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# *THE EFFECTS AND CONTROL OF COAGULASE NEGATIVE STAPHYLOCOCCAL MASTITIS IN DAIRY CATTLE IN P.E.I.*

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

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Thomas Jeffrey Davidson

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## ABSTRACT

This project, an investigation of coagulase negative Staphylococcal (CNS) mastitis in Prince Edward Island involved two studies.

In the first study, a cohort of 84 cows at 7 farms were quarter sampled eight times during the year. The quarter prevalence of CNS mastitis varied from 4.8% to 6.4% in the first five months of lactation and increased to 14.2 to 16.6% in the last four months of lactation. The geometric mean somatic cell count (SCC) for quarters infected with CNS and uninfected quarters were 90 x $10^3$ and 64 x $10^3$ respectively. The 2 month new infection risk of CNS was 9.0% while the 2 month elimination risk was 74.4%. Infection with CNS did not alter the risk of subsequent infection with S. aureus. CNS mastitis significantly increased SCC. No significant effect on milk production was detected.

There was no significant difference between treatment with Orbenin Dry Cow or Novodry Plus Dry Cow with respect to new infection rate or elimination rate over the dry period. New infection rates were 2.8% and 5.3% for Orbenin Dry Cow and Novodry Plus Dry Cow respectively. The elimination rate was 92% for both products.

In the second study, 57 farms involving 1688 cows were randomly chosen from all herds in P.E.I. Composite samples were collected from all cows milking. The overall prevalence of CNS infection was 24.5%. The prevalence of CNS infections was highest in the first and second lactation and lower but constant in lactations greater than two. The geometric mean SCCs for CNS infected quarters and

uninfected quarters were $108 \times 10^3$ and $69.2 \times 10^3$ respectively. Infection with CNS resulted in a significant increase in geometric mean SCC. No significant differences were found between the herd prevalence of CNS infection in herds using dry cow treatment and not using dry cow treatment or between herds using teat dip and not using teat dip.

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I am grateful to my wife, Charlotte, for giving me the encouragement and strength to change career paths and putting up with me while I did. My children and I started school on the same day in September, 1988: Jessica into grade 1, John into kindergarten and I into graduate school. Thank God I finished my studies before them.

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# CHAPTER 1

## 1. INTRODUCTION

Coagulase negative Staphylococci (CNS) are the organisms most frequently isolated from milk samples of lactating cows (1,2). In the past, little was known about the epidemiology of CNS intramammary infections (IMI) because CNS were classified as minor pathogens and considered insignificant in mastitis control programmes (3,4,5,6,7,8,9,10,11). In the past 3 years, it was noted in some dairy herds in Prince Edward Island (P.E.I.), that there was a moderately elevated bulk tank somatic cell count (300,000-400,000) and relatively few isolations of major pathogens. The only organisms consistently isolated were CNS. These observations stimulated this study, it's purpose being to determine the prevalence and significance of intramammary infection with CNS in P.E.I. dairy herds.

Studies have shown that CNS IMI significantly increase somatic cell count (SCC) (5,7,10,12). Studies examining the relationship between milk production and CNS IMI have not been consistent. Most studies report a negative influence on milk production (6,10,13). However, one study showed no relationship (14) and another reported a positive effect on milk production (15). A number of studies have reported that CNS IMI has a protective effect against infection with major pathogens such as Staphylococcus aureus and Streptococcus agalactiae (4,9,16,10,17,18,19,20)

Dry cow therapy and teat dipping have been reported to reduce the prevalence of CNS IMI (3,21,22,23).

Little work has been done on the prevalence of CNS IMI in P.E.I. or any other part of Canada. Studies in other parts of the world have shown the quarter prevalence to vary from 7.2% to 14.3% (24,3,7,21,22). The new infection risk and spontaneous elimination risk also varied between studies (1,6,7,8,22).

The objectives of this study were 1) to determine the new infection and spontaneous elimination risk of CNS infections throughout the lactation, 2) to determine the influence of CNS infection on subsequent infections with S. aureus, 3) to demonstrate the influence of CNS infection on SCC and milk production, 4) to determine if there were differences between dry cow therapies in the prevention and elimination of CNS infections over the dry period, 5) to report the effect of teat dipping and dry cow therapy on the prevalence of CNS mastitis and 6) to determine the prevalence of CNS mastitis in P.E.I.

# CHAPTER 2

## 2. A COHORT STUDY OF COAGULASE NEGATIVE STAPHYLOCOCCAL (CNS) MASTITIS IN SELECTED P.E.I. DAIRY HERDS

### 2.1 INTRODUCTION

In P.E.I., there appeared to be an abundance of coagulase negative Staphylococci (CNS) in a number of dairy herds. This study examined selected dynamics of this organism in these herds.

In the United States, there are discrepancies in reports examining the prevalence of CNS mastitis throughout lactation of dairy cows. Various studies have reported that the prevalence increases (6), decreases (8,22) and remains constant (7) throughout lactation. The spontaneous elimination rate of CNS during lactation also varied from 15.5% to 40% in studies in the United States and France (1,6,25). The protective effect of CNS on the rate of infection of major pathogens has been studied with varying results (4,5,9,16,10,17,18,19,20). CNS has been shown to increase the somatic cell count (SCC) in infected quarters in most studies (7,16,10,12). CNS infections have had a detrimental effect on milk production in most studies (6,10,13) but have also been shown to have no effect (14) and to increase production (15).

The objectives of this study were to determine the new infection and spontaneous elimination rates of CNS throughout the lactation, to determine the influence of CNS infection on subsequent infections with S. aureus and to investigate the influence of CNS infection on SCC and milk production in selected dairy herds in P.E.I.

## 2.2 MATERIALS AND METHODS

### Selection of Herds and Cows

Seven dairy herds within a 30 km. radius of Charlottetown, P.E.I. were selected to participate in this study. Herds were selected on the basis of an apparently elevated prevalence of coagulase negative Staphylococcus mastitis, current participation in a production and SCC recording programme (Dairy Herd Analysis Service (DHAS) or Record of Performance (ROP)), a willingness to participate and cooperate in the study and an established client-doctor relationship with the principal investigator.

In these herds, 84 cows were due to freshen between May and August 1988. Heifers were not included because they would be unable to provide all the samples required (predrying and drying off samples).

There was no history of Streptococcus agalactiae on these farms for at least 5 years. At the beginning of the study, bulk tank samples from all the farms were taken weekly for 3 weeks. All these samples were negative for S. agalactiae. The herds were assumed to be free of this organism for the duration of the study.

**Sampling schedule**

Each cow was scheduled to be quarter sampled eight times during the study. Samples were: predry sample (S1), taken one week before drying off; dry sample (S2), taken at the time of drying off; fresh sample (S3), taken within one week after freshening; post fresh sample (S4), taken between one and two weeks after freshening; 2-3 month sample (S5), taken 2 to 3 months after freshening; 4-5 month sample (S6), taken 4 to 5 months after freshening; 6-7 month sample (S7), taken 6 to 7 months after freshening and the 8-9 month sample (S8), taken 8 to 9 months after freshening. Sampling was completed by June, 1989.

**Collection of samples and data**

Sampling was performed by a veterinary student from May 1988 to September 1, 1988 and by the primary investigator for the remainder of the project. For S1 and S2, the cows were milked before collection of the samples. Immediately after the removal of the milking equipment, the teat ends were scrubbed with alcohol and quarter samples were obtained in an aseptic manner. For S3 to S8, foremilk was used for sampling purposes. After the producer had washed and dried the teats, the teat ends were scrubbed with alcohol and four streams of foremilk were discarded before collection of the samples in an aseptic manner.

A copy of the ROP, DHAS, or ADLIC (Atlantic Dairy Livestock Improvement Committee) report closest to the sampling date was obtained. It

included milk production, the fat content of the milk, lactation number and the number of days the cow was milking.

**Bacteriology**

Samples were taken directly from the farm to the Diagnostic Microbiology Laboratory at the Atlantic Veterinary College. Platinum loops were used to transfer 0.01 ml of milk aseptically from the milk vials to a blood agar plate (Columbia agar base, 5% defibrinated sheep's blood). This plate was then incubated at 35 degrees C for 24 hours. After the transfer of the milk to the blood agar plates, the milk vials were sent to the P.E.I. Provincial Dairy Laboratory for somatic cell count determination by the Fossomatic Somatic Cell Counter: Model 215 (A/SN. Foss Electric, 69, Slangerupgade Dk-3400 Hillerod, Denmark). If a delay in this transfer was anticipated, the samples were kept in a refrigerator. The maximum delay was 24 hours.

Figure 1 shows the flowchart used to identify the various organisms. A gram stain was performed on each sample. Gram negative rods and Gram positive rods were identified as such and not classified further. A catalase test was performed on gram +ve cocci to differentiate catalase negative Streptococcus sp. from catalase positive Staphylococcus and Micrococcus. Streptococci were not further identified. The tube coagulase test was performed on all catalase positive cocci. Coagulase positive cocci were classified as Staphylococcus aureus. Coagulase negative cocci were classified as either micrococci or coagulase negative

Staphylococci by colony characteristics. Number of colonies of all organisms were recorded.

**Species Identification**

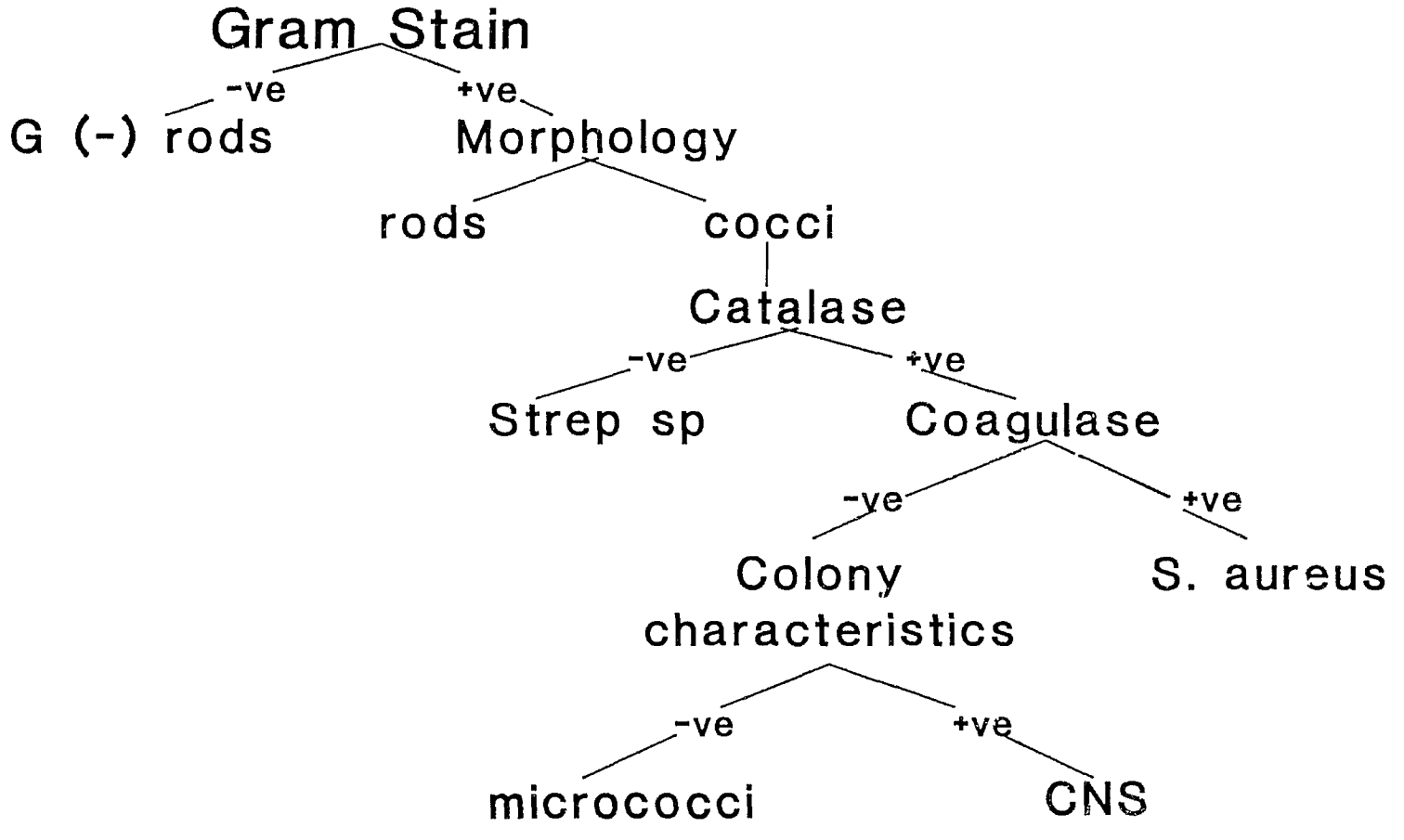
Between July 18 and August 2, 1988, all isolates of Staphylococci were saved for species identification. These cultures were frozen on trypticase soy agar (TSA) slants and analyzed in January, 1989 using Staph Ident (API Laboratory Products Ltd., St-Laurent, Quebec) and repetition of the tube coagulase test. In February and March 1989, 8 coagulase +ve Staphylococci samples and 100 coagulase -ve Staphylococci samples were tested in a similar manner.

**Classification of Culture Results**

The method of determining the number of colonies needed to establish a CNS infection was to compare colony count criteria ($\geq$1 colony, $\geq$2 colonies, $\geq$5 colonies) to a gold standard. The gold standard was defined as positive culture results for CNS in both S1 and S2. CNS $\geq$2 colonies had the most acceptable combination of sensitivity, specificity and overall correct classification and was selected as the criterion for a positive culture (Appendix A). Based on previously published criteria, a minimum of 2 colonies and 5 colonies were required for positive infection status for S. aureus and Gram -ve rods respectively (26).

Culture results were classified as follows. A culture was classified as Coagulase negative Staphylococcus-pure (CNSP) if it had at least 2 colonies of CNS with no other growth. A culture was classified as Coagulase negative Staphylococcus-all (CNSA) if it had at least 2 colonies of CNS with no more than

# COHORT STUDY Figure 1



one other colony type isolated. A culture was classified as S. aureus (SA2) if it had at least 2 colonies of S. aureus. A culture was classified as Gram -ve rods (GN5) if it had at least 5 colonies of gram -ve rods. Streptococci (non-agalactiae) and Gram +ve rods were classified as contaminants and samples yielding only these organisms were recorded as having no significant growth (NSG). If three or more colony types were isolated from a sample, the sample was considered to be contaminated (CON). However, if S. aureus was isolated, then it was a positive culture even if three different colony types were isolated (27).

**Statistical Analysis**

Data were entered and stored in dBASE III Plus (28), a database management software package. Statistical analyses were conducted using Minitab Version 7.1 (29) on U.P.E.I.'s mainframe computer (VAX).

The chi-square statistic was used to determine significant differences between the prevalences, by stage of lactation, of CNSP and CNSA. This statistic was also used to determine the significance of CNS infection on subsequent infections with S. aureus.

The new infection and spontaneous elimination risks between consecutive samples (approximately two months apart) were calculated for CNSA, CNSP and SA2. The numerator of the new infection risk was the percentage of uninfected quarters in the initial sample that became infected in the subsequent sample. The denominator was the percentage of uninfected quarters in the initial sample. The numerator of the elimination risk was the percentage of infected quarters in the

initial sample that spontaneously eliminated the infection in the subsequent sample. The denominator was the percentage of infected quarters in the initial sample. All rates were expressed as percentages and were "two month risks" (i.e. the percentage of new cases over a two month period among quarters at risk).

The effect of a CNS infection in a quarter on the subsequent infection rate of SA2 in that quarter was calculated. The numerator was the percentage of quarters infected with CNS in the initial sample that became infected with SA2 in the subsequent sample. The denominator was the percentage of quarters infected with CNS and uninfected with SA2 in the initial sample.

Cow level bacteriologic results were calculated from quarter level bacteriologic results for use in production analysis. These results were cumulative from each cow's quarter results.

## 2.3 RESULTS

All eight samples were collected and processed on 56 of the 84 cows in the cohort. Of the remaining 28 cows with missing samples, 15 missed one sample and 8 missed two samples. The main reason for missing data was the lack of opportunity to collect S1 and S8 before the cows were dried off.

Production data obtained for the sample dates for each cow included milk production, fat content of the milk, lactation number and number of days in lactation. To correctly relate production data to bacteriological data, the only samples used in the production analyses were those samples whose production test

day was no more than seven days from the collection of the milk samples. Data from 146 samples were used for production analyses.

A total of 64 isolates of Staphylococci were saved in July and August, 1988 for species identification. Of the 24 initially identified to be coagulase +ve, 17 were subsequently identified as coagulase -ve and not S. aureus. The 40 cultures initially identified as CNS were confirmed to be CNS. Samples were also saved in February and March 1989 for species identification. The 8 initially identified as coagulase +ve were S. aureus, and the 100 initially identified as coagulase -ve were CNS. The species and number of isolations of CNS confirmed in the above samples are in Appendix B.

The quarter prevalence of organisms by sample is given in Table I. The prevalence of CNSP and CNSA was relatively constant throughout the first five months of lactation, ranging from 4.8% and 13.1% for S3 to 6.4% and 16.7% for S6 for CNSP and CNSA respectively. The prevalence increased in the sixth to ninth month of lactation to 16.6% and 25.3% for S7 and 14.2% and 27.4% for S8 for CNSP and CNSA respectively. S1 and S2 had prevalences of 6.5% and 7.8%, and 17.5% and 20.0% respectively, for CNSP and CNSA respectively. There were significant differences in the prevalence of CNSP by stage of lactation ($p<0.001$) and CNSA by the stage of lactation ($p<0.001$).

The mean SCC for S5 to S8 are presented in Table I. Somatic cell counts were not used for S1 and S2 because samples were taken after the quarters were milked. These stripping samples would not yield reliable estimates of the SCC

(30). Counts for S3 and S4 were also eliminated as these samples were taken within two weeks of freshening, thus rendering them invalid (27). The mean SCC increased from S5 to S8 arithmetically (136.8 x $10^3$ to 390.3 x $10^3$) and geometrically (31.8 x $10^3$ to 126.6 x $10^3$).

The mean SCC for culture results is given in Table II. The overall arithmetic mean SCC for CNSP and CNSA were 291.2 x $10^3$ and 251.0 x $10^3$ respectively. Mean SCC for CNSP and CNSA were both minimally higher than mean SCC for NSG.

The 2 month new infection and elimination risks are given in Table III. The infection risk is greater between S6 to S7 (CNSP-14.7%, CNSA-22.5) and S7 to S8 (CNSP-11.5%, CNSA-24.0%) than between S4 to S5 (CNSP-4.0%, CNSA-9.3%) and S5-S6 (CNSP-6.7%, CNSA-14.4%). The overall 2 month infection risk for CNSP and CNSA was 9.0% and 17.0% respectively.

The 2 month elimination risk ranged from 52.4% to 100% for CNSP and 60.0% to 72.4% for CNSA. The overall 2 month elimination risk for CNSP and CNSA was 74.4% and 64.7% respectively.

The overall rate of new infection with SA2 was 2.2%. The new infection rate for SA2 was 2.7% and 1.3% for quarters previously infected with CNSP and CNSA respectively. The new infection rate for SA2 was 2.0% and 2.5% for quarter previously not infected with CNSP and CNSA respectively. There were no significant differences between these rates.

TABLE I
PREVALENCE OF ORGANISMS (QUARTER BASIS) IN AN 84 COW COHORT STUDY IN P.E.I.

| | $S_1$ (Predry) | $S_2$ (Dry) | $S_3$ (Fresh) | $S_4$ (Post Fresh) | $S_5$ (2-3 m) | $S_6$ (4-5 m) | $S_7$ (6-7 m) | $S_8$ (8-9 m) |
|---|---|---|---|---|---|---|---|---|
| # of Samples | 308 | 320 | 336 | 332 | 320 | 312 | 308 | 288 |
| CNS Pure (CNSP) | 6.5% | 7.8% | 4.8% | 4.2% | 4.7% | 6.4% | 16.6% | 14.2% |
| CNS All (CNSA) | 17.5% | 20.0% | 13.1% | 9.9% | 10.9% | 16.7% | 25.3% | 27.4% |
| S. aureus ($SA_2$) | NA | NA | NA | NA | 3.8% | 5.8% | 3.6% | 2.8% |
| Gram - ve R. (GN5) | 1.3% | 1.3% | 0.6% | 0.9% | 0.3% | 0.3% | 0.0% | 0.3% |
| Contam. | 7.8% | 8.1% | 9.2% | 8.7% | 14.7% | 3.2% | 7.8% | 8.0% |
| No growth | 66.9% | 63.4% | 74.1% | 78.6% | 69.7% | 75.0% | 63.6% | 61.1% |
| SCC - (x 1000) | NA | NA | NA | NA | 136.8 | 175.1 | 217.2 | 390.3 |
| SCC - Geometric (x 1000) | NA | NA | NA | NA | 31.8 | 45.8 | 79.9 | 126.6 |

## TABLE II

**THE ARITHMETIC AND GEOMETRIC MEAN SCC BY ORGANISMS FROM AN 84 COW (1228 SAMPLES) COHORT STUDY IN P.E.I.**

| | **Arithmetic mean SCC X1000 $S_5$-$S_8$** | **Geometric mean SCC X1000 $S_5$-$S_8$** |
|---|---|---|
| CNS Pure (CNSP) | 291 | 90 |
| CNS All (CNSA) | 251 | 75 |
| S. aureus ($SA_2$) | 920 | 375 |
| Gram - ve R. (GN5) | 44 | 43 |
| No growth | 186 | 64 |

## TABLE III

## THE 2 MONTH NEW INFECTION RISK AND ELIMINATION RISK FOR 320 QUARTERS IN A COHORT STUDY IN P.E.I.

| | S4→S5 | S5→S6 | S6→S7 | S7→S8 |
|---|---|---|---|---|
| **Coagulase Negative Staph-Pure (CNSP)** | | | | |
| Both - ve (%) | 91.7 | 89.1 | 79.9 | 75.0 |
| New Infection (%) | 3.8 | 6.4 | 13.8 | 9.7 |
| Elimination (%) | 3.5 | 4.5 | 3.3 | 11.5 |
| Both + ve (%) | 1.0 | 0 | 3.0 | 3.8 |
| 2 Month Infection Risk (%) | 4.0 | 6.7 | 14.7 | 11.5 |
| 2 Month Elimination Risk (%) | 77.8 | 100.0 | 52.4 | 75.2 |
| **Coagulase Negative Staph-All (CNSA)** | | | | |
| Both - ve (%) | 81.3 | 76.3 | 64.8 | 57.3 |
| New Infection (%) | 8.3 | 12.8 | 18.8 | 18.1 |
| Elimination (%) | 7.6 | 7.4 | 9.9 | 15.3 |
| Both + ve (%) | 2.9 | 3.5 | 6.6 | 9.0 |
| 2 Month Infection Risk (%) | 9.3 | 14.4 | 22.5 | 24.0 |
| 2 Month Elimination Risk (%) | 72.4 | 67.9 | 60.0 | 63.0 |
| **S. Aureus (SA2)** | | | | |
| Both - ve (%) | 95.6 | 92.6 | 92.8 | 93.8 |
| New Infection (%) | 2.5 | 3.5 | 1.3 | 2.4 |
| Elimination (%) | 0.6 | 1.9 | 3.6 | 2.4 |
| Both + ve (%) | 1.6 | 1.9 | 2.3 | 1.4 |
| 2 Month Infection Risk (%) | 2.5 | 3.6 | 1.4 | 2.5 |
| 2 Month Elimination Risk (%) | 27.3 | 50.0 | 61.0 | 63.2 |

S4 - 1-2 weeks postpartum
S5 - 2-3 months postpartum
S6 - 4-5 months postpartum
S7 - 6-7 months postpartum
S8 - 8-9 months postpartum

The natural log of the SCC (lnSCC) was regressed on CNSP and CNSA separately to determine if CNSP or CNSA had a significant influence on SCC. Cow, sample number, lactation number, farm of origin, SA2 infection and GN5 infection were controlled in the regression. CNSP and CNSA had a significant positive influence on log SCC. The coefficients for CNSP and CNSA in the regression equation were 0.2721 (p=0.024) and 0.1813 (p=0.049) respectively. Overall $R^2$ was 29.6% and 29.5% for models containing CNSP and CNSA respectively.

Daily milk production was regressed on CNSP and CNSA separately controlling for lactation, SA2 infection, days in milk and farm to determine if CNSP or CNSA had an influence on milk production. CNSP and CNSA did not have a significant influence on milk production. The coefficients for CNSP and CNSA were 0.2085 (p=0.794) and 0.7712 (p=0.327) respectively.

## 2.4 DISCUSSION

There are no standard criteria for the diagnosis of CNS infections in the bovine udder. For this reason CNS was evaluated by two criteria, CNSP and CNSA. Most analyses were based on results from a single sample so it was necessary to come up with a minimum number of colonies required for a diagnosis of CNS. Based on results from one set of paired samples, two colonies provided the best combination of sensitivity, specificity and overall correct classification.

The reason for the misclassification of organisms in the samples saved for speciation in July and August, 1988 was not determined. It may not have been determined due to the lapse of time between initial identification of the organisms and verification procedures (4-5 months). Other cultures were not saved so further investigation of the problem was not possible. All avenues of investigation were explored with no satisfactory results. These included the possibility of contamination of laboratory materials, identification errors and human errors. It was concluded that identification of S. aureus was not reliable during the summer of 1988. Starting in September,1988, a medical microbiologist processed all the samples and verification of results in February and March 1989 confirmed identification by the medical microbiologist was correct. All the samples of S1 and S2 and approximately one-half the samples of S3 and S4 were processed before September. S. aureus was not used in the analysis of data from S1, S2 and S3. During this period, CNS prevalence was probably underestimated by approximately 12% due to misclassification of CNS as S. aureus.

The overall prevalence of CNSP (8.0%) and CNSA (17.4%) are similar to values reported from other studies. In a clinical trial involving 16 herds in Vermont, the prevalence of CNS in teat dipped and non teat dipped quarters was 7.2% and 11.0% respectively (3). The criterion for infection was positive duplicate foremilk samples. Another study in Britain involving 30 herds, found a 13.4% prevalence of CNS in herds not practising teat dipping and dry cow treatment, and a 6.3% prevalence in the same herds after 3 years of teat dipping and dry cow

treating (21). The criterion for infection was two consecutive isolations of CNS from samples taken one week apart. All the herds in the current study practised teat dipping and dry cow treatment. A five year study of several herds in Sweden found 7.6% of quarters infected with a pure culture of CNS (24). The University of Kentucky dairy herd had a CNS quarter prevalence of 14.3% (7). Criteria for infection in this study was not given, making the findings difficult to interpret. Comparison of results among studies can be difficult because of the lack of common criteria for infection status (25).

Prevalence of CNSP was relatively constant prepartum and throughout the next lactation except for an increase at 6-7 months. The University of Kentucky dairy herd demonstrated a constant prevalence throughout the lactation (7). Two University of Wisconsin dairy herds reported a steady increase in CNS prevalence from 13% at calving to 27% at lactation end (6) while a study in the Ohio Agricultural Research and Development Center (OARDC) dairy herd showed CNS prevalence greatest at parturition (15%) then to decrease throughout the lactation to drying off (3-6%) (22). A study in a commercial herd in Louisiana reported an increase in prevalence of S. epidermidis but a decrease in total CNS infections throughout the lactation (8). The prevalence of CNS and S. epidermidis, and species composition of CNS was not reported in this study, making interpretation difficult. The dramatic differences between studies in changes in CNS prevalence throughout the lactation may be due to different dry cow treatment practices and/or to a different species composition of CNS (25).

The overall 2 month new infection risks for CNSP and CNSA were 9.0% and 17.0% respectively. The new infection risk increased during the lactation. Studies have shown infection risks to remain fairly constant during the lactation. In the results from the two University of Wisconsin herds, infection risks were constant but no values were presented (6). One previous study involving 24 heifers in Iowa, showed the 1 month infection risk to be fairly constant throughout lactation at approximately 12% (1).

The overall 2 month elimination risk for CNSP and CNSA of 74.4% and 64.7% respectively during lactation was considerably higher than other studies. In the University of Wisconsin study, where the criterion for infection rate was two cultures positive for CNS one week apart and the criterion for elimination was two cultures negative for CNS one week apart, the elimination risk over the lactation was 15.5% (6). This low rate may be due to the strict criteria for elimination of CNS. In another study involving two commercial herds in France, the 3 week elimination risk was 34% (25). The criterion for elimination risk was similar to the University of Wisconsin study except that sampling took place every three weeks. In this study, foremilk samples were taken without discarding the first strippings. These strippings are expected to contain more CNS than the rest of the milk (32). The species composition in first strippings compared to other samples and the frequency of sampling may account for the different elimination risks. In the study involving 20 heifers in Iowa, the one month elimination risk was 40% (1). The criterion for new infections was the repetition of the isolation

of CNS after it was first isolated and an elevated SCC count. The study did not define the criterion for elimination. The species composition of CNS in the various studies in all likelihood vary (33), which could account for the different persistences (1). Different persistences would account for the differences in elimination risks (7,25).

The influence of CNS infections in protecting the quarter from S. aureus infections was not demonstrated. The failure to demonstrate this may have due to the small number of quarters previously infected with CNS which subsequently became infected with S. aureus. There are numerous studies showing a protective effect from infection with major pathogens by CNS. A study in 1966 involving a herd in Great Britain suggested that mildly pathogenic CNS might protect the udder from becoming infected by more pathogenic bacteria (4). In a controlled laboratory experiment in Britain, quarters infected with S. epidermidis were less susceptible to infection with E.coli or S. agalactiae (9,16). The authors explained this as being due to an increased SCC. In contrast, a study based on a single Ohio dairy herd showed that the rate of environmental streptococcal infections was greater in quarters infected with CNS (5). Rates of coliform infections were neither enhanced nor reduced in quarters infected with CNS. Results from trials in France and Sweden have shown a protective effect of CNS against experimental infections with S. aureus (10,17,18,19,20). The mechanism of this protection in one study was stated as being due mainly to an increase in SCC caused by CNS (10). A study at the Hill Farm Research Station dairy farm in Louisiana

suggested that CNS afforded little resistance to S. aureus infections (35).

Infection with CNSP and CNSA had a significant positive influence on lnSCC. Other studies concur with this finding. LnSCC was approximately two-times higher in CNS infected quarters than in uninfected quarters in studies done in Ohio, Kentucky, Sweden and Wisconsin (5,7,10,12). LnSCC was 58 x $10^3$, 216 x $10^3$ and 67 x $10^3$ for uninfected quarters, and 105 x $10^3$, 500 x $10^3$ and 122 x $10^3$ for infected quarters respectively (7,10,12). In a seventeen cow study in England, S. epidermidis caused lnSCC to increase from three to ten times that of uninfected quarters (16). In another English herd, most species of CNS did not cause an increase in lnSCC (4).

CNSP had a greater effect on lnSCC than CNSA. Predicted SCC for a quarter uninfected with CNS, infected with CNSA and infected with CNSP were 87 x $10^3$, 102 x $10^3$ and 115 x $10^3$ respectively in a midlactation cow.

Infection with CNSP and CNSA did not have a significant influence on milk production. The coefficients for both were positive, indicating a possible trend to higher production in CNS infected cows. In the regression equation, CNSP and CNSA added 0.21 kg and 0.77 kg respectively to mean daily production. A 30 herd study in Virginia also demonstrated this trend (15). S. epidermidis was positively correlated (r=0.27) to production. An 85 herd study in Pennsylvania showed no significant correlation between prevalence of CNS and herd production (14). Most studies have shown a negative influence of CNS on milk production (6,10,13). A study in two University of Wisconsin herds reported a 2.9% decrease

in milk production in CNS infected cows (6). There was a 8.7% milk production decrease during the lactation in cows infected with CNS for the duration of the lactation compared to uninfected cows. A Swedish study involving 35 cows from 11 herds demonstrated a decrease in milk yield of 10.3% in quarters infected with CNS compared to corresponding healthy quarters (10). Another study with 24 commercial dairy herds in New York reported a reduction in milk production of approximately 700 kg in second lactation and older cows infected with CNS or C. bovis (13). The prevalence study in Chapter 4 also showed a trend to reduced milk production. The trend of a positive influence of CNS on milk production was unexpected as infection with CNS increased SCC in this study. The reason for this result could not be satisfactorily explained although the following explanations may exist: a) the time interval of one to seven days between production data collection and bacteriological data collection may have affected the true relationship b) the sample size may have been too small to detect a difference and c) the bacteriologic data was collected on a quarter basis while the production data was recorded on a cow basis.

# CHAPTER 3

# 3. COMPARING TWO DRY COW TREATMENTS ON THE NEW INFECTION AND ELIMINATION RATES OF COAGULASE NEGATIVE STAPHYLOCOCCUS (CNS) OVER THE DRY PERIOD

## 3.1 INTRODUCTION

Treatment of all cows at drying off with an effective antibiotic is one recommended procedure for all dairy herds on P.E.I. From personal observation, some herds in P.E.I. have a relatively high prevalence of coagulase negative Staphylococci (CNS) infections. The elimination of existing CNS infections over the dry period and the prevention of new CNS infections over the same period may be of benefit in a mastitis control programme. The two antibiotic preparations most commonly used in P.E.I. are a) novobiocin and penicillin (Novodry Plus, Upjohn Company, Animal Health Division, Orangeville, Ontario) and b) cloxicillin (Orbenin, Ayerst Laboratories, Montreal, Quebec).

The objective of this study was to determine if there were differences between the use of Novodry Plus and Orbenin in the prevention of new CNS infections and the elimination of existing CNS infections over the dry period.

## 3.2 MATERIALS AND METHODS

### Selection of Herds and Cows

The procedures for selecting herds and cows for participation in this study are described in Chapter 2. The cows in each herd were systematically allocated to the two study groups. One group was dry treated with Novodry Plus and the other was dry treated with Orbenin. The first cow in each herd was randomly assigned to one of the two study groups. Cows in that herd were then placed alternately into each study group.

### Sampling schedule

Each cow was scheduled to be quarter sampled four times during the study. Samples were: predry sample (S1), taken one week prior to drying off; dry sample (S2), taken at the time of drying off; fresh sample (S3), taken within one week after freshening and post fresh sample (S4), taken within one week after S3. Sampling was completed by September, 1989.

### Collection of Samples

Sampling was performed by a trained veterinary student and the principal investigator. For S1 and S2, the cows were milked prior to collection of the samples. Immediately after the removal of the milking equipment, the teat ends were scrubbed with alcohol and quarter samples were obtained in an aseptic manner. After S2 was collected, the cow was dry cow treated aseptically using individual tubes. The teats were then teat dipped. For S3 and S4, foremilk was used for sampling purposes. After the producer had washed and dried the teats,

the teat ends were scrubbed with alcohol and four streams of foremilk were discarded prior to collection of the samples in an aseptic manner.

**Description of Products**

The two dry cow preparations used were Novodry Plus and Orbenin. Novodry Plus contains 200,000 IU procaine G penicillin and 400 mg novobiocin in a 10 ml suspension. Orbenin Dry Cow contains 500 mg benzathine cloxacillin in a 10 ml suspension.

**Bacteriology**

Bacteriologic procedures used are described in chapter 2. Results were recorded as coagulase negative Staphylococci, S. aureus, Streptococci spp, Gram +ve rods and Gram -ve rods.

**Species Identification**

Between July 18 and August 2, 1988, all Staphylococci isolates were saved for species identification. These cultures were frozen on trypticase soy agar (TSA) slants and analyzed in January, 1989.

**Classification of Culture Results**

The method of determining the number of colonies needed to establish a CNS infection is outlined in Chapter 2. Two criteria were used for classifying infected quarters. The first required at least 2 colonies of CNS with no other growth. Denoted by CNSP, only S2 and S3 were used with this criteria because S2 was the sample taken at drying off and S3 was the sample taken closest to freshening. The second required the isolation of at least one colony of CNS from

two samples taken one week apart. Denoted by CNSB, samples taken before the dry period were S1 and S2 (S1-2), and samples taken after the dry period were S3 and S4 (S3-4).

Data were entered and stored in dBASE III Plus (28), a database management software package. Statistical analysis was conducted by Minitab Version 7.1 (29). Infection and elimination rates of CNS over the dry period were calculated for each study group. The chi-square test statistic was employed to identify any significant differences between the groups.

The new infection rate of CNSP and CNSB was calculated in both study groups. The numerator for the new infection rate of CNSP was the number of quarters uninfected with CNSP in S2 that became infected in S3. The denominator was the number of quarters uninfected with CNSP in S2. The numerator for the new infection rate of CNSB was the number of quarters uninfected with CNSB in S1-2 that became infected in S3-4. The denominator was the number of quarters uninfected with CNSB in S1-2.

The elimination rate of CNSP and CNSB was calculated in both study groups. The numerator for the elimination rate of CNSP was the number of quarters infected with CNSP in S2 that became uninfected in S3. The denominator was the number of quarters infected with CNSP in S2. The numerator for the elimination rate of CNSB was the number of quarters infected with CNSB in S1-2 that became uninfected in S3-4. The denominator was the number of quarters infected with CNSB in S1-2.

## 3.3 RESULTS

Species identification and distribution was described in Chapter 2. Due to misclassification of an undetermined number of CNS as S. aureus, CNS prevalence was probably underestimated by approximately 12%.

The prevalence of CNSP was 7.8% and 4.8% for S2 and S3 respectively. The prevalence of CNSB was 12.2% and 7.4% for CNS1-2 and CNS3-4 respectively.

Table IV shows the new infection rate and elimination rate of CNSP and CNSB for both products. The new infection rate of CNSP over the dry period (S2 to S3) was 2.8% and 5.3% for Orbenin and Novodry Plus respectively. The new infection rate of CNSB over the same period was 7.5% and 7.9% for Orbenin and Novodry Plus respectively. Both classifications of CNS showed no significant difference between products ($p>0.25$).

The elimination rate of CNSP over the dry period was 92.3% and 91.7% for Orbenin and Novodry Plus respectively. The elimination rate of CNSB over the same period was 90.5% and 95.2% for Orbenin and Novodry Plus respectively. Both classifications of CNS showed no significant difference between products.

## 3.4 DISCUSSION

The lack of independence between quarters of the same cow and between samples of the same quarter was not considered when calculating new infection and elimination rates. This probably increased the probability of demonstrating a

## TABLE IV

THE NEW INFECTION AND ELIMINATION RATES OF COAGULASE NEGATIVE STAPHYLOCOCCAL MASTITIS OVER THE DRY PERIOD USING DIFFERENT DRY COW THERAPIES

| | CNSP[1] | | CNSB[2] | |
|---|---|---|---|---|
| | New Infection Rate | Elimination Rate | New Infection Rate | Elimination Rate |
| Novodry Plus | 5.3% | 91.7% | 7.9% | 95.2% |
| Orbenin | 2.8% | 92.3% | 7.5% | 90.5% |

1. CNSP = Isolating at least 2 colonies of coagulase negative Staphlococci with no other growth.

2. CNSB = Isolating at least 1 colony of coagulase negative Staphylococci from 2 samples taken 1 week apart.

significant difference between the products. With high p-values observed, it is unlikely that this would have changed the conclusions.

The elimination rate for untreated quarters over the dry period could not be determined from this study. The value of blanket dry cow treatment was well recognized in these herds and hence all cows were dry treated. The elimination rate for CNS in untreated cows over the dry period has been 72.7% in studies in a Kentucky herd (38), and 65% in the Beltsville Agricultural Research Center dairy herd (39). Results in herds may vary with species distribution of CNS. The elimination rate of quarters treated with either product used in the study was considerably higher (91-95%), suggesting that both products were effective in eliminating existing CNS infections over the dry period. There was no significant difference between Orbenin and Novodry in eliminating CNS infections over the dry period.

The new infection rate of CNS in untreated cows over the dry period could not be determined for the reasons given above. In a study involving a single herd in Iowa, the new infection rate for CNS in untreated cows over the dry period was 12% (1). The criteria for infection was 2 or more isolations of CNS from different milk samples and an elevated SCC. These criteria were slightly more stringent than the present study where the new infection rate for CNSB was 7.5 and 7.9%. With the criteria used in the current study, the former study (1) would most likely have had a higher infection rate. Dry cow treatment with Orbenin or Novodry Plus appears to decrease the new infection rate of CNS over the dry period.

There was no significant difference between Orbenin and Novodry Plus in preventing new CNS infections over the dry period.

# CHAPTER 4

# 4. PREVALENCE STUDY OF COAGULASE NEGATIVE STAPHYLOCOCCAL MASTITIS ON P.E.I.

## 4.1 INTRODUCTION

The prevalence of coagulase negative Staphylococci (CNS) mastitis on P.E.I. has not been documented. Clinical experience has indicated a high prevalence in some herds despite fairly strict criteria for diagnosis of CNS infection by the Diagnostic Microbiology Laboratory at the Atlantic Veterinary College (minimum of 10 colonies and a positive California Mastitis Test). This study was carried out to determine the prevalence of CNS mastitis in P.E.I. and selected dynamics associated with it.

The prevalence of CNS mastitis on a quarter basis has varied from 7.2% to 14.3% in studies conducted in the United States and Britain (24,3,7,21,22). The literature has reported the prevalence of CNS mastitis to be greatest in first lactation heifers (7,21,22). In most studies, CNS mastitis has been shown to increase the somatic cell count (SCC) in affected quarters (5,7,16,10,12). Studies examining the effect of CNS infection on milk production have been contradictory. CNS mastitis

has decreased milk production in the majority of studies (6,10,13) but has also been reported to have no effect (14) and to increase production (15). Both teat dipping and dry cow therapy have been reported to decrease the prevalence of CNS mastitis (3,41,21,22,23).

The objectives of this study were to determine the prevalence of CNS mastitis in P.E.I., both in general and by parity, to determine the influence of CNS mastitis on SCC and milk production, and to demonstrate the effect of teat dipping and dry cow therapy on the prevalence of CNS mastitis.

## 4.2 MATERIALS AND METHODS

### Selection of Herds

The Prince Edward Island Milk Marketing Board provided the names and addresses of 70 dairy producers from across P.E.I. This list was randomly selected by computer from a total of 520 registered producers shipping milk.

### Sample and Data Schedule

Each herd was sampled once between May and August, 1989. This sampling occurred prior to the morning milking.

At the herd visit, a composite milk sample was obtained from each cow producing milk. Following the producer's routine udder preparation, each teat end was scrubbed with alcohol. Four streams of foremilk were discarded and approximately equal volumes of milk were aseptically obtained from each quarter into a single sterile vial. The producer was asked if teat dipping and dry cow therapy

were routinely used in the herd. If they were used, the formulations of the products were recorded.

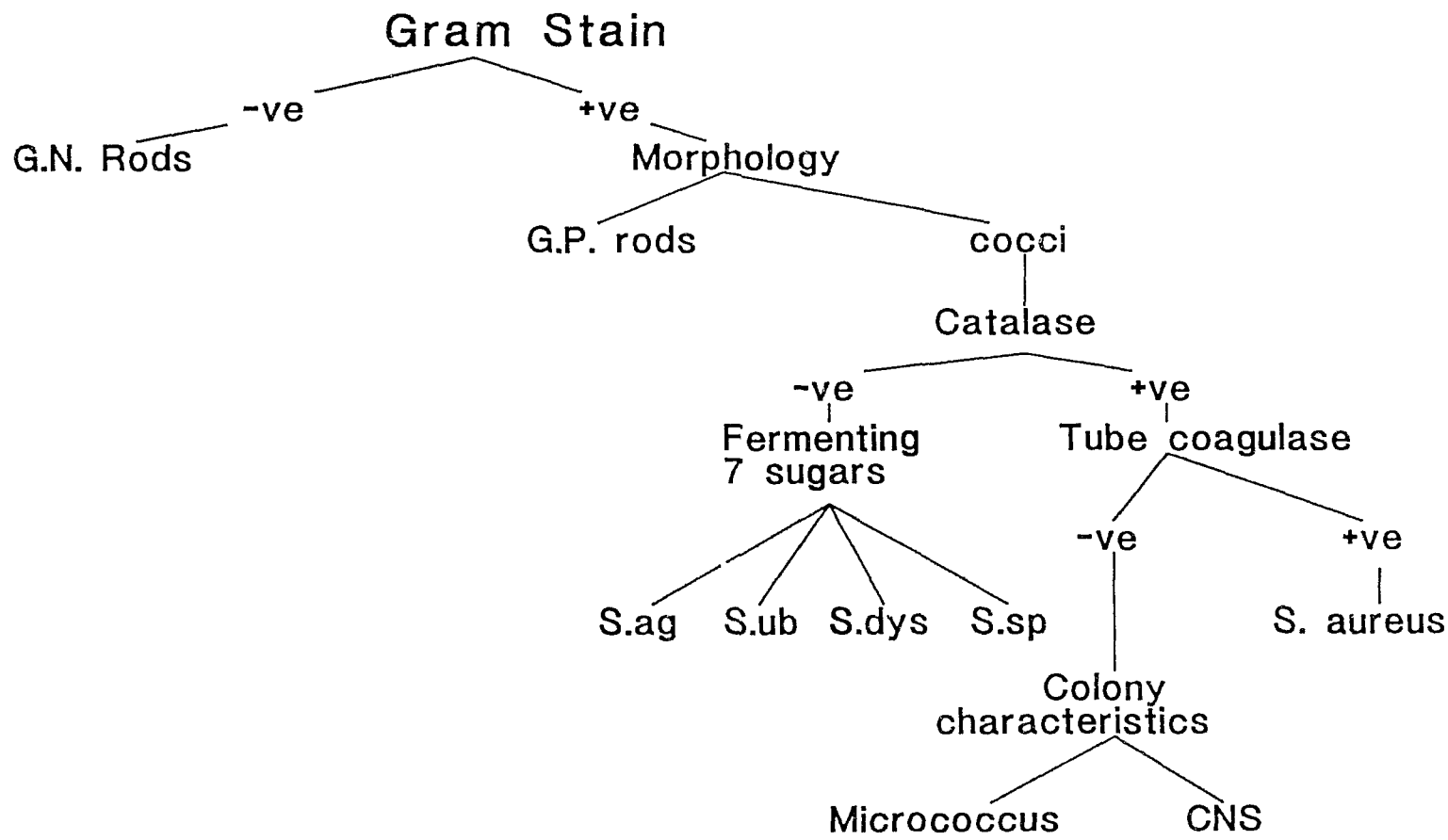
Subsequent to the visit, the producer was asked if the herd was enroled in the Atlantic Dairy Livestock Improvement Committee (ADLIC) programme. If the herd was enroled, a copy of the ADLIC report closest to the sampling date was requested. The data in the report were on a cow basis for the day of the ADLIC test. It included milk production, the fat content of the milk, lactation number and the number of days the cow was milking.

**Bacteriology**

The processing of the samples was outlined in Chapter 2.

Figure 2 shows the flowchart used to identify the various organisms. A gram stain was performed on each sample. Gram +ve rods and Gram -ve rods were identified as such and not classified further. A catalase test was performed on gram +ve cocci to differentiate catalase -ve Streptococcus spp. from catalase +ve Staphylococcus and Micrococcus. Streptococci were further classified as S. agalactiae, S. uberis, S. dysgalactiae and Streptococcus spp. by the fermentation reaction to the following sugars: treholose, sorbitol, mannitol, salicin, lactose, raffinose and inulin. The tube coagulase test was performed on all catalase +ve cocci. Coagulase +ve isolates were classified as Staphylococcus aureus. Coagulase -ve isolates were classified as either Micrococcus or Staphylococcus spp. (coagulase negative Staphylococcus) by colony characteristics. Colony numbers of all organisms were recorded.

# PREVALENCE STUDY Figure 2



**Species identification**

During the study, cultures were saved from every coagulase +ve Staphylococcus sample and systematically from every fourth coagulase -ve Staphylococcus sample. Fifty samples from each group were then randomly selected for species identification. Species identification was determined by testing with MicroScan (Baxter Corporation, Mississauga, Ontario), a microbiological identification system and by repeating the tube coagulase test.

**Classification of Culture Results**

The method of determining the number of colonies required to establish a CNS infection is outlined in Chapter 2 and Appendix A.

Culture results were classified as follows. A culture was classified as Coagulase negative Staphylococcus-pure (CNSP) if it had at least 2 colonies of CNS with no other growth. A culture was classified as Coagulase negative Staphylococcus-all (CNSA) if it had at least 2 colonies of CNS with no more than one other colony type isolated. A culture was classified as S. aureus (SA2) if it had at least 2 colonies of S. aureus. A culture was classified as Gram -ve rods (GN5) if it had at least 5 colonies of gram -ve rods and no more than one other colony type. A culture was classified as Streptococcus agalactiae (SAG2) if it had at least 2 colonies of S. agalactiae. A colony was classified as S. dysgalactiae (SDYS5) if it had at least 5 colonies of S. dysgalactiae and no more than one other colony type isolated. A colony was classified as S. uberis (SUB5) if it had at least 5 colonies of S. uberis and no more than one other colony type isolated. Gram+ve rods and streptococci, other

than S. agalactiae, S. dysgalactiae or S. uberis, were classified as contaminants and samples yielding only these organisms were recorded as having no significant growth (NSG). If three or more colony types were isolated from a sample, the sample was considered to be contaminated (CON). However, if S. aureus or S. agalactiae were isolated, then it was a positive culture even if three or more different colony types were isolated (27).

**Statistical Analysis**

Data were entered and stored in dBase III Plus (28), a database management software package. Statistical analyses were conducted using Minitab Version 7.1 (29). Analysis of variance and simple linear regression were used to determine significant differences between the prevalence of CNSP and CNSA in herds that teat dipped compared to herds that did not teat dip and between herds that dry cow treated compared to herds that did not dry cow treat.

Simple linear regression was used to determine significant differences between the prevalences, by lactation number, of CNSP and CNSA. Multiple linear regression analyses were conducted to determine if CNSP and CNSA had a significant effect on the natural log of the SCC (lnSCC) and milk production.

## 4.3 RESULTS

Seventy dairy herds were randomly selected for participation in this study. Sampling was not done on 9 herds for the following reasons: five herds declined

participation in the study, two herds were in the process of liquidation and two herds could not be contacted.

The number of farms in each study group for teat dip treatment and dry cow treatment is listed in Appendix C. Sixty-one herds were sampled during the study. Four herds were eliminated from the study because of gross contamination of the milk samples at the time of collection. This was manifested by the growth of Gram +ve rods in over 50% of the samples taken. Sixteen hundred and eighty-eight cows on 57 farms were involved in the CNS prevalence analyses.

Twenty farms including 645 cows supplied the study with an ADLIC report. To correctly relate production data to bacteriological data, the only herds used in the production analyses were herds whose ADLIC test day was no more than seven days from the collection of the milk samples. Thus, data from four farms containing 144 cows were used for production analyses.

A total of 50 isolates of coagulase positive Staphylococci and 50 isolates of coagulase negative Staphylococci were identified by species. Of the 50 cultures initially identified to be coagulase +ve, 34 were identified as coagulase +ve and S. aureus, 14 were identified as coagulase +ve and not S. aureus and 2 were identified as coagulase -ve and CNS. Of the 14 colonies identified as coagulase +ve and not S. aureus, 9 were S.hyicus hyicus and 5 were S.intermedius. Of the 50 cultures initially identified to be coagulase -ve, 40 were identified as coagulase -ve and CNS, 4 were identified as coagulase -ve and Micrococcus and 6 were identified as coagulase

+ve and S. aureus. The species and number of cultures verified as Staphylococcus are in Appendix D.

The prevalence of infection with CNSP, CNSA, SA2, SUB5, SDYS5, GN5 and SAG2 is given in Table V. The corresponding arithmetic and geometric mean somatic cell count (SCC) is also given in Table V. The prevalences of CNSP, CNSA and SA2 were 24.5% (95% Confidence Interval= (21.8%, 27.3%)), 38.3% (95% Confidence Interval= (34.6%, 42.0%)) and 14.1% (95% Confidence Interval= (11.2%, 18.5%)) respectively. The geometric mean SCCs for CNSP, CNSA, SA2 and NSG were 108 X $10^3$, 125.0 X $10^3$, 367.5 X $10^3$ and 69.2 X $10^3$ respectively. The prevalence of organisms in milk samples submitted as components of whole herd samples to the Atlantic Veterinary College (AVC) Microbiology Laboratory in 1989 is also included in Table V. Prevalences for CNSP and CNSA from the AVC Microbiology Laboratory were not applicable because a different criterion was used to classify infections with CNS in routine diagnostic samples.

Table VI gives the prevalence of CNSP and CNSA by lactation number. The prevalence of CNSP was highest in the first and second lactation (36.1% and 35.7%) and was relatively constant from the third lactation to the seventh or higher lactation. The prevalence ranged from 19.5% in the seventh or higher lactation to 28.9% in the fifth lactation. The prevalence of CNSA was relatively constant in all the lactations, ranging from 32.3% in the sixth lactation to 38.0% in the fourth lactation. There was a significant difference in the prevalence of CNSP by parity but not in the prevalence

of CNSA by parity. The coefficients for CNSP and CNSA in the regression equations were -2.45 (p=0.026) and -0.5893 (p=0.191) respectively.

The lnSCC was regressed on CNSP and CNSA separately, controlling for farm, SA2 and lactation number to determine if CNSP or CNSA had a significant influence on lnSCC. Infection with both CNSP and CNSA had a significant positive influence on lnSCC. The coefficients for CNSP and CNSA in the regression equation were 0.513 (p=0.000) and 0.6108 (p=0.000) respectively. The overall $R^2$ was 20.0% and 21.4% for the models incorporating CNSP and CNSA respectively.

Analysis of variance (GLM) showed no significant differences between the herd prevalence of CNSP and CNSA in herds using a teat dip product (regardless of formulation) and not using a teat dip product. The coefficients for CNSP and CNSA in the simple linear regression equations were -0.01313 (p=0.730) and -0.04420 (p=0.374) respectively.

Analysis of variance showed no significant differences between the herd prevalence of CNSP and CNSA in herds using dry cow treatment and not using dry cow treatment. The coefficients for CNSP and CNSA in the simple linear regression equation were -.03784 (p=0.366) and -.09589(p=0.077) respectively.

The effect of infection with CNSP and CNSA on milk production was investigated. Milk production was regressed on CNSP and CNSA separately, controlling for farm, lactation number, SA2 infection and days in milk. Infection with CNSP and CNSA did not have a significant influence on milk production. The

## TABLE V

## PREVALENCE OF ORGANISMS IN A 1688 COW STUDY IN P.E.I.

| Organism | No. + ve Samples | Prevalence[4] | Prevalence P.E.I Herd Samples[2] | Arithmetic[1] SCC X10[3] | Geometric[1] SCC X10[3] |
|---|---|---|---|---|---|
| CNSP | 414 | 24.5% | NA | 378.8 | 108.2 |
| CNSA | 646 | 38.3% | NA | 477.1 | 125.0 |
| SA2 | 238 | 14.1% | 7.9% | 963.1 | 367.5 |
| SUB5 | 9 | 0.5% | 2.0% | 491.8 | 346.8 |
| SDYS5 | 1 | 0.06% | 0.6% | NA | NA |
| GN5 | 3 | 0.18% | 0.16% | NA | NA |
| SAG2 | 1 | 0.06% | 3.2% | NA | NA |
| NSG[3] | 716 | 42.4% | NA | 300.7 | 69.2 |
| CON | 102 | 6.04% | NA | NA | NA |

1 Arithmetic + geometric means not given if <5 positive samples.
2 Prevalence of organisms in herd samples submitted to AVC Microbiology Laboratory in 1989.
3 Includes 3 samples identified as C. bovis and 1 sample of A. pyogenes.
4 All positive samples in CNSP are also in CNSA. Therefore, total prevalence is greater than 100%.

## TABLE VI

**PREVALENCE OF CNS BY LACTATION NUMBER IN AN 1688 COW STUDY IN P.E.I.**

| | | PREVALENCE | |
|---|---|---|---|
| **Lactation #** | **# Cows** | **CNSP (%)** | **CNSA (%)** |
| 1 | 200 | 36.1% | 36.5% |
| 2 | 156 | 35.7 | 37.8 |
| 3 | 97 | 24.4 | 33.0 |
| 4 | 71 | 22.4 | 38.0 |
| 5 | 49 | 28.9 | 34.7 |
| 6 | 31 | 24.0 | 32.3 |
| 7+ | 41 | 19.5 | 34.1 |

coefficients for CNSP and CNSA in the regression equation were -1.311 (p=0.32) and -0.813 (p=0.513). Overall $R^2$ was 37% for both models.

## 4.4 DISCUSSION

When compared to the MicroScan results, it appeared there was some misclassification in the original identification of the Staphylococcus. Staphylococcus hyicus hyicus and S.intermedius, as well as S. aureus can react positively to the coagulase reaction (43). Twenty-eight percent of the coagulase +ve staphylococci in this study were S.hyicus hyicus or S.intermedius. This would have contributed to an overestimation in the prevalence of S. aureus and an underestimation in the prevalence of CNS. Twelve percent of the cultures originally identified as coagulase -ve were coagulase +ve, S. aureus on retesting. The reason for the variation in the coagulase test results was undetermined. This would have contributed to an overestimation in the prevalence of CNS and an underestimation in the prevalence of S. aureus.

Assuming the same frequency of misclassification that was observed in the subsample processed for species identification was also present in the rest of the study samples, the effects of the misclassification on the prevalence estimates can be determined. The identification of CNS was misclassified in 16 of 50 coagulase +ve subsamples, resulting in an overestimation of CNS, and misclassified in 10 of 50

coagulase -ve subsamples, resulting in an underestimation of CNS. Since the prevalence of coagulase -ve Staphylococcus in this study was approximately twice the prevalence of coagulase +ve Staphylococcus, the effect of misclassification of coagulase -ve samples was approximately twice that of coagulase +ve samples. Therefore the prevalence of CNS was slightly underestimated.

The identification of S. aureus was misclassified in 16 of the 50 coagulase +ve subsamples, resulting in an underestimation of S. aureus, and misclassified in 6 of 50 coagulase -ve subsamples, resulting in an overestimation of S. aureus. Due to the fact that the effect of misclassification of coagulase -ve samples was approximately twice that of coagulase +ve samples, the prevalence of S. aureus was also slightly underestimated.

The overall prevalence of CNSP (24.5%) and CNSA (38.3%) was considerably higher than those reported in other studies (24,3,7,21,22). The prevalence of CNS in these studies, which were reviewed in Chapter 2, ranged from 6.3% to 14.3%. The reason for this discrepancy may have been that the current study was conducted on a composite cow sample basis while the other studies were done on a quarter sample basis.

The prevalence of CNSP was greatest in first and second lactation cows and consistently lower in later lactations. A study involving 105 cows in Kentucky reported first lactation heifers having the highest prevalence of CNS and second lactation heifers having the lowest (31). A 30 herd study in England reported a constant quarter prevalence of 9 to 17% in herds not practising dry cow therapy or

teat dipping (21). After three years of teat dip and dry cow therapy, there was a higher quarter prevalence in first lactation animals (8.8%). The prevalence in second lactation animals was 2.2%. This gradually increased to 9% in animals having 8 or more lactations. A study involving 222 cows in the University of Kentucky dairy herd also had the greatest quarter prevalence in first lactation cows (18.7%) and the lowest prevalence in second lactation animals (9.2%). The prevalence ranged from 12 to 17% in other lactations (7). A study involving the Ohio Agricultural Research and Development Center dairy herd reported twice as many infections in first lactation animals than other animals (22). The level of infection was not given in this study, preventing evaluation of the magnitude of the difference. The differences between the prevalence of CNS in first and second lactation animals may be due to the practice of dry cow therapy. First lactation animals are not able to benefit from this procedure.

Infection with CNSP and CNSA had a significant positive influence on the lnSCC. The mean lnSCC was approximately 47 X $10^3$ in uninfected udders and 79 X $10^3$ in udders infected with CNS. The majority of studies reviewed in Chapter 2 also reported an approximate twofold lnSCC increase in infected quarters (5,7,16,10,12). The cohort study in Chapter 2 reported a significant influence of CNSP on lnSCC however the magnitude of the effect was not as great as in this prevalence study. The mean lnSCC was 42 X $10^3$ for uninfected quarters and 56 X $10^3$ for quarters infected with CNSP in the cohort study.

There was no significant difference between the herd prevalence of CNSP and CNSA in herds that teat dipped compared to herds that did not teat dip. The coefficient in the regression equation was negative, suggesting a trend to decreased herd prevalence associated with the use of teat dip. A study involving 30 herds in England reported significant reductions in CNS in herds practising both dry cow therapy and teat dipping (21). Two studies, one involving 16 herds in Vermont (3,22) and the other involving 152 cows in the Department of Dairy and Animal Science herd at the Pennsylvania State University (23), demonstrated that teat dipping significantly reduced prevalence on a quarter basis. However the study in Vermont used teat dip on a herd basis but analyzed the data on a quarter basis. This would overestimate the statistical significance of the effect of the teat dip because of the larger sample size. Analyses of effects of teat dip in this study were based on herd level data. Because analyses were conducted on a herd basis, and only 57 herds were involved in the current study, the failure to demonstrate the significance of teat dipping on herd CNS prevalence may have been due to insufficient sample size.

There was no significant difference between the herd prevalence of CNSP and CNSA in herds that used dry cow therapy and herds that did not use dry cow therapy. A study involving 156 cows in Kentucky reported that dry cow therapy significantly reduced CNS infections (40). The failure to demonstrate the significance of dry cow treatment on herd CNS prevalence in the current study may have been due to insufficient sample size due to analyses on a herd basis or incomplete data regarding cow selection criteria for dry cow therapy in the herds that dry cow treated.

Infection with CNSP or CNSA did not have a statistically significant effect on milk production although the coefficients suggested a slight negative effect. The majority of studies that were reviewed in Chapter 2 demonstrated that CNS had a significant negative effect on milk production (6,10,13). The cohort study in Chapter 2 reported a non-significant trend towards increased milk production with CNS infection. Because cows infected with CNS significantly increased lnSCC in the current study, and increased lnSCC is known to decrease production, it was expected that CNS infection would significantly decrease milk production. The reasons for this not occurring could be twofold. Firstly, because of the criteria limiting the analyses to farms providing both production and bacteriological data, only 4 farms and 144 cows were involved in the analyses. This may have been too small a sample size to demonstrate a significant relationship. Secondly, the time interval of one to seven days between production data collection and bacteriological data collection may have masked an effect.

# CHAPTER 5

## 5.0 SUMMARY

The quarter prevalence of CNS infection in the cohort portion of the project (Chapter 2) was similar to other studies. However, the cow prevalence of CNS in the prevalence study (Chapter 4) was considerably higher. One reason for this discrepancy may have been that the prevalence study was conducted on a composite cow sample basis while the cohort and other studies were done on a quarter sample basis. The estimate of CNS prevalence in P.E.I. in the prevalence is more accurate than the estimate in the cohort study because the sample population was randomly selected in the prevalence study.

The prevalence of CNS was relatively constant throughout the lactation except for an increase at 6-7 months. There is no consistency in the literature with regard to the effect of stage of lactation on the prevalence of CNS. The dramatic differences between studies from different locations may be due to different dry cow treatment practices and/or a different species composition of CNS.

The prevalence of CNS was greatest in first and second lactation cows. Most studies have reported a higher prevalence in first lactation cows but not second lactation animals. This may be because first lactation animals do not benefit from dry cow therapy. In the current study, the reason for the high prevalence in second lactation cows was not determined.

The new infection rate for CNS increased over the course of the lactation. Other studies have shown infection rates to remain constant. The overall elimination rate was considerably higher than other studies. Species variation of CNS in the various studies could have accounted for the varied infection and elimination rates.

Infection with CNS did not appear to protect the quarter from subsequent infection with S. aureus. In other studies, CNS infection did afford some protection. A small sample size may have made this detection difficult.

Infection with CNS had a significant positive influence on the geometric mean SCC. The geometric mean SCC in CNS infected quarters was 1½ to 2 times that of uninfected quarters. Milk production however, was not significantly influenced by infection with CNS. Most studies have shown CNS to significantly increase SCC and decrease milk production. The analysis of the effect of CNS on production were limited to very few herds which would have made detection of an effect quite difficult.

No significant differences were detected between quarter treatment with Orbenin Dry Cow or Novodry Plus Dry Cow with respect to new infection rate or elimination rate over the dry period.

No significant differences were detected between the herd prevalence of CNS infection in herds using dry cow therapy and not using dry cow therapy, or between herds using teat dip and not using teat dip. However, very small sample sizes for these analyses would have made detection of differences very difficult.

The results from this project supported the classification of CNS as a minor pathogen in mastitis control programmes. Infection with CNS significantly increased SCC and could have also decreased milk production. Although CNS mastitis may not decrease milk production to a great extent on the cow level, it might decrease P.E.I.'s total milk production markedly because of the high prevalence of CNS mastitis.

## APPENDIX A

## SENSITIVITIES, SPECIFICITIES AND CORRECT CLASSIFICATION OF CNS INFECTIONS IN AN 84 COW STUDY IN P.E.I.

| | # of CNS Colonies | | |
|---|---|---|---|
| | ≥1 | ≥2 | ≥5 |
| Sensitivity | 100% | 78% | 53% |
| Specificity | 75% | 83% | 91% |
| Correct Classification | 81% | 84% | 87% |

The gold standard was defined as positive culture results for CNS in both S1 and S2.

Three criteria were compared to the gold standard; ≥1 colonies of CNS, ≥2 colonies of CNS and ≥5 colonies of CNS.

For each criteria, all the quarters in S1 and S2 were compared to the gold standard in the corresponding quarter. The sensitivity, specificity and correct classification was computed for each criteria.

## APPENDIX B

## SPECIATION BY STAPH IDENT OF A SUBSET OF COAGULASE NEGATIVE STAPHYLOCOCCAL ISOLATES FROM AN 84 COW STUDY IN P.E.I.

| | |
|---|---|
| Staphylococcus hominis | 25 |
| S. sciuri | 24 |
| S. xylosus | 18 |
| S. epidermidis | 17 |
| S. warneri | 16 |
| S. capitis | 16 |
| S. saprophytius | 11 |
| S. hemolyticus | 10 |
| S. simulans | 5 |
| S. cohnii | 5 |
| S. intermedius | 4 |
| S. warnei | 4 |
| S. hyicus | 1 |
| **Total # of CNS Speciated** | **157** |

## APPENDIX C

## STUDY GROUPS FOR TEAT DIP TREATMENTS AND DRY COW TREATMENTS

## IN A 57 FARM PREVALENCE STUDY IN P.E.I.

### Teat Dip Treatments

| # of Farms | Study Group |
|---|---|
| 16 | None used |
| 1 | Iodine |
| 27 | Chlorhexidine |
| 4 | Quaternary ammonium compounds |
| 9 | Dodecyl Benzene Sulfonic Acid (DDBSA) |

### Dry Cow Treatments

| # of Farms | Study Group |
|---|---|
| 12 | None used |
| 19 | Orbenin |
| 12 | Penicillin and Novobiocin |
| 6 | Penicillin |
| 8 | Product unknown |

## APPENDIX D

## SPECIATION BY MICROSCAN OF A SUBSET OF STAPHYLOCOCCAL ISOLATES FROM A 1688 COW PREVALENCE STUDY IN P.E.I.

| Coagulase (+)ve | |
|---|---|
| S. aureus | 40 |
| S. hyicus hyicus | 9 |
| S. intermedius | 5 |
| | 54 |

| Coagulase (-)ve | |
|---|---|
| S. hyicus hyicus | 12 |
| S. simulans | 7 |
| S. xylosus | 7 |
| S. hemolyticus | 5 |
| S. cohnii | 3 |
| S. epidermidis | 2 |
| S. hominis | 2 |
| S. warneri | 2 |
| S. lentus | 1 |
| S. sciuri | 1 |
| Micrococcus | 4 |
| | 46 |

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