gland and cannot survive for long periods of time in the environment.[10] The most likely source of new herd infections is the addition of infected cows to the milking herd. Once infection becomes established within the herd it can spread rapidly, especially if milking hygiene and machine maintenance is not adequate. Many P.E.I. dairy producers do not raise there own replacement heifers and as a result must purchase replacement animals. Addition of these potentially infected cattle would be a risk factor for new herd infections.

4.4 EVALUATION OF AN "ON FARM" EXTENSION PROGRAM FOR HERD LEVEL ERADICATION OF *S. agalactiae*

Despite the fact that extension programs to decrease the prevalence of subclinical mastitis appear to be widespread in the developed world and herd level eradication programs for *S. agalactiae* have been shown to be efficacious and cost effective, it remains a prevalent cause of mastitis. Previous studies had attempted to evaluate the effectiveness of extension programs by monitoring the adoption rates of control procedures published by such organizations as the National Mastitis Council.[11,12,13,14] Evaluation of extension effectiveness in this study was assessed with a defined outcome variable: herd level eradication of *S. agalactiae*.

After adjustment for misclassification bias, herds in the "on farm" extension program were 4.62 times more likely to eradicate the infection than a group of positive control herds. A survey of participating producers indicated that most herds that successfully eradicated followed a certain pathway. The extension program led producers

to consult with their local veterinarian or udder health technician. After consultation many herds had whole herd cultures to identify infected cattle and subsequently undertook eradication procedures..

Targeted extension programs with an "on farm" educational component appeared to initiate a chain of events, which culminated in herd level eradication of *S. agalactiae*. The program stimulated producers to seek further professional assistance, which was the initial step to achieve the desired end result.

4.5 EVALUATION OF A "MAIL OUT" EXTENSION PROGRAM FOR HERDS LEVEL ERADICATION OF *S. agalactiae*

The number of herds successfully eradicating *S. agalactiae* was not significantly different between farms in the more intensive "on farm" extension program and the "mail out" group. Despite this fact, there appeared to be a substantially higher success rate in the "on farm" group. Herd numbers for this evaluation were low and the study may not have had enough power to discern a difference, if, in fact, a difference did exist.

4.6 SUMMARY

Streptococcus agalactiae was identified as an important cause of subclinical mastitis in the P.E.I. dairy industry. Bulk tank milk samples were used and the prevalence of herd level infection was established to be 18.9% in December of 1992. Infection was associated with several factors which indicate financial loss both for the

P.E.I. dairy industry as a whole, and more particularly for individual infected herds. The incidence of new herd infections was estimated to be 3.51 per 100 herds per year. The most likely source of new infections is the addition of purchased infected cows to the milking herd. Extension efforts appeared to lead producers to seek further professional aid and this was the initial step in a chain of events leading to herd level eradication. No significant difference was observed between "on farm" and "mail out" extension programs, although intensive "on farm" programs may be superior.

4.7 REFERENCES

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APPENDIX A
(adapted from Kirk and Mellenberger)

PRODUCER EDUCATION MATERIAL FOR HERDS
SELECTED TO BE IN THE "ON FARM" EXTENSION PROGRAM

Streptococcus agalactiae or Strep. ag. as it is often called is a common cause of mastitis in dairy cows. It is not unusual to find up to 60% of cows within an infected herd to have the disease. The majority of cases are subclinical, that is, they don't cause the changes in milk that we normally associate with mastitis. It is estimated that a farmer treats only one clinical case (changes in milk) for every 20 to 40 subclinical infections.

What effect does Strep. ag. have on milk quality and production

Strep. ag. infection causes an increase in Somatic Cell Count in infected cows. This can lead to a high somatic cell count in the bulk tank and penalties to the farmer for poor milk quality.

Strep. ag., even when not causing changes in the milk, can lead to a decrease in milk production. An infected cow may produce up to 40% less milk then she would if she were free of infection.

Your herd has been identified by sampling of your bulk tank milk as having this disease.

Where can Strep. ag. be found on the farm?

Infected cows are the source of Strep. ag. The bacteria can survive for a very long time in the mammary gland of infected cows but lives only for a very short time outside the udder. The purchase of infected cows and spread within the herd is the number one way to get a serious herd-wide infection. "Clean" cows become infected when they contact the milk of infected cows during milking. The infection is spread by the milking machine, milkers hands or the use of a common wash cloth or sponge. Infection may also be spread among group penned heifer calves that are fed infected milk and suckle on each others mammary glands. The infection can remain in the udder until the heifer calves and may lead to blind quarters and decreased milk production.

What Management steps should you take to eliminate this problem on your farm?

First and foremost you should consult your local veterinarian. He or she is the expert with respect to conditions on your farm, and may wish to modify the outlined program to meet your specific needs. Depending on your immediate situation you should follow one of two routes. If you are in a penalty situation, with respect to bulk tank somatic cell count, you should consider the following steps.

Milk quality penalty situation

Day 1

Cull all cows that are greater than 5 years old that have had a SCC of 1,600,000 for greater for three months. In consultation with your veterinarian, treat all quarters of all cows with a prepackaged mastitis product containing penicillin (consult with your local veterinarian as to an appropriate choice). Follow the package directions with respect to treatment schedule and milk withdrawal time.

Day 21

Have milk cultures performed on all cows in the herd including cows and heifers which have calved since Day 1.

Day 25

Based on the culture results of Day 21 retreat any animals with a positive culture for Strep. ag. Use the same drug as the day one treatment. By now approximately 90% of the animals infected on Day 1 will have been cured. Follow the label directions for treatment schedule and milk withdrawal times. Change the order of milking, if possible, so that these cows are milked last.

Day 46

Reculture the milk from all cows that were positive on Day 25 plus any cows and heifers that have calved since Day 21. If any cows are still positive after two treatments (this should be less than 1% of the herd) they should be culled. If it is not possible for them to be culled, they should be milked last and treated at dry off again. At 5 days after calving the cow should have a milk culture. If it is positive for Strep. ag. the cow must be culled.

APPENDIX A

Non-penalty situation for milk quality

If your herd is Strep. ag. positive, but you are not in a penalty situation with respect to bulk tank somatic cell count, you should implement the following program.

Day 1 Cull all cows greater than 5 years old with a SCC of more than 1,600,000 for three months. Consult your local veterinarian and have milk cultures performed on all cows to determine which ones are positive for Strep. ag.

Day 5 Segregate all positive cows so that they can be milked last. Treat all quarters of all positive cows with a prepackaged mastitis product containing penicillin (consult with your local veterinarian for an appropriate choice). Follow the labelled treatment schedule and milk withdrawal times.

Day 21 Repeat the milk cultures on all cows.

Day 25 Treat all cows that are positive on this milk culture with the same drug as used on Day 5 (90% or more of the original cases should now be cured). Follow the labelled treatment schedule and withdrawal times.

Day 46 Reculture the cows that were positive on Day 21 plus any cows that were added to the milking line since that time. If any cows remain positive they should be culled (this should be less than 1% of the herd). If it is not possible to cull them they should be milked last and treated at dry off time. At 5 days after calving cows in this group should have a milk culture, and, if it is positive for Strep. ag., she should be culled.

Is it possible for your herd to become reinfected if you complete this program?

Once the infection has been eliminated off the farm it can only be reintroduced by purchasing infected cattle. If one cow that is infected with for Strep. ag. is allowed to enter or remain in the herd it can spread throughout the herd within a few months. This is especially true if milking technique or machine function is not adequate. You should discuss these issues with your veterinarian or udder health technician.

APPENDIX A

Long term follow up

In order to derive the maximum benefit from a Strep. ag. eradication program it is essential to follow it up with an udder health management scheme. Some key points are listed below, however, you should contact your veterinarian and udder health technician for more details.

(1) Use an approved post-milking teat-dip.
(2) Wash and dry teats using individual paper towels.
(3) Use a vacuum shut-off before removing the milking machine.
(4) Dry treat all quarters on all cows unless advised otherwise by your veterinarian.
(5) Become involved in a milk testing program (ADLIC) where individual cow monthly SCC are available.
(6) Segregate known infected cows (high SCC or positive milk culture) and milk these cows last.
(7) Have regular equipment checks by an independent udder health technician and perform routine maintenance of the system between these checks.
(8) Cull all cows with SCC chronically higher than *1,000,000.*
(9) House calves individually if you are going to feed whole milk.
(10) Have milk cultures done on newly purchased cows and heifers before they are added to the milking line.

COSTS

The cost of elimination of Strep. ag. from your herd and the implementation of "good" milking procedures to help prevent re-infection are far outweighed by the value of increased milk production. The return on investment is usually in the range of 4:1. For every dollar you invest in this program you should see $4 in benefits. With the farm economy in its present state I don't know any other investment that will have such a marked positive effect.

APPENDIX B

LEGEND AND CRITERIA FOR CLASSIFICATION OF BACTERIA ISOLATED ON MODIFIED EDWARDS® MEDIA

Fields recorded

(1)	Sample number (corresponds with the producers shipping number)	
(2)	Isolate number (each colony type was given a isolate number)	
(3)	Growth	0 = no growth 1 = 1-10 colonies 2 = 10-100 colonies 3 = >100 colonies
(4)	Colour	0 = gun-metal brown 1 = Light blue 2 = Blue 3 = Royal blue
(5)	Morphology	0 = mucoid 1 = distinct margins
(6)	Size	0 = < 1 mm 1 = 1-3 mm 2 = > 3 mm
(7)	β enhancement (CAMP)	0 = negative 1 = unclear zone 2 = clear zone
(8)	Esculin hydrolysis	0 = negative 1 = positive
(9)	Isolate classification	0 = negative 1 = suspicious 2 = highly suspicious

Criteria for isolate classification

Negative(0)	**Suspicious(1)**	**Highly suspicious(2)**
Growth (0) or, Growth (1,2,3) and Colour (0) and β haemolytic zone (0) and Esculin (1)	Growth (1,2,3) and Colour (1,2,3) or β haemolytic zone (1,2) or Esculin (0)	Growth (1,2,3) and Colour (1,2,3) and β haemolytic zone (2)

APPENDIX C

LEGEND AND CRITERIA FOR CLASSIFICATION OF BACTERIA ISOLATED ON MODIFIED GBS® MEDIA

Fields recorded

(1) Sample number (corresponds with the producers shipping number)

(2) Isolate number (each colony type was given a isolate number)

(3) Growth
0 = no growth
1 = 1-10 colonies
2 = 10-100 colonies
3 = >100 colonies

(4) Colour
0 = cream
1 = light orange
2 = orange
3 = intense rust

(5) Morphology
0 = mucoid
1 = distinct margins

(6) Size
0 = < 1 mm
1 = 1-3 mm
2 = > 3 mm

(7) Isolate classification
0 = negative
1 = suspicious
2 = highly suspicious

Criteria for isolate classification

Negative(0)	**Suspicious(1)**	**Highly suspicious(2)**
Growth (0) or, Colour (0) and morphology (1)	Growth (1,2,3) and morphology (0)	Growth (1,2,3) and Colour (1,2,3)

APPENDIX D

Estimates of sensitivity of the culture protocol
for isolation of *S. agalactiae* from bulk tank milk

(A)

Five estimates of the sensitivity of individual bulk tank milk culture protocols using modified Edwards® and GBS® media

	December, 1992 census		June, 1994 census		January, 1993 subsample[a]
	culture 1	culture 2	culture 1	culture 2	
positive herds	60	52	38	42	26
positive on series	77	77	57	57	40
estimate of sensitivity	.78	.68	.67	.74	.65

(B)

Estimate of the sensitivity of the testing protocol[b] when 2 culture procedures are interpreted in parallel

Estimate of the sensitivity of a single culture procedure[b]	0.70		
Formula[c] for calculation of the combined sensitivity of two tests interpreted in parallel	$\alpha_1 + \alpha_2 - \alpha_1\alpha_2$		
Combined sensitivity	.70 + .70 - (.70 x .70)	=	0.91

[a] 40 of 42 herds which had conflicting results between culture 1 and 2 in December, 1992 were re-cultured
[b] One modified GBS® and one modified Edwards® bulk tank milk culture
[c] Where α_1 and α_2 are the sensitivities of the two tests

APPENDIX D

(C)

Validation of the assumption of independence of tests

The assumption of independence of the two tests can be justified by comparing the observed agreement between the two tests in the December, 1992 culture series and a calculation of the expected agreement based on the sensitivity of the test.

Expected agreement

The expected agreement is equal to the proportion of herds expected to have 2 positive results plus the proportion of herds expected to have 2 negative tests based on the sensitivity of the test.

Formula[a]	$\alpha^2 + (1-\alpha)^2$		
Expected agreement if the sensitivity of a test is 0.70	$0.70^2 + (1 - 0.70)^2$	=	.58 or 58%

Observed agreement[b]

Number positive on both culture series	35		
Number positive on at least one culture	77		
Observed agreement	$\frac{35}{77}$	=	.45 or 45%

The observed agreement is less than expected based on the sensitivity of the test and therefore the assumption of independence of tests is reasonable.

[a]Where α is the estimated sensitivity of the test
[b]Based on the December, 1992 census data

APPENDIX E

Costs associated with *S. agalactiae* infection in Prince Edward Island dairy herds

Table 1.

Cost of elevated BTSCC in herds with *S. agalactiae* over culture negative herds in the December, 1992 census

culture status	N	average[a] lost production	decrease milk per month[b]	milk price[c] per l	value of lost milk (month)	value of lost milk (year)
positive	77	5.46%	1139 l	$.4542	$517.33	$6,208
negative	375	2.03%	423 l	$.4614	$195.17	$2,242
difference		3.43%	716 l	N/A	$322.16	$3,866

Calculated costs

Estimate of prevalence of *S. agalactiae* in 1992	=	18.9%
Estimated cost of elevated BTSCC associated with *S. agalactiae*	=	($3,866 x .189 x 452) $336,110

[a] Calculated in each herd using monthly BTSCC and based on a loss of 2% for each 100 x 10^3 BTSCC above a 200 x 10^3 threshold. See reference 8 Chapter 2.
[b] Average P.E.I. dairy herd shipping 23.1 l/day from each of 30.1 cows for 30 days
[c] Calculated from proportion of industrial and fluid herds in each group. The industrial milk price in 1992 was $.4389/l. Eighty percent of fluid producers production was paid at the industrial price and 20% at a price of $.6204/l.

APPENDIX E

Table 2.

Cost associated with the 2¢ a litre penalty and loss of fluid premium due to BTSCC above threshold values[a] in herds with *S. agalactiae*

S. agalactiae	N	number of penalties	expected penalty numbers[b]	number of penalties in excess of expected	total value of excess penalties (5 risk periods)	total value of excess penalties (year)
positive (industrial)	45	23	5	18	$7,506[c]	$16,513[c]
positive (fluid)	32	12	2	10	$11,740[d]	$25,828[d]
negative (industrial)	126	13				
negative (fluid)	249	17				

Calculated costs

Average cost of penalties on infected farms above the cost in negative herds	=	$550
Cost of BTSCC penalties associated with *S. agalactiae* infection on P.E.I. at a herd prevalence of 18.9%	=	($550 x .189 x 452) $47,817

[a] Greater than 500,000 for fluid producers and 750,000 for industrial producers
[b] Calculated from the rate of penalization in the culture negative group and the proportion of fluid versus industrial producers
[c] 2¢ per litre of milk shipped in the penalized month. The value of such a penalty in the average P.E.I. herd was $417.
[d] $417 associated with the basic penalty plus $757 due to loss of fluid premium

APPENDIX E

Table 3.

Cost associated with the 2¢ per litre penalty and loss of fluid premium due to standard plate count elevation above threshold levels[a] in herds with *S. agalactiae*

S. agalactiae infection status	N	penalty number	expected penalty numbers[b]	number of penalties in excess of expected	value of excess penalties (10 risk periods)	value of excess penalties (year)
positive (industrial)	45	15	3	12	$2,502[c]	$6,005[c]
positive (fluid)	32	0	0[d]	0	$0	$0
negative (fluid)	249	4				
negative (industrial)	126	7				

Calculated costs

Average cost of penalties on infected farms above the cost in negative herds	=	$78
Cost of SPC penalties associated with *S. agalactiae* infection on P.E.I. at a herd prevalence of 18.9%	=	($78 x .189 x 452) $6,781

greater than 50,000 cfu/ml for fluid producers and 200,000 cfu/ml for industrial producers

[b] Calculated from the rate of penalization in the culture negative group and the proportion of fluid versus industrial producers

[c] 2¢ per litre of milk shipped in the penalized period. The value of such a penalty in the average P.E.I. herd was $208.50.

[d] The expected value was 0.5. It is unlikely that infection has a sparing effect on SPC

APPENDIX E

Table 4.

Cost associated with loss of fluid status[a] in the August 1, 1992 to July 31, 1993 dairy year and *S. agalactiae* infection status.

S. agalactiae infection status	N	number of herds losing fluid status	expected number[b] losing fluid status	number of penalties in excess of expected	value of excess penalties (16 month duration)[c]	value of excess penalties (year)
positive	32	7	1	6	$12,112[d]	$9,084[d]
negative	249	7				

Calculated costs

Cost to the P.E.I. dairy industry per year of the loss of fluid status associated with *S. agalactiae* infection = $54,504

[a] Due to receiving four or greater monthly BTSCC above 500,000. The program did not start until the 1992-1993 dairy year was partially over. To more closely estimate the economic affect, calculations are based on the complete year.

[b] Based on the proportion of herds in the culture negative group losing fluid status.

[c] The average herd loses fluid status in month 8 of the dairy year. They lose the fluid premium for the remainder of that year (4 months) and the following year.

[d] Value is based on the average P.E.I. herd (30.1 cows milking 23.1 l/day) losing fluid premium ($757/month).

APPENDIX F

TELEPHONE SURVEY OF MASTITIS CONTROL
PRACTICES ON SELECTED FARMS

PRODUCERS NAME/DAIRY NUMBER

ADDRESS/TELEPHONE NUMBER _____

_____

(1)	Do you teat dip your cows after milking?	No _____	Sometimes _____	Always _____
(2)	Do you treat you cows with "Drycow" Medication in the quarters at dry off?	No _____	Some cows _____	All cows _____
(3)	Have you had your milking machinery serviced in the last...	6 months _____	1 year _____	Greater than 1 year _____
(4)	Have you consulted your veterinarian or udder health technician because of high somatic cell counts or Strep. ag. mastitis in the last year?	No _____	Veterinarian _____	Udder health technician _____
(5)	Have you sent milk samples to the lab to determine the type of mastitis that is present on your farm in the last year?	From individual cows _____	From all cows in the herd? _____	From the bulk tank milk? _____

APPENDIX G

Table 1.

Chi-square statistic and relative risk of changes in *S. agalactiae* culture status of infected herds[a] participating in an "on farm" extension program versus herds not participating in the extension program and not informed of their infection status

		culture negative herds	culture positive herds	total
"on farm" extension program	observed	19	27	46
	expected	14.90	31.10	
infected[a] control herds	observed	4	21	25
	expected	8.10	16.90	
total		23	48	71

uncorrected Chi-square = 4.74 *p-value* = 0.0295
Degrees of freedom = 1

Relative Risk (RR)[b] = 2.58
Taylor series 95% confidence limit = 0.99<RR<6.76

[a] Infection status determined in a December, 1992 census of all herds shipping milk in P.E.I. The specificity of the culture protocol was 100%.
[b] Relative risk and confidence interval computed in Epi Info see text (page 125) for further explanation

APPENDIX G

Table 2.

Relative risk, after adjustment for misclassification bias,[a] of changes in *S. agalactiae* culture status of infected herds[b] participating in an "on farm" extension program versus herds not participating in the extension program and not informed of their infection status

		culture negative herds	culture positive herds	total
"on farm" extension program	adjusted cell frequency[b]	16.33	29.67	46
infected[a] control herds	adjusted cell frequency[c]	1.92	23.08	25
total		17.25	52.75	71

Relative Risk (RR)[d] = 4.62
Taylor series 95% confidence limit = 2.25<RR<9.51

[a] See reference 15 Chapter 3 for formula
[b] Infection status determined in a December, 1992 census of all herds shipping milk in P.E.I. The specificity of the culture protocol was 100%.
[c] Adjusted for nondifferential misclassification of disease status. The specificity of the test was 1 and the sensitivity was estimated to be .91.
[d] Relative risk and confidence interval computed in Epi Info see text (page 125) for further explanation

APPENDIX H

Results of the telephone survey of 109 program, positive control and negative control herds and *S. agalactiae* status in December, 1993 (see Appendix F for survey questions)

			program herds		positive control herds		negative control herds
		# of herds	*S. ag.* +ve	*S. ag.* -ve	*S. ag.* +ve	*S. ag.* -ve	
teat dip	always	90	16	18	12	4	40
	sometimes	4	1	0	2	0	1
	never	15	7	1	3	0	4
dry cow therapy	all cows	57	9	9	9	3	27
	selective	40	10	10	5	0	15
	none	12	5	0	3	1	3
equipment last serviced	< 6 mo.	50	8	8	6	3	25
	> 6 mo. < 1 year	35	8	7	6	0	14
	> 1 year	24	8	4	5	1	6
consulted professional	veterinarian	30	7	12	2	2	7
	udder health technician	7	2	1	2	0	2
	both	2	0	2	0	0	0
	none	70	15	4	13	2	36
milk culture	whole herd culture	14	1	11	1	0	1
	individual cow cultures	41	7	4	7	1	22
	bulk tank culture	1	0	0	0	0	1
	none	53	16	4	9	3	21

APPENDIX I

(A)

Association between program and consultation with udder health management professionals

		Professional Consultation		
		yes	no	
Extension Program	yes	24	19	43
	no	6	15	21
		30	34	64

Relative risk = 1.95

$\chi^2 = 4.20$

p value < 0.05

Conclusion:

Herds that were involved in the extension program where 1.95 times more likely to consult a professional for help due to elevated SCC or subclinical mastitis than positive control herds

(B)

Association between extension program and the use of whole herd milk cultures among herds that did not consult with udder health professionals

		Whole Herd Culture		
		yes	no	
Extension Program	yes	0	19	19
	no	0	15	15
		0	34	34

Risk of culture in both the program and control herds was 0

Conclusion:

The probability of carrying out a whole herd culture was 0 in both the extension program and control herds if the herd owner had not consulted with an udder health management professionals. As a result, there is no evidence of a direct pathway from program to culture.

APPENDIX I

(C)

Association between consultation with udder health professionals and the use of whole herd culture controlling for the effect of the extension program

Herds were stratified on whether or not they had been part of the "on farm" extension program or positive control group and categorized by professional consultation and whole herd culture.

Program Herds

		Whole Herd Culture		
		yes	no	
Professional Consultation	yes	12	12	24
	no	0	19	19
		12	31	43

Relative risk undefined

$\chi^2 = 13.18$

p value < 0.001

Positive Controls

		Whole Herd Culture		
		yes	no	
Professional Consultation	yes	1	5	6
	no	0	15	15
		1	20	21

Relative risk undefined

$\chi^2 = 2.63$

p value = .1

Stratified Analysis

Mantel-Haenszel summary $\chi^2 = 12.74$

p value < 0.01

Mantel-Haenszel weighted relative risk = undefined

Conclusion:

The relative risk of culture in the consultation and non-consultation herds was undefined because the probability of culture in the non-consulting group was 0. However, there is clearly a strong association between consulting and carrying out a whole herd culture since 13 of the 30 herds (43%) that had professional consultation carried out a whole herd culture while none of the herds that did not consult carried out a culture.

(D)

Effect of consulting on the outcome variable eradication

		Eradication		
		yes	no	
Professional Consultation	yes	17	13	30
	no	6	28	34
		23	41	64

Relative risk = 3.21

$\chi^2 = 10.54$

p value < .01

Effect of consulting on the outcome variable eradication controlling for program

Herds were stratified on whether or not they had been part of the "on farm" extension program or positive control group and categorized by professional consultation and eradication

Program Herds

		Eradication		
		yes	no	
Professional Consultation	yes	15	9	24
	no	4	15	19
		19	24	43

Relative risk = 2.97

$\chi^2 = 7.39$

p value < 0.01

Positive Controls

		Eradication		
		yes	no	
Professional Consultation	yes	2	4	6
	no	2	13	15
		4	17	21

Relative risk = 2.50

$\chi^2 = 1.11$

p value = .29

Stratified Analysis

Mantel-Haenszel summary $\chi^2 = 6.70$
p value < 0.01
Mantel-Haenszel weighted relative risk = 2.87

Conclusion:
The effect of consulting was the same in the positive controls as in the program herds.

(E)

Relative risk of eradication of *S. agalactiae* as a result of consulting a professional if whole herd culture was not performed

		Eradication			
		yes	no		Relative risk = 1.89
Professional Consultation	yes	6	12	18	$\chi^2 = 1.63$
	no	6	28	34	
		12	40	52	*p value* > 0.05

Conclusions:
There was no significant association between consulting and eradication among herds that did not do a whole herd culture. Amongst herds that did not carry out a whole herd culture, the probability of eradication appeared higher if the herd had received professional consultation (6/18 or 33%) compared to herds that did not receive consultation (6/30 or 18%). However, this difference was not statistically significant.

(F)

Effect of whole herd culture on the outcome variable eradication controlling for consultation with an udder health professional

Herds were stratified on whether or not they had consulted an udder health professional and categorized by whole herd culture and eradication

		Eradication		
		yes	no	
Whole Herd Culture	yes	11	2	13
	no	6	11	17
		17	13	30

Herds consulting an udder health professional

Relative risk = 2.40

$\chi^2 = 7.30$

p value < 0.01

		Eradication		
		yes	no	
Whole Herd Culture	yes	0	0	0
	no	6	28	34
		6	28	34

Herds not consulting a professional

Because no herds in the non-consulting group used whole herd culture the relative risk of eradication due to this practice cannot be calculated

Stratified Analysis

Mantel-Haenszel summary $\chi^2 = 5.25$

p value < 0.05

Mantel-Haenszel weighted relative risk = 2.40

Conclusion:

The risk of culture in both the extension program and control herds was 0 if they did not consult with udder health management professionals (see section B). As a result, the pathway culture --> eradication can be described by examining the effect of culture on eradication controlling for professional consultation. Because no herds in the consultation negative group did whole herd cultures the summary relative risk (RR) of eradication due to culture is the same as the RR in the consulting herds. Whole herd culture was associated with herd level eradication of *S. agalactiae*.

APPENDIX I

(G)

Association of the extension program and herd level eradication of *S. agalactiae* among herds that did not consult an udder health professional or do whole herd cultures

		Eradication		
		yes	no	
Extension Program	yes	4	15	19
	no	2	13	15
		6	28	34

Relative risk = 1.58

$\chi^2 = 0.34$

p value < 0.56

Conclusion:

There is no direct association between the extension program and herd level eradication of *S. agalactiae*. The previously observed association (see Appendix G) is mediated by the use of professional consultants and whole herd culture.

APPENDIX J

In order to compare the response to the "on farm" and "mail out" extension programs the rate of spontaneous elimination in the positive control herds was measured. Between December, 1992 and December, 1993 this rate was 16%.

Table 1.
Response by Prince Edward Island dairy producers with *S. agalactiae* infected herds to a "mail out" extension program compared to the response of a similar group of producers to an "on farm" extension program.

	number of herds	number of herds eliminating *S. agalactiae*	number of herds expected to eliminate *S. agalactiae*[a]	number at risk	number assumed in response to program
"on farm" program	46	19	7.36[a]	38.64	11.64 (30.1%)[c]
"mail out" program	20	5	1.60[b]	18.40	3.40 (18.5%)[c,d]

[a] 12 month risk period for spontaneous eliminations

[b] 6 month risk period for spontaneous eliminations

[c] Proportions are not significantly different from each other ($z = 0.93$, $p > 0.05$)

[d] Standard Error (9.0%)