THE RELATIONSHIP BETWEEN MANAGEMENT FACTORS, ANTIMICROBIAL SENSITIVITY AND PREVALENCE OF STAPHYLOCOCCAL MASTITIS PATHOGENS IN A RANDOM SAMPLE OF PRINCE EDWARD ISLAND DAIRY HERDS

A thesis
submitted to the Graduate Faculty
in Partial Fulfilment of the Requirements
for the Degree of
Master of Science
in the Department of Health Management
Faculty of Veterinary Medicine
University of Prince Edward Island

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Charlottetown, P.E.I.
August, 1992

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18 August, 1992

Examiners

Date 10 September, 1992

ABSTRACT

This study, an investigation of coagulase positive (CPS) and coagulase negative (CNS) staphylococcal mastitis in 44 randomly selected dairy herds in Prince Edward Island (PEI) had three major objectives. These are:

1. to survey dairy management factors and determine the effect of management factors on the herd prevalence of staphylococcal mastitis pathogens.
2. to survey antimicrobial sensitivity of Staphylococcus isolates from the study herds, and assess which management factors affect sensitivity of staphylococcal mastitis pathogens to these antimicrobials.
3. to determine if significant correlations exist between the prevalence of staphylococcal mastitis pathogens and their in vitro antimicrobial sensitivity.

Information on dairy management practices and antibiotic use was sought through a mail out questionnaire. A response rate of 77% was realized. Adoption rates of 79% and 48% for post-milking teat dipping and dry cow therapy for all cows in a herd were observed. Cloxacillin benthazine and Oxytetracyline hydrochloride were the most widely used dry cow products, on 48% and 32% of farms respectively. Seventy eight percent of the farms used products containing beta lactams, 48% of these contained Penicillin G.

Weighted least squares multiple regression was used to relate the within herd prevalence of CPS or CNS to management factors and, separately, to dry cow products. For CPS, farms that dipped teats after milking were significantly associated with low prevalence. The number of times the milking machine was checked had a weak positive correlation with prevalence. Dry cow products used had no association with the prevalence of CPS. Freestall and tiestall versus loose housing, high-line milking system versus bucket milking, and pre-partum teat dipping were associated with low prevalence of CNS. Farms that used a dry cow product containing procaine penicillin G and novobiocin sodium were associated with low prevalence of CNS, while farms that used any dry cow product containing novobiocin were associated with high prevalence.

All CPS isolates and a sub-sample representing 25% of CNS isolates were tested for sensitivity to 13 antimicrobials using the disc diffusion method. Among CPS, sensitivity was above 91% to all the antimicrobials tested except penicillin G, ampicillin, neomycin, and polymyxin B. Among the CNS, sensitivity was above 91% except penicillin G, ampicillin, polymyxin B, tetracycline, and sulphamethoxazole / trimethoprim. There was between farm variation in sensitivity for both CPS and CNS. When farm factors were taken into account, the proportions of CPS sensitive to tetracycline, sulphamethoxazole / trimethoprim, and nitrofurantoin were significantly higher than CNS. The proportions of CNS sensitive to neomycin and polymyxin B were significantly higher than CPS. Sensitivity to penicillin G, ampicillin, and erythromycin was strongly affected by farm level factors.

Weighted least squares multiple regression was used to relate the proportions of CPS and CNS sensitive to penicillin G or tetracycline to management factors and, separately, to dry cow products. Among CPS, post-milking teat dipping was associated with high sensitivity to penicillin G and tetracycline. The milking herd proportion culled because of mastitis had a negative correlation with sensitivity for tetracycline. Neither the herd proportion receiving dry cow therapy nor the dry cow products used had any association with CPS sensitivity to Penicillin G or tetracycline. Among the CNS, post-milking teat dipping, dipping milking cluster between cows and the herd proportion receiving dry cow therapy had positive correlations with sensitivity to penicillin G. The milking herd proportion treated for clinical mastitis and the herd proportion receiving dry cow therapy had negative correlations with CNS sensitivity for tetracycline, while the herd proportion culled because of mastitis had a positive correlation. Dry cow products used predicted CNS sensitivity to penicillin G and tetracycline.

The association between the proportions of CPS or CNS sensitive to penicillin G and tetracycline in vitro and the herd prevalence were sought after taking into account farm factors using the partial correlation coefficient. No significant associations were found.

ACKNOWLEDGMENTS

I thank Dr. Liz Spangler for her supervision and endurance throughout this study. Her enthusiasm and constructive criticism were invaluable in refining the final product. I thank the members of my supervisory committee, Drs. Bob Curtis, Ian Dohoo, Alan Donald, Fred Kibenge, Tim Ogilvie, and Pam Ruegg for their guidance and expertise. I offer special thanks to Dr. Alan Donald for going above and beyond the call of duty to assist me with statistical analysis and interpretation. I also offer special thanks to Dr. Pam Ruegg for her insight in the dairy industry, and to Dr. Ian Dohoo for his guidance in the study design.

I thank the Kenyan and Canadian governments for providing me with the opportunity to pursue a masters degree under the General Training Fund (GTF) program and for funding this project.

I thank Dr. Jeff Davidson for the materials provided from his project as well as for being a valuable source of information regarding the dairy industry in Prince Edward Island (PEI).

I thank the participating dairy farmers of PEI for their cooperation in providing the required data for this project.

Special thanks go to Joanne Daley, staff of AVC central services, and staff of diagnostic laboratory in providing technical assistance in bacteriology.

I would also like to express my gratitude to the staff of Health Management department for always being so willing to provide assistance whenever I needed it.

I am indebted to Estelle Mugford for her friendship and support.

I am very grateful to my parents through whom the pursuit of excellence in education became a priority to me. Therefore, in thanks, I dedicate this work to them.

Finally, I wish to thank my twin sons, Chibeu and Nyikuri, for being so patient in waiting for me to return to them.

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ABBREVIATIONS

ADLIC Atlantic Livestock Improvement Corporation
AVC Atlantic Veterinary College
BCA Breed Class Average
PEI Prince Edward Island.

1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Mastitis, the inflammation of the mammary gland due to the effects of infection by bacteria or mycotic pathogens, is of great financial loss in dairy cows. In the US, direct losses to the producer have been estimated at $200 per cow annually (Philpot, 1984). Reduced milk production accounts for 70% of the total loss (Philpot, 1984). Subclinical mastitis caused by *Staphylococcus* species is responsible for a large proportion of reduced milk production. Other losses include milk discarded after treatment (8%), drugs and veterinary expenses (8%), and death and premature culling (14%) (Philpot, 1984).

Staphylococcus aureus long been recognized as a major mastitis pathogen (Dodd, 1983). Intramammary infections (IMI) due to *S. aureus* can be present in one of 3 forms, namely, peracute, clinical, or subclinical. The subclinical form is the most common. IMI due to *S. aureus* are difficult to eradicate with antibiotic therapy and often vacillate between subclinical and clinical forms, both of which are characterised by chronic reactions (Anderson, 1982). *S. aureus* is contagious, so control measures are based on practices that: 1) prevent cow to cow and quarter to quarter spread; 2) prevent teat injuries and teat canal penetration; 3) deal with residual contamination and colonization of teats and teat lesions; 4) reduce the duration of infection; and 5) eliminate the source of infection from the herd (Anderson, 1982).

Intramammary infections due to other *Staphylococci* (coagulase negative *Staphylococci* (CNS)) are classified as minor (Hogan et al, 1987; Hogan et al, 1988 Jarp, 1991). IMI due to CNS are generally subclinical although a mild form of clinical mastitis can occur (Jarp, 1990). The subclinical form is characterised by elevated somatic cell counts and a decrease in milk production (Natzke et al, 1972; Timms and Schultz, 1987; Davidson, 1990). CNS are regarded as potential primary udder pathogens, but may have a protective effect against the major mastitis pathogens (Bramley, 1978; Linde, 1982; Rainhard and Poutrel, 1988; Mathews et al, 1990). The protective effect is attributed to the increased polymorphonuclear leucocyte content of milk in quarters infected rather than any direct inhibition produced by CNS (Bramley, 1978; Linde, 1982). The CNS consist of many species and do not readily conform to either of the classifications as contagious or environmental, but, occupy an intermediate category as skin flora opportunists (Hogan et al, 1987). Control measures for CNS therefore, combine the measures for both contagious and environmental pathogens. Environmental control measures include: 1) pre-milking udder and teat preparation; and 2) a clean and dry environment (Smith, 1986; Hogan et al, 1987; Pankey, 1987).

In Prince Edward Island (PEI), the prevalence of IMI due to coagulase positive *Staphylococci* (CPS) (*S. aureus*) was estimated at 14.1% and CNS (without any other bacterial isolate) at 24.5% (Davidson, 1990). The study reported that IMI due to CNS significantly increased SCC and could also have reduced milk production.

1.2 Literature review

The members of the genus *Staphylococcus* are widely distributed in nature. Devriese (1990) has recently reviewed how they are associated in many diverse and specialized forms with healthy and diseased farm animals and with most common pet animals. *Staphylococci* are divided into coagulase positive *Staphylococci* (CPS) and coagulase negative *Staphylococci* (CNS) based on ability to produce coagulase.

CPS are major mastitis pathogens in cattle. The main CPS species is *S. aureus*. Some strains of *S. hyicus* are also coagulase positive. CPS colonize the teat and udder skin following injury. *S. aureus* will persist for several months in teat lesions and other body locations (McDonald, 1984). The source of infection for *S. aureus* is injured teat ends or other skin surfaces, including milkers' hands, and the subclinical, chronically infected glands that are more common in older cows within each herd (McDonald, 1984). Cow-to-cow spread is by objects contaminated with milk during the milking process (McDonald, 1984; Etgen et al, 1987). For CPS, it is recognized that over an extended period of time the proportion of cows infected in a herd is determined by both new infection rate and duration of infections (Dodd, 1986; Craven, 1987).

Although CPS are the major mastitis pathogens (Moore and Heider, 1984; Bushnell, 1984; Dodd, 1986; Pankey et al, 1987; Mathews et al, 1988), CNS are the bacteria groups most frequently isolated from mammary secretions of lactating cows (Brown and Scherer, 1978 Devriese and De Keyser, 1980), dry cows and pregnant heifers (Oliver and Mitchell, 1983). The percent quarters infected with CNS is

greatest at parturition and decreases dramatically early into lactation (Hogan et al, 1987).

The CNS most commonly encountered in IMI in cattle are *S. xylosus*, *S. epidermidis*, and *S. haemolyticus* (Devriese, 1979; Hinkley et al, 1985; Devriese 1990). Jarp (1991) reported *S. simulans* to be the predominant species isolated from both clinical and subclinical mastitis. Other strains of CNS encountered are *S. chromogenes, S. warneri*, and some strains of *S. hyicus*. *S. haemolyticus, S. warneri*, and *S. epidermidis* colonize teat ducts and teat apex of cows (Devriese, 1990). The rest of the CNS are considered normal, resident inhabitants of the skin that colonize the teat skin and external orifice of the teat canal and increase in number with injury. The distribution of the CNS species in IMI was reported to be similar in clinical and subclinical mastitis (Jarp, 1991; Hodges et al, 1984). The study by Jarp (1991) noted no significant difference between the clinical status of cows associated with various CNS species. Thus, bacterial species among the CNS is of minor importance for the severity of mastitis. The importance of IMI due to CNS relates to high risk of infection, long duration, changes in milk composition, and losses in milk production (Timms and Schultz, 1987).

Exposure of udders to mastitis pathogens is recognized to play an important role in determining the rate of infection (McDonald, 1984; Dodd, 1986). Milking hygiene and milking machine management are important in this regard. Stripping the first streams of milk onto a strip cup or floor detects abnormalities such as flakes, clots or wateriness. Washing teats with water containing disinfectant and or pre-

dipping teats in a teat dip reduces teat skin micro-flora. This micro-flora reduction technique coupled with single-service towels to dry the teats, attaching the milking unit when the udder and teats are well dried, and dipping the milking cluster in disnifectant solution between cows are important in preventing spread from infected quarters and teat lesions to the teat skin of all cows in a herd (Bushnell, 1984; Guterbock et al, 1984; Jarrett, 1984a). The adequate use of a properly functioning milking machine minimizes forced penetration of micro-organisms caused by vacuum fluctuations (O'Shea and O'Callaghan, 1978). Among udder preparation practices, Galton et al (1984) reported that the use of water hose and pre-dipping followed by drying with single-service paper towels significantly reduced both coliform and CNS, while the addition of disinfectant in wash water was of marginal significance. In another study, Galton et al (1986) reported that pre-milking teat dipping with either of iodophor, sodium hypochlorite or dodecyl benzene sulfonic acid (DDBSA) followed by manual drying of teats significantly reduced total bacteria in milk. Pankey et al (1986) reported pre-dipping with 0.1% iodine significantly reduced environmental pathogens. Among milking machine functions, Osteras and Lund (1988a), reported good udder health to be associated with the following functions: good technical condition of the liner, vacuum at 36-39 cm Hg, correct rate of pulsator, efficient functioning of the vacuum regulator, no limping of the pulsator, no pronounced undulations in the milking pipeline, adequate preparation time, no over-milking, slow milking rate, and no air admission during application of the teat cups. In a study on housing, Osteras and Lund (1988b) reported good udder health to be

associated with good indoor climate, good insulation of animal housing with double-glazed windows, extended stall length, good hoof care, and rubber mats on stall floors. A study by Fox and Hancock (1989) reported no value in segregating and milking cows infected with CPS last as a control measure against IMI due to CPS.

Post-milking teat dipping with germicides was recognized in the late 1950's as a means of dealing with residual contamination and colonization of teat ducts and lesions (Dodd, 1983). Studies have confirmed the important role played by post-milking teat dipping in reducing IMI and teat canal infections due to CPS (Hogan et al, 1987; Nickerson et al, 1990; Fox et al, 1991). A study by Hogan et al (1987) found the prevalence of CNS to be lower in herds using chlorhexidine or iodophor than in herds using linear dodecyl benzene or control herds. The study further observed that different teat dips affected the distribution of CNS species. Nickerson et al (1990) reported that post-milking teat dipping with either iodophor or a fatty acid plus lactic acid teat dip had no effect on teat canal infections due to CNS. Pre-partum teat dipping has been reported to offer no protection against infection by either CPS or CNS (Schultze, 1985; Mathews et al, 1988).

Less than 50% of new infections arising during lactation are manifest by clinical signs and are, therefore, recognizable (Dodd, 1986). Bacteriological cure rates for clinical mastitis due to CPS in lactating cows with intramammary infusion is low, even when combined with systemic treatment (Moore and Heider, 1984). The treatment of subclinical mastitis due to *Staphylococci* during lactation is economically unjustified because of the low cure rates, high costs in losses of milk sales due to

antibiotic withholding time and additional costs associated with disease detection (Craven, 1987).

The administration of long acting antibiotics to all quarters of all cows at the time of drying off is expected to cure existing infections and, additionally, to prevent new infections during the early part of the dry period (McDonald, 1984; Dodd, 1986; Nickerson and Owens, 1990). Antibiotics administered to non-lactating cows have been designed to create a prolonged local concentration of the drug (Nickerson and Owens, 1990). Prolonged duration of antibiotic in the udder is ideal for preventing new infections during the first week of the dry period, one of the high risk times of infection. The advantage of dry cow treatment (DCT) is that there are no losses caused by discarded milk, and the possibility of antibiotic-contaminated milk reaching the consumer is minimal (Nickerson and Owens, 1990). Reports from experimental studies on the effectiveness of DCT in reducing IMI due to CPS show varying degrees of success. Batra (1988) reported a micro-biologic elimination rate of 94.1% in quarters infected with CPS. Buddle et al (1987) reported a protective effect by DCT, and observed that cows infected in 3-4 quarters with CPS and *Streptococcus uberis* before drying off had a higher susceptibility to reinfection in subsequent lactation than cows infected in two or fewer quarters. The study further noted that quarters infected with CPS prior to dry cow therapy were very susceptible to new infections in the following lactation. Browning et al (1990) reported a significantly lower infection rate during the dry period in cows that had all quarters infused than in cows in which only the infected quarters were infused. During the following lactation

period, the infection rate was significantly lower in cows in which only the infected quarters were infused than in cows that had all quarters infused. Further, the study did not find any difference in new infection rate during the dry period and early lactation between uninfected cows receiving and not receiving dry cow therapy. Dry cow therapy has been reported to eliminate all IMI due to CNS (McDonald, 1984). In PEI, Davidson (1990) reported micro-biologic elimination rates of over 90% for CNS for 2 different dry cow preparations.

Drying off abruptly is the recommended procedure when cows receive dry cow therapy (Dodd, 1983). A study by Natzke et al (1974) demonstrated that either method of drying off (abrupt cessation or intermittent milking) is satisfactory in treated cows. The study also reported that intermittent milking in cows not receiving dry cow therapy is advantagious over the abrupt cessation.

Due to the unsatisfactory therapeutic cure rates achieved with IMI due to CPS, culling of cows with chronic infections is necessary as part of a mastitis control program (Dodd, 1986). Thus, culling eliminates the source of infection from a herd. Osteras and Lund (1988b) reported that culling cows with chronic udder infections was associated with low prevalence of CPS.

The variation in the response of *Staphylococcal* infection to therapy is striking. Sandholm et al (1990) classified antibiotic failures into one of six categories: 1) resistance to antibiotics; 2) failure of antibiotics to reach the site of infection in adequate concentrations (Mercer and Teske, 1977; Soback, 1987; Craven and Anderson, 1979; Ziv, 1980: Owens and Nickerson, 1990; Craven and Anderson, 1984;

Owens and Watts, 1987; Ziv, 1980); 3) formation of L-forms of bacteria during treatment (Sears et al, 1987; Owens, 1988: Owens and Nickerson, 1989); 4) bacterial dormancy; 5) inhibition of phagocytosis by some antibiotics (Ziv, 1983; Nickerson et al, 1986; Paape et al, 1990); and 6) reinfection from residual bacteria not cleared by the antibiotic during treatment (Sandholm et al, 1990). Antimicrobial resistance is a survival mechanism by which bacteria survive exposure to an antimicrobial agent (Timoney et al, 1988). Natural resistance implies an intrinsic property in an organism that confers resistance. Acquired resistance implies that an organism has obtained, by one mechanism or another, the means to survive in the presence of antibiotics (Timoney et al, 1988; Sandholm et al, 1990; Tyler et al, 1992). These mechanisms are genetically determined and involve a modification of protein synthesis and enzyme activity. Chromosomes and plasmids are the two structures that confer resistance. Both consist of double-strand DNA and both are associated with bacterial cell inner membrane at some time (Timoney et al, 1988).

Chromosomal resistance depends on a mutation in the bacterial genes that leads to resistance to a particular antimicrobial agent. Antimicrobial agents act only as selective agents that allow the resistant mutants to emerge either by a single step (nitrofurans, rifampin, and streptomycin) or sequential mutations (penicillin and tetracycline). Resistance from single step mutations are more important clinically than resistance from sequential mutations because of their higher frequency of occurrence. Chromosomal resistance to antimicrobials is more common among Gram-positive pathogenic bacteria, and possibly occurs among Staphylococci

(Timoney et al, 1988).

In plasmid-mediated resistance (R factor), the genesis and transfer of resistance is dependent on the presence of an antimicrobial agent (Timoney et al, 1988; Sandholm et al, 1990; Tyler et al, 1992). This is the most common type of resistance and by far the most important from a clinical point of view. Each plasmid may contain 20-500 genes that carry resistance to a number of different antimicrobial agents plus specific virulence factors (Timoney et al, 1988). The transfer of resistance is accomplished by either transformation, conjugation, or transduction. In transformation, naked DNA passes from the donor to the recipient through the growth medium. This process is not thought to occur among the *Staphylococci* although it is more common in Gram positive bacteria than in Gram negative bacteria. Conjugation occurs most frequently in Gram-negative bacteria and results in a direct cell-to-cell passage of plasmid genetic information. Among the *Staphylococci*, resistance transfer is mainly by transduction. This is a process whereby bacteriophages carry plasmids between bacteria, transfering the genetic ability to confer resistance (Timoney et al, 1988; Sandholm et al, 1990; Tyler et al, 1992).

Penicillin has been used in the treatment of mastitis for over 40 years. Penicillin resistance among the *Staphylococci* was reported as a possible explanation for unsatisfactory results from mastitis therapy (Dodd, 1983; Sandholm et al, 1990). Resistance to penicillin is the result of beta-lactamase production. Studies have shown a correlation between resistance and beta-lactamase production among the *Staphylococci* (Craven et al, 1986; Owens and Watts, 1988). The emergence and

subsequent transfer of resistance among the *Staphylococci* is not confined to penicillins, but, has spread to other antibiotics, including broad spectrum antibiotics, such as tetracyclines. Failure to control mastitis is often blamed on the indiscriminate use of antibiotics without evidence of the causative organism(s) or of their sensitivity to antibiotics (Wright, 1977). Although the correlation between antimicrobial sensitivity results *in vitro* and clinical response in mastitis is poor (Mercer, 1976), knowledge of common disease agents and their sensitivity profiles can help in making a successful empirical decision about which antibiotic to use. Furthermore, if a mastitis pathogen is resistant *in vitro* to an antimicrobial, the probability of successful therapy with the antimicrobial is greatly reduced (Davidson et al, 1982; Levy, 1991). Antimicrobial resistance has been reported among CPS and CNS isolated from both clinical and subclinical mastitis (Bishop et al, 1980; McDonald and Anderson, 1981; Davidson et al, 1982; Hinckley et al, 1985; Francis and Carroll, 1986; Mackie et al, 1988; Owens and Watts, 1988; Trinidad et al 1990). Resistance, and in particular multiresistance, propagates where antibiotics are being overused and where poor hygiene is practised. It is also noted that the probability that an illness will be caused by a resistant strain will increase as the numbers of resistant pathogens (and resistance genes) increase in the environment (Levy, 1991). It is therefore speculated that the spread of resistant *Staphylococci* in a particular herd will depend on the dairy management practices.

1.3 Summary and objectives

Subclinical mastitis due to *Staphylococci* in dairy cattle causes economic losses for the producer. Practical control is based on improved management practices. Surveys on adoption rate of management practices have been carried out elsewhere in Canada (Meek et al, 1978; Godkin, 1989) and in USA (Burton et al, 1988). Observational studies have been conducted elsewhere that have looked at the relationship between management factors and the prevalence of CPS (Dargent-Molina et al, 1988; Godkin, 1989; Hutton et al, 1991). No similar studies have been conducted for CNS, nor for studies relating prevalence of CPS and CNS to the type of dry cow products used. No literature exists on the relationship between herd level antibiotic sensitivity and management factors nor the correlation with herd prevalence of staphylococcal mastitis pathogens.

The evaluation of the current control methods for mastitis is difficult because mastitis is a complex of many intramammary infections (Dodd, 1986). Mastitis is a dynamic disease with spontaneous recoveries, constant re-infections, and variable susceptibilities of cows to reinfection that make the assessment of therapeutic results difficult (Sandholm et al, 1990). The adoption rates of proven control practices vary from place to place. More work from field studies is required to validate and contrast the existing management measures. The problem of antibiotic resistance may emanate from the very control measures that were instituted to eliminate mastitis. Little research has been done on how sensitivity profiles of the organisms relate to prevalence and management factors. It is anticipated that exploration into these areas will add to our understanding of the impact of dairy management

practices on the prevalence of staphylococcal intramammary infections in dairy cows.

The first objective of this study was to survey dairy management practices used on a random sample of PEI dairy herds, and to determine the effect of management factors on the prevalence of staphylococcal pathogens, both coagulase positive and coagulase negative. The second objective was to survey antimicrobial sensitivity of staphylococcal isolates from the study herds, and assess which management factors affect sensitivity of staphylococcal mastitis pathogens to these antimicrobials. The third objective was to determine whether there is a correlation between sensitivities to different antimicrobials and the prevalence of staphylococcal pathogens after accounting for management factors.

2. A SURVEY OF DAIRY MANAGEMENT PRACTICES

2.1 Introduction

Practical methods for the control of mastitis have long been documented and practised, yet mastitis is still a major problem on most dairy farms. Control measures for contagious mastitis pathogens (*S. aureus* (CPS) and *Streptococcus agalactiae*) are based on practices that: 1) prevent cow-to-cow and quarter-to-quarter spread; 2) prevent teat injuries and teat canal penetration; 3) deal with residual contamination and colonization of teats and teat lesions; 4) reduce the duration of infection; and 5) eliminate the source of infection from a herd (Anderson, 1982).

For environmental mastitis pathogens (coliform and *Streptococcus* species other than *Streptococcus agalactiae*), control is based on the provision of a clean and dry environment (Smith, 1986; Hogan et al, 1987; Pankey, 1987). Control measures for the intermediate category / skin flora opportunists *Staphylococcus* species (CNS) other than *S. aureus*, combine the measures for both contagious and environmental pathogens.

Surveys of dairy management practices carried out elsewhere in Canada (Meek et al, 1981; Godkin, 1989) and in USA (Burton et al, 1988) have reported varying frequencies of adoption among specific dairy practices. The objective of this first part of the study is to describe the frequency of adoption of management factors that control mastitis in Prince Edward Island (PEI). The factors covered the broad categories of milk production, farming experience, housing and calf rearing, milking

management, dry cow management, and antibiotic use.

2.2 Materials and methods

2.2.1 Herd selection

A sample of 57 PEI dairy herds was selected randomly from a list of 520 registered producers shipping fluid and industrial milk on Prince Edward Island. The sample was originally selected for a prevalence survey of staphylococcal mastitis pathogens (Davidson, 1990).

2.2.2 Survey

Information on dairy management practices and antibiotic use was sought through a mailout questionnaire in May 1990. Prospective participants were informed in writing that individual responses were strictly confidential, and that only a summary of the results at the project level would be made available to those who requested a copy. Initial mail contact with prospective participants was followed by a mailing of the questionnaire. Two additional mailings were sent to non-respondents. In August 1990, those who had not responded and those with incomplete questionnaires were contacted and interviewed by telephone. Information regarding milk production for the province was sought from Atlantic Dairy Livestock Improvement Corporation (ADLIC).

The questionnaire covered general factors such as herd size and production,

housing of calves, milk cows and dry cows, calving management, calf rearing and replacement practices, milking management, and dry cow management. A copy of the questionnaire is in Appendix A. A description of lactating and dry cow products as entered in the data base are in Appendices B and C respectively.

2.2.3 Analysis

Data were entered and stored in dBase III Plus (Ashton Tate). Descriptive statistics were performed in Minitab version 7.1 (Minitab, Inc.). Graphs were drawn in Lotus 1-2-3 (Lotus Development Corporation Release 2.2).

For categorical and ordinal data, frequency tables of count and percent were constructed. For continuous data, graphical summaries, the mean, standard deviation (SD), median, and range were determined.

2.3 Results

Forty four farmers (77%) responded to the survey. Two farmers withdrew because they had gone out of the dairy industry and were unwilling to answer for the time they were involved with dairying. Ten other farmers declined to participate and one could not be contacted.

Responses to general demographic variables are displayed in Figures 1 and 2. The mean farming experience on the 44 farms was 23 years (SD 12 years, median 20 years, range 2 to 49 years). The majority of farmers had at least 20 years of experience. The mean milking herd size on 43 farms was 33 cows (S.D 14 cows,

median 30 cows, range 12 to 75 cows). Modal milking herd size was 21-30 cows. About half of the total respondents (20) were members of ADLIC. Of these, only 13 farms (29% of the total respondents) provided information on BCA milk production. The mean BCA milk was 149.5 (SD 15.59, median 152, range 105 to 167). The 95% confidence interval for the mean was 140.11 to 158.96. The daily milk production per cow based on 19 respondents was 20.86 kg (SD 5.34 kg, median 22 kg, range 10 to 28 kg). Based on this, the mean lactation milk production was 6363.35 kg. The 95% confidence interval for the mean was 5578 kg to 7149 kg.

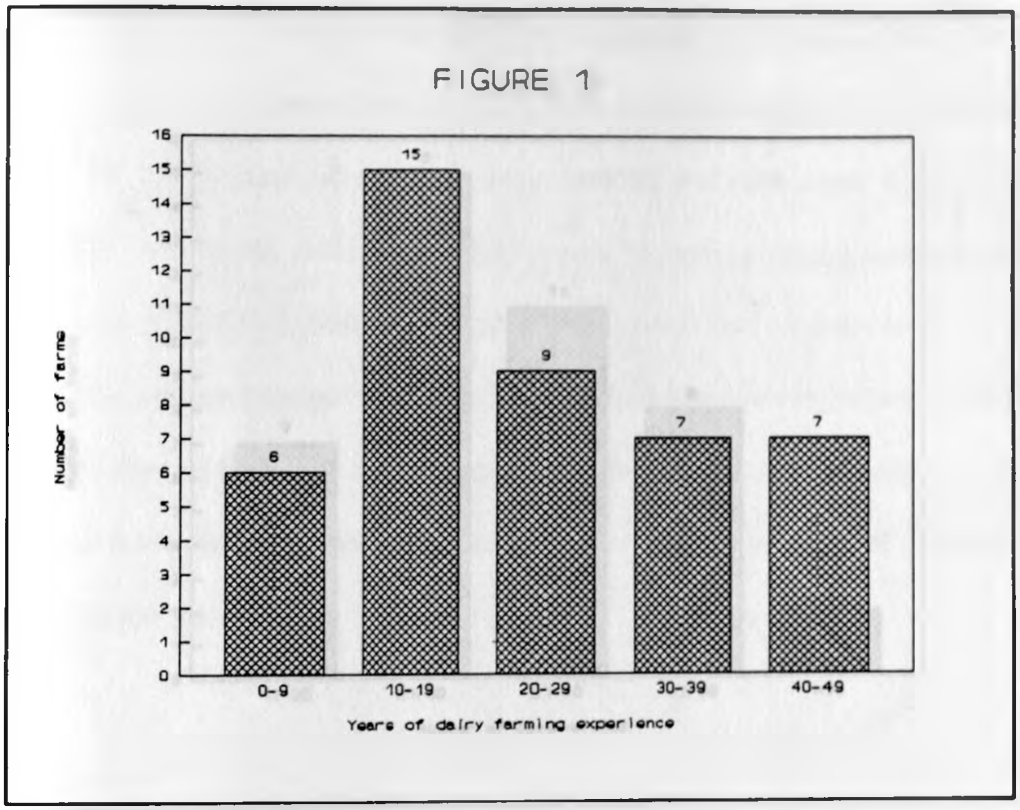

Figure 1. Years of dairy farming experience on 44 randomly selected Prince Edward Island dairy farms (mean = 23 years, SD = 12 years, median = 20 years, range = 2 to 49 years).

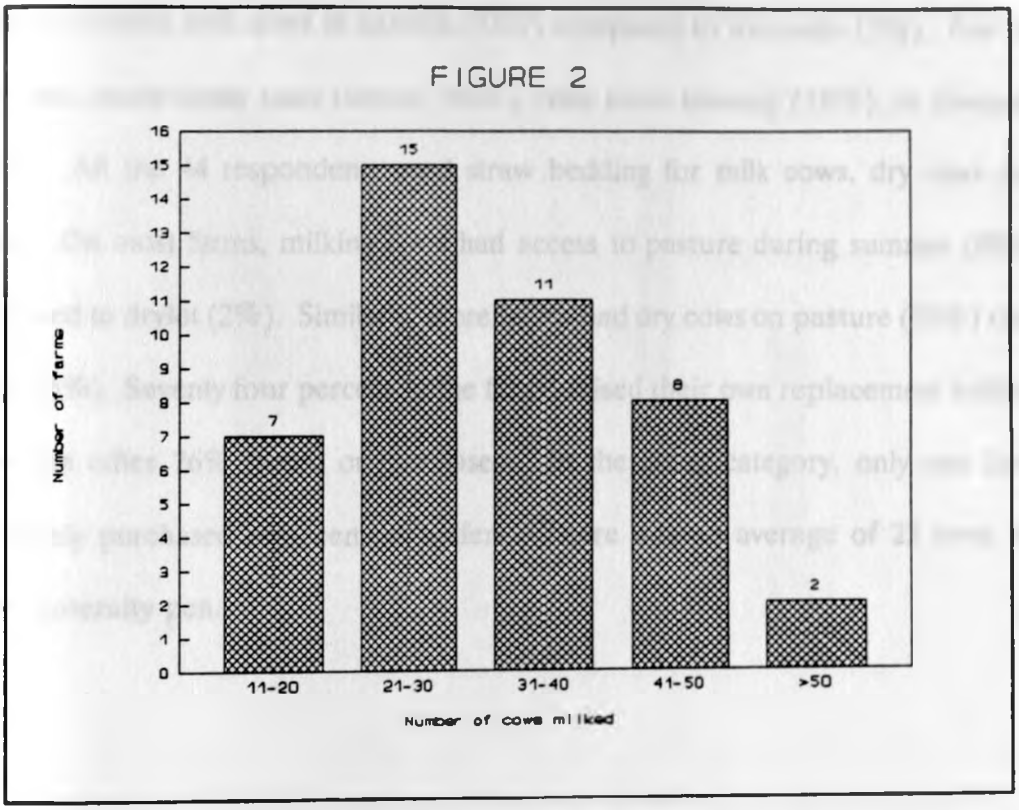

Figure 2. Milking herd size on 43 randomly selected Prince Edward Island dairy farms (mean = 33 cows, SD = 14 cows, median = 30 cows, range = 12 to 75 cows).

Reported housing and calf rearing practices are presented in Figure 3. Nearly all farms housed milk cows in tiestalls (93%) compared to freestalls (7%). For dry cows also, more farms used tiestalls (68%), than loose housing (18%), or freestalls (14%). All the 44 respondents used straw bedding for milk cows, dry cows and calves. On most farms, milking cows had access to pasture during summer (98%) compared to drylot (2%). Similarly, more farms had dry cows on pasture (95%) than drylot (5%). Seventy four percent of the farms raised their own replacement heifers, while the other 26% raised or purchased. In the latter category, only one farm exclusively purchased replacement heifers. There was an average of 23 cows for every maternity pen.

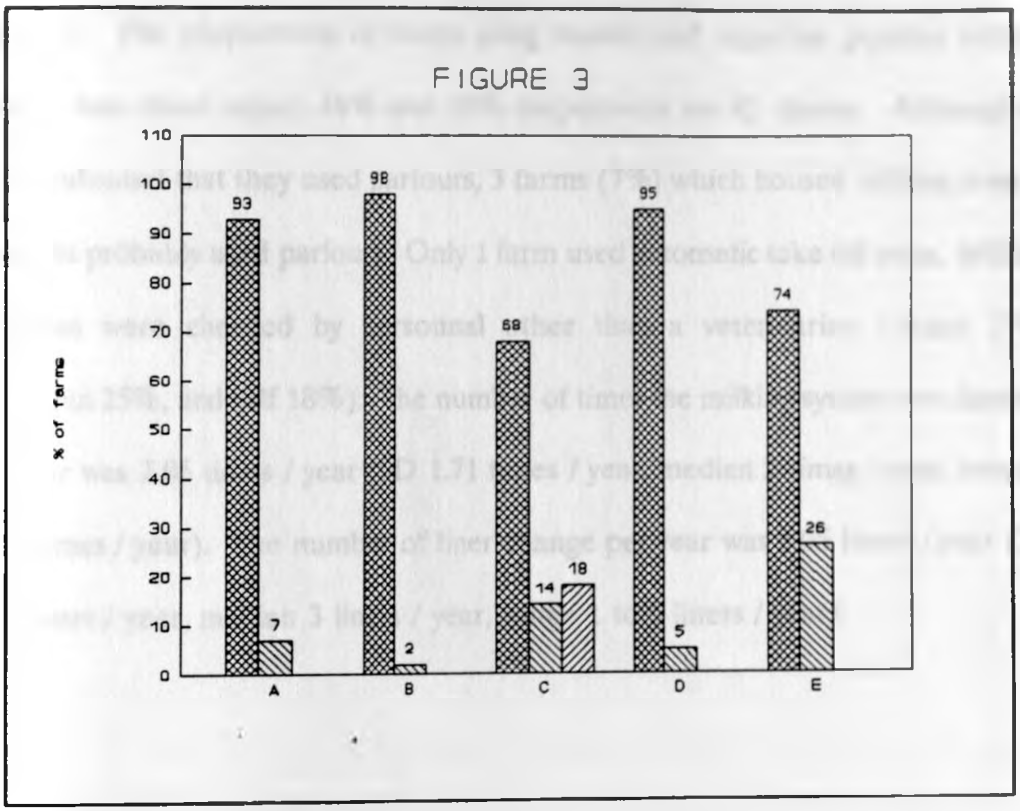

Figure 3. Housing and Calf rearing practices on a random sample of Prince Edward Island dairy farms.

A Milking cow barn (Tiestall* / Freestall**) on 44 farms.
B Outdoor access for milking cows (Pasture / Yard) on 44 farms.
C Dry cow barn (Tiestall / Freestall / Loose housing***) on 44 farms.
D Outdoor access for dry cows (Pasture / Yard).
E Heifer source (Self / Purchase).

* First labelled bar
** Second labelled bar
*** Third labelled bar

Responses to selected aspects of milking system function are presented in Figure 4. The proportions of farms using bucket and high-line pipeline milking systems was about equal, 48% and 52% respectively on 42 farms. Although no farms indicated that they used parlours, 3 farms (7%) which housed milking cows in freestalls probably used parlours. Only 1 farm used automatic take off units. Milking machines were checked by personnel other than a veterinarian (dealer 57%, technician 25%, and self 18%). The number of times the milking system was checked per year was 2.05 times / year (SD 1.71 times / year, median 2 times / year, range 0 to 10 times / year). The number of liner change per year was 3.05 liners / year (SD 1.08 liners / year, median 3 liners / year, range 1 to 6 liners / year).

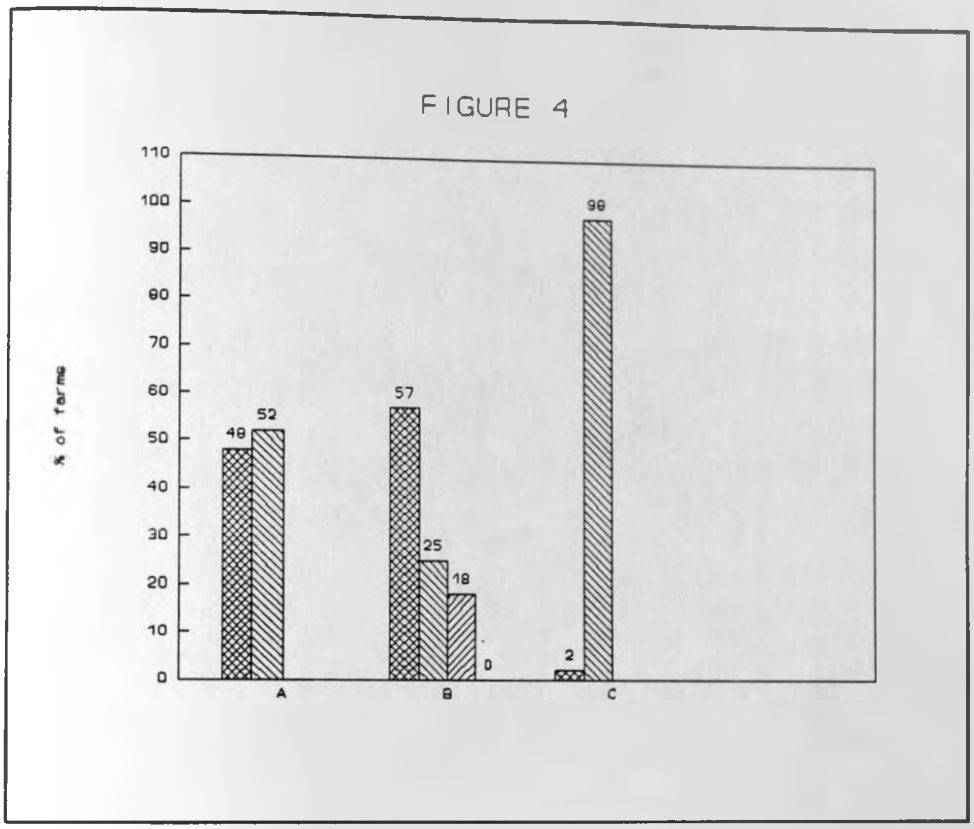

Figure 4. Selected aspects of milking system function on a random sample of Prince Edward Island dairy farms.

A Type of milking system (Bucket* / High-line pipeline**) on 42 farms.
B Person who checked the milking system (Dealer / Technician / Self***/Veterinarian****) on 40 farms.
C Used automatic take offs (Yes / No) on 42 farms.

* First labelled bar
** Second labelled bar
*** Third labelled bar
**** Fourth labelled bar

Responses to pre-milking udder hygiene practices are presented in Figure 5. Only 14% of the farms stripped cows before milking versus 86% which did not. More farms washed and / or pre-dipped teats before milking (88%), added disinfectant in wash water (85%), and used disposable paper towels (83%) in comparison to those which did not. The type of disinfectant added in wash water was about even between chlorhexidine (52%) and iodine (48%).

Responses to post-milking udder hygiene practices are presented in Figure 6. Post-milking teat dipping was practised on 79% of the farms. Chlorhexidine based teat dips were used by 65% of these farms, linear dodecyl benzene sulfonic acid (LDBSA) (19%), and iodine dips (16%). Over half (56%) of the farms that teat dipped used a squeeze bottle to apply, while 26% and 18% used a cup and spray respectively. Among those using either a squeeze bottle or cup, 32% changed the teat dip at every milking or daily, 45% changed occasionally, while 24% did not change. Dipping the milking cluster in disinfectant solution between cows was practised by 36% of the farms.

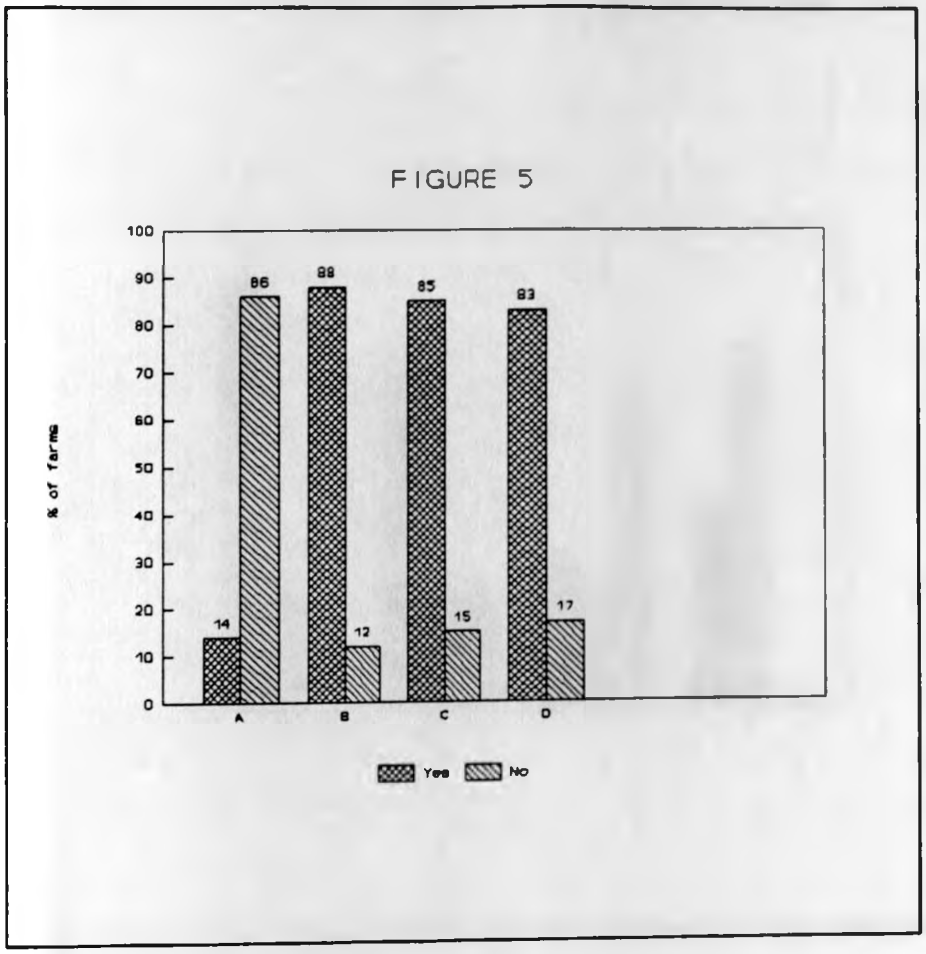

Figure 5. Pre-milking udder hygiene on a random sample of Prince Edward Island dairy farms.

A Cows stripped (on 41 farms)
B Teats washed and or dipped (on 42 farms)
C Added disinfectant in wash water (on 40 farms)
D Used disposable paper towels (on 42 farms)

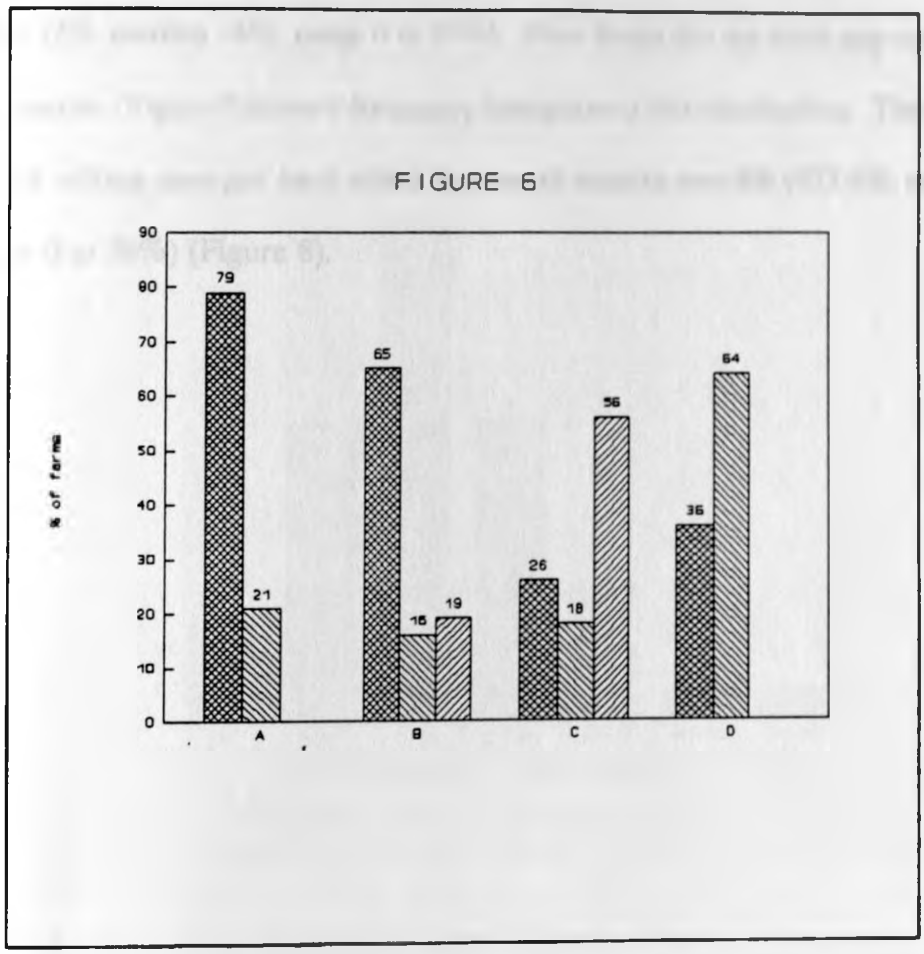

Figure 6. Post-Milking Udder Hygiene on a random sample of Prince Edward dairy farms.

A Teats dipped (Yes* / No**) on 42 farms
B Dip product used (Chlorhexidine / Iodine dip / Linear dodecyl benzene sulfonic acid***) on 31 farms.
C How dip is applied (Cup / Spray / Squeeze bottle) on 34 farms.
D Milking cluster dipped between cows (Yes / No) on 42 farms

* First labelled bar
** Second labelled bar
*** Third labelled bar

The mean percent of milking cows per herd treated for clinical mastitis was 18% (SD 12%, median 14%, range 0 to 53%). Nine farms did not treat any cows for clinical mastitis. Figure 7 shows a frequency histogram of this distribution. The mean percent of milking cows per herd culled because of mastitis was 8% (SD 8%, median 5% range 0 to 30%) (Figure 8).

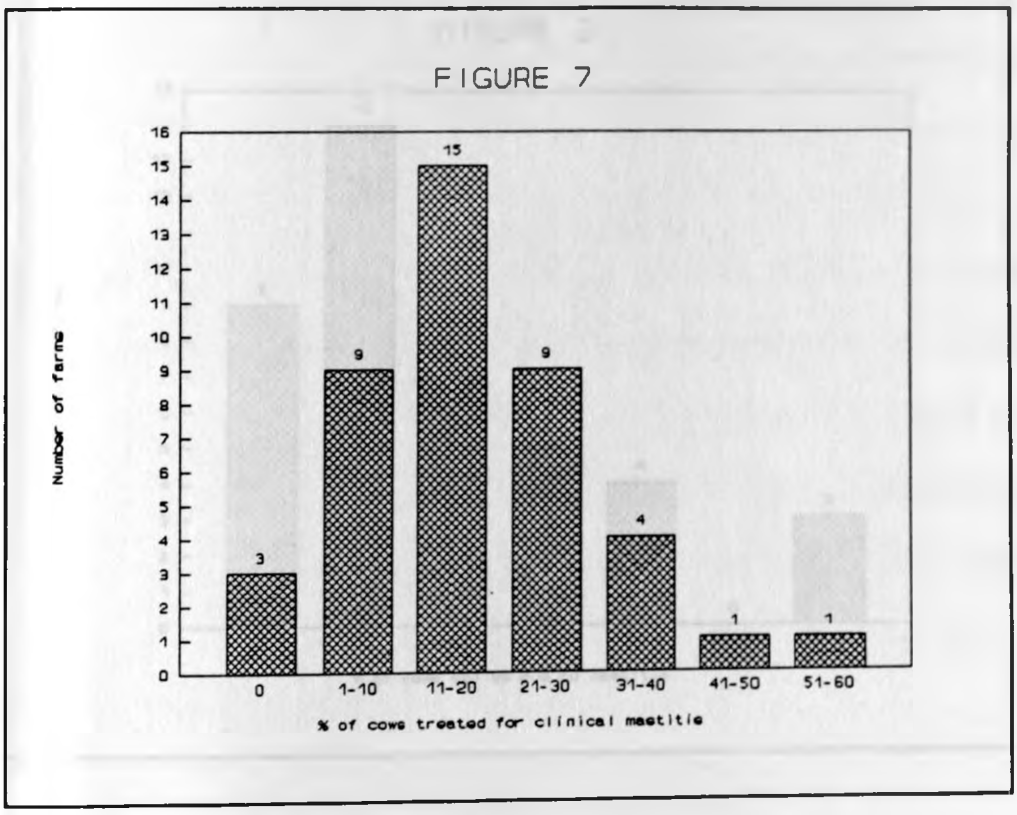

Figure 7. Proportion (%) of cows in milking herd treated for clinical mastitis (mean = 18%, SD = 12%, median = 14%, range = 0-53%).

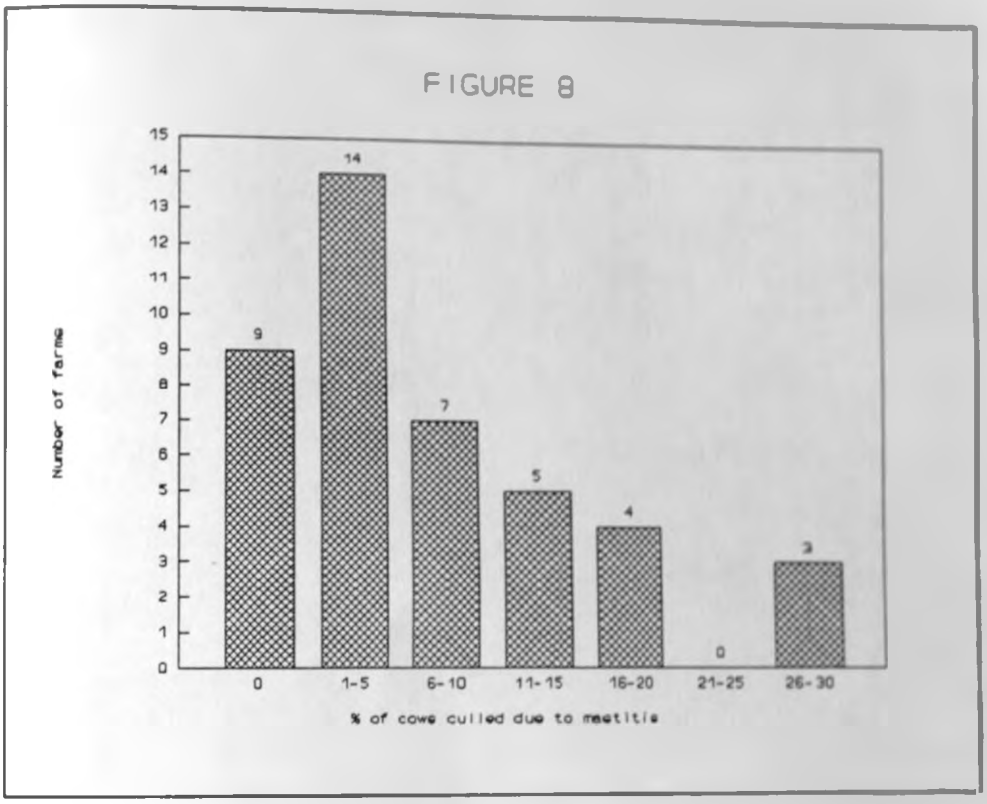

Figure 8. The milking herd proportion (%) culled due to mastitis (mean = 8%, SD = 8%, median = 5%, range = 0-30%).

The percent of the herd receiving dry cow therapy was 67.25% (SD 40.70%, median 95%, range 0 to 100%). This is depicted in Figure 9 as a frequency histogram. The distribution is bi-modally skewed with herds tending towards an all or none approach to dry cow therapy. Nearly one half of the respondents to this question (19) treated the whole herd at drying off. Nine farms (20% of the total respondents) treated less than 20% of the herd. The other 12 farms, representing 27% of the total respondents infused between 20-99% of the herd. The results of other dry period management practices are presented in Figure 10. A majority of the farms disinfected teats prior to infusing dry cow therapy compared to those which did not (89.74% versus 10.26%). More farms dried off cows by intermittent milking (81.40%) than by the abrupt method (18.60%). Only 30.77% of the farms teat dipped prior to calving.

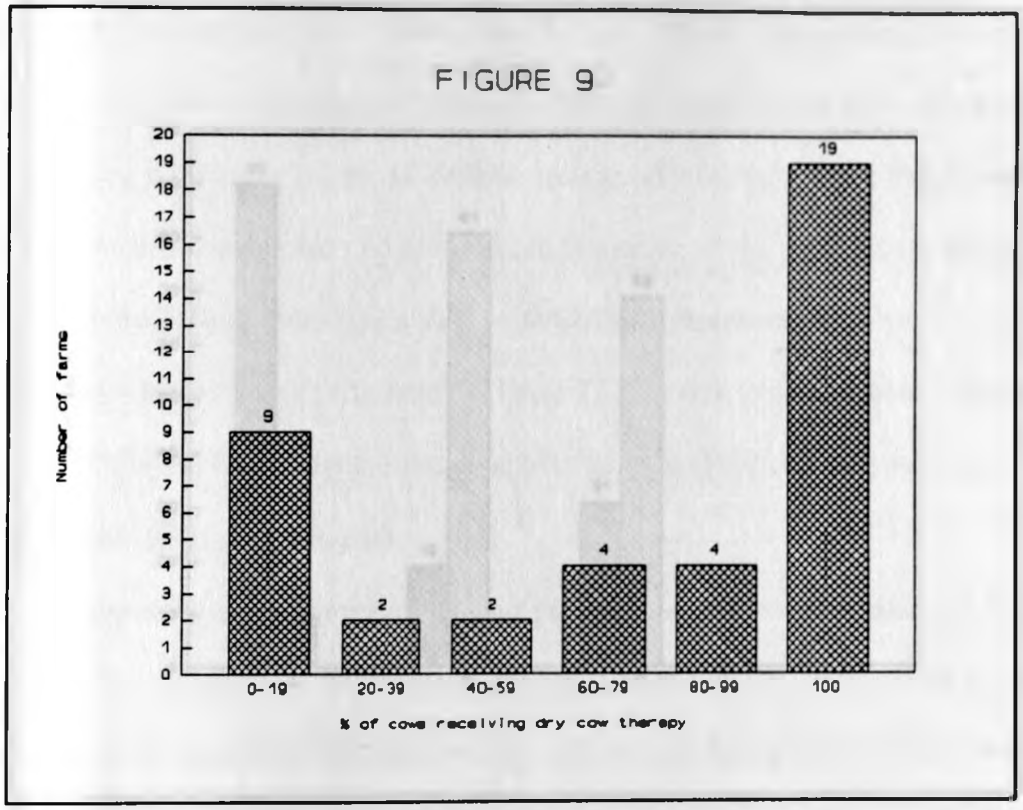

Figure 9. Proportion (%) of herd receiving dry cow therapy on 40 randomly selected Prince Edward Island dairy farms (mean = 67.25%, SD = 40.70%, median = 95%, range = 0-100%).

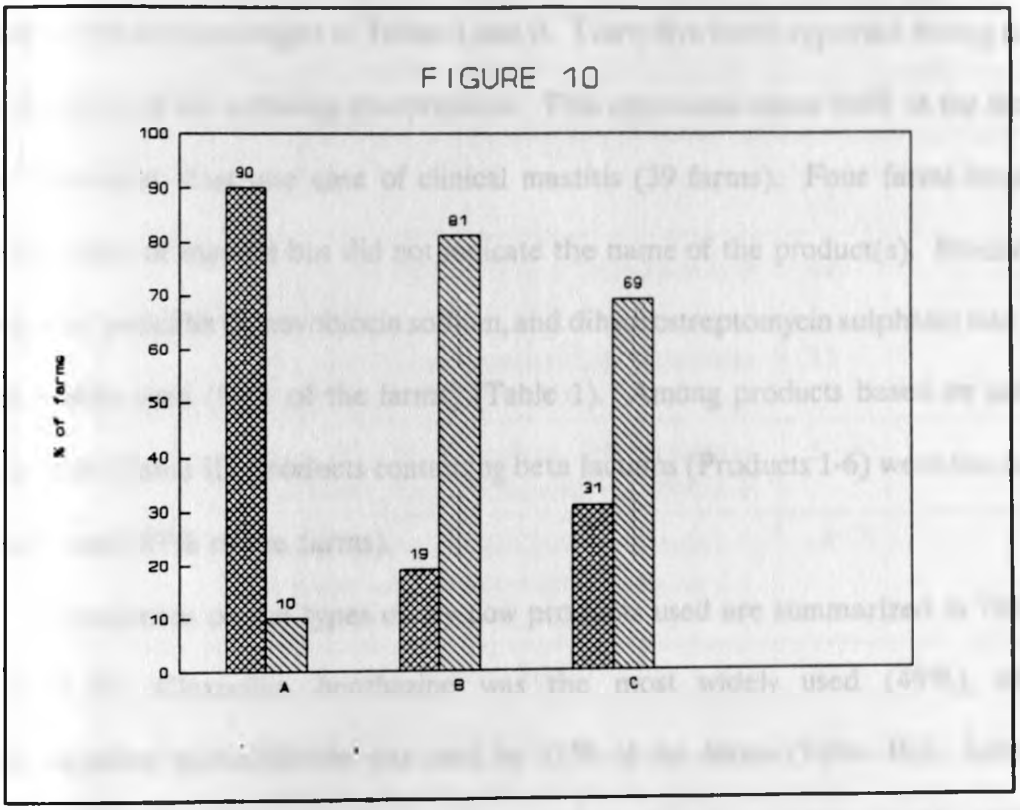

Figure 10. Dry cow management practices on a random sample of Prince Edward Island dairy farms.

A Disinfection prior to infusion (yes* / No**) on 39 farms.
B Dry off method (Abrupt / Gradual) on 43 farms.
C Teats dipped before calving (Yes / No) on 39 farms.
* First labelled bar
** Second labelled bar

Responses pertaining to the types of lactating cow products used are summarized as percentages in Tables I and II. Thirty five farms reported having used one or more of the lactating cow products. This represents about 90% of the farms that treated at least one case of clinical mastitis (39 farms). Four farms treated clinical cases of mastitis but did not indicate the name of the product(s). Product 4 (procaine penicillin G, novobiocin sodium, and dihydrostreptomycin sulphate) was the most widely used (64% of the farms) (Table 1). Among products based on active ingredient (Table II), products containing beta lactams (Products 1-6) were the most widely used (87% of the farms).

Responses on the types of dry cow products used are summarized in Tables III and IV. Cloxacillin benthazine was the most widely used (49%), while oxytetracycline hydrochloride was used by 32% of the farms (Table III). Seventy eight percent of the farms used products containing beta lactams (Table IV), 43% of these contained penicillin G.

TABLE I. LACTATING COW PRODUCTS USED ON 35 RANDOMLY SELECTED PRINCE EDWARD ISLAND DAIRY FARMS

Product Code	Active Ingredients	Response Yes (%)
Product 1	Procaine Penicillin G and Novobiocin sodium	13
Product 2	Penicillin G potassium, Streptomycin sulphate, and Bacitracin	15
Product 3	Penicillin G potassium, Streptomycin sulphate, Bacitracin, and Polymyxin B	5
Product 4	Procaine Penicillin G, Novobiocin sodium, and Dihydrostreptomycin sulphate	64
Product 5	Cephapirin sodium base	44
Product 6	Cloxacillin sodium	18
Product 7	Oxytetracycline hydrochloride	41
Product 8	Erythromycin	8

TABLE II. LACTATING COW PRODUCTS BASED ON ACTIVE INGREDIENT USED ON 35 RANDOMLY SELECTED PRINCE EDWARD ISLAND DAIRY FARMS

Product Code	Active Ingredient	Response Yes (%)
Product 9	Any product containing Novobiocin	69
Product 10	Any product containing Penicillin G	77
Product 11	Any product containing a Beta lactam	87
Product 12	Any product containing Streptomycin	72

TABLE III. DRY COW PRODUCTS USED ON 37 RANDOMLY SELECTED PRINCE EDWARD ISLAND DAIRY FARMS

Product Code	Active Ingredients	Response Yes (%)
Dry Cow Product 1	Novobiocin sodium	32
Dry Cow Product 2	Procaine Penicillin G and Novobiocin sodium	11
Dry Cow Product 3	Procaine Penicillin G, Novobiocin sodium, and Dihydrostreptomycin sulphate	37
Dry Cow Product 4	Procaine Penicillin G and Dihydrostreptomycin sulphate	3
Dry Cow Product 5	Procaine Penicillin G, Streptomycin sulphate, Neomycin, and Polymyxin B	0
Dry Cow Product 6	Cloxacillin Benthazine	49
Dry Cow Product 7	Cephapirin Benthazine	19
Dry Cow Product 8	Oxytetracycline hydrochloride	32

TABLE IV. DRY COW PRODUCTS BASED ON ACTIVE INGREDIENT USED ON 37 RANDOMLY SELECTED PRINCE EDWARD ISLAND DAIRY FARMS

Product Code	Active Ingredient	Response Yes (%)
Dry Cow Product 9	Any product containing Novobiocin	43
Dry Cow Product 10	Any product containing Penicillin G	43
Dry Cow Product 11	Any product containing a Beta lactam	78
Dry Cow Product 12	Any product containing Streptomycin	32

2.4 Discussion

The results of some of the practices surveyed in this study can be compared and contrasted with the recommended procedures for appropriate mastitis control program, existing production averages and other surveys done elsewhere. The study realized a response rate of 77%. Although it was possible that information from the 23% non-respondents could bias the formal random herd selection process used to identify the total study population, the response rate achieved was better than other published dairy management mail surveys which have reported response rates between 50 and 70% (Meek et al, 1981; Burton et al, 1988).

Results of this survey compared favorably with provincial average figures for milking herd size and milk production. The average milking herd size reported here, 33 cows (SD 14 cows) compares well with the provincial average for 1989 (31 cows) (Dairy Profile PEI, 1991). The average lactation milk production, 6363.35 kg (95% C.I 5578 kg, 7149 kg) did not differ significantly from the provincial (6827 kg) and ADLIC farms (7030 kg) figures for 1989 (ADLIC, 1989). The mean BCA milk for ADLIC farms in this study, 149.5 (95% C.I 140.11, 158.96) did not differ significantly from the average for ADLIC farms in 1989 (152) (ADLIC, 1989).

The majority of the farms surveyed practised good pre-milking hygiene. The high rates of compliance with accepted pre-milking hygiene practices may be due to farmer awareness of the existing regulations in PEI that make udder washing and the use of disposable paper towels mandatory. This in itself may have biased the information among those who responded that they carried out these pre-milking

practices. The observation that only 14% of the farms stripped cows before milking indicates unawareness about mastitis detection procedures among the majority of the farms. The adoption rate for post-milking teat dipping was only 79% despite the proven benefits. Elsewhere in Canada, rates of 54% and 80% have been reported (Meek et al, 1981: Godkin 1989). In the USA, Burton et al (1988) reported a teat dipping rate of 75%.

Among milking machine function and maintenance procedures, it was noted that personnel other than the veterinarian checked the milking system. It could not be ascertained whether this was an individual preference for farmers or a lack of involvement by their veterinarians.

The number of cows treated for clinical mastitis expressed as percent of cows milked in the herd was 18%. . Since less than 50% of new infections arising during lactation are manifest by clinical signs (Dodd, 1986), the number of new infections would be higher than 18 cases / 100 cows / year. Only 14% of the farms stripped cows before milking. Stripping foremilk aids in the early detection of clinical mastitis. In this study, less than half of the total respondents (43%) administered dry cow therapy to all cows at drying off. Further, only 19% dried off cows abruptly, the procedure recommended when dry cow therapy is applied (Dodd, 1983). This level of adoption may be a reflection of the perceived gain versus cost. It is possible that some farmers are not aware of the importance of the subclinical form of mastitis. Elsewhere in Canada, comparable rates for dry cow therapy have been reported, 37% and 66% (Meek et al, 1981; Godkin 1989). In the USA, Burton et al (1988) reported

93% of farmers surveyed used dry cow therapy but less than half of farmers using dry cow therapy infused all quarters of all cows.

The annual culling rate of 8% due to mastitis was above the recommended maximum culling rate due to mastitis of 6% on farms with appropriate mastitis control programs (McDonald, 1984). Enrolment in Somatic Cell Count (SCC) Program such as provided by ADLIC may help in the identification of chronically infected cows due for culling and thereby eliminate the source of infection. Therefore the 47% of the respondents who were members of ADLIC are the only ones likely to have identified chronically infected cows.

In conclusion, the study realized a response rate of 77%. The response rate could have led to bias, but the results did not depart substantially from provincial averages, therefore the selection bias is probably small. The results of the study indicate that the rates of adoption of pre-milking udder hygiene practices are higher than either post-milking teat dipping or dry cow therapy for all cows. Other studies have reported similarly low rates of adoption with regards to these two latter practices. The study did not assess the farmers perception and knowledge of mastitis. Nonetheless, it is apparent that the desire to work in a clean environment and yield an unadulterated product is shared by many. This is reflected in high rates of adoption of pre-milking hygiene practices. However, knowledge of the Provincial Dairy Act Regulations may have biased this observation. Conversely, the relatively low rates of adoption of post-milking teat dipping and dry cow therapy, despite the documented emphasis, may indicate unawareness about the actual role of these

procedures or they may indicate more varied perceptions. While the onus to adopt or discontinue specific mastitis control procedures lies with the farmer, it is unlikely that farmers will achieve maximum benefits if they are not made fully aware of the importance of mastitis, and how each specific control procedure helps mitigate this disease.

3. THE RELATIONSHIP BETWEEN DAIRY MANAGEMENT PRACTICES INCLUDING DRY COW PRODUCTS USED AND PREVALENCE OF STAPHYLOCOCCAL MASTITIS PATHOGENS

3.1 Introduction

Improving dairy management practices is recognized as the only practical approach to minimize intramammary infections (IMI) (Dodd, 1983). However, the interaction of management and environmental factors can increase the teats' exposure to mastitis pathogens and aid the pathogens in traversing the teat canal and gaining access to the secretory epithelium of the udder (Philpot 1984). Coagulase positive *Staphylococci* (CPS) are contagious; infections are often chronic, vacillate between subclinical and clinical forms and are difficult to eradicate with antibiotic therapy (Anderson, 1982). *Staphylococcus* species other than *Staphylococcus aureus* (coagulase negative *Staphylococci* (CNS)) are partly contagious and partly environmental. Intramammary infections due to CNS are important because of high risk of infection, long duration, compositional changes in milk and lost milk production (Timms and Schultz, 1987). In Prince Edward Island (PEI), the prevalence of CPS was estimated at 14.1% and CNS at 24.5% (Davidson, 1990).

Most studies have evaluated specific mastitis control measures. Galton et al (1984), Galton et al (1986), and Pankey et al (1986) have conducted studies on pre-milking udder hygiene practices. Osteras and Lund (1988a) have evaluated milking machine function. Studies on environmental factors have been conducted by Osteras

and Lund (1988b). Post-milking teat dipping as a management tool for the control of contagious mastitis pathogens has been studied by Hogan et al, (1987), Nickerson et al, (1990), and Fox et al, (1991). The possible role of pre-partum teat dipping in the control of mastitis pathogens has been evaluated by Schultze (1985), and Mathews et al, (1988). Buddle et al, (1987), Batra (1988), and Browning et al, (1990) have studied the effectiveness of intramammary antibiotic therapy at drying off. Studies addressing all management practices simultaneously are needed to validate the efficacy of the existing management practices. Such studies have been conducted for CPS IMI (Dargent-Molina et al, 1988; Godkin, 1989; Hutton et al, 1991). No similar studies have been conducted for CNS IMI. No studies have been done to relate prevalence or incidence of staphylococcal mastitis pathogens to the type of dry cow products used at drying off.

The objective of this part of the study is to relate the prevalence of staphylococcal (CPS and CNS) mastitis pathogens to dairy management practices and, separately, to the antibiotic products used at drying off. The frequency of adoption of dairy management practices and antibiotic use were described in Chapter Two.

3.2 Materials and methods

3.2.1 Sample collection and storage

A sample of 57 PEI dairy herds was selected randomly from a list of 520 registered producers shipping fluid and industrial milk in PEI. Composite cow milk samples were collected from each milking cow for a total of 1,647 samples. The

materials and methods used to isolate microorganisms from these samples have been described previously (Davidson, 1990). Figure 11 shows the flow chart used to identify the various organisms. Briefly, following culture on 5% blood agar plates, organisms were initially identified as Gram positive cocci. A catalase test was performed on Gram positive cocci to differentiate catalase negative *Streptococcus* species from catalase positive *Staphylococcus* and *Micrococcus*. 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. To establish infection status, a culture was classified as coagulase negative *Staphylococci* (CNS) if it had at least 2 colonies of coagulase negative *Staphylococci* with no other growth. A culture was classified as *Staphylococcus aureus* (CPS) if it had at least 2 colonies of *Staphylococcus aureus* with or without other bacterial growth. All isolates of *Staphylococci* classified as coagulase positive *Staphylococci* (CPS) and a sub-sample representing 25% of other *Staphylococci* isolates classified as coagulase negative *Staphylococci* (CNS) were frozen on slants at -20°C in the Summer of 1989. Each sample was identified by farm, cow, and the date of collection. A total of 419 samples were stored.

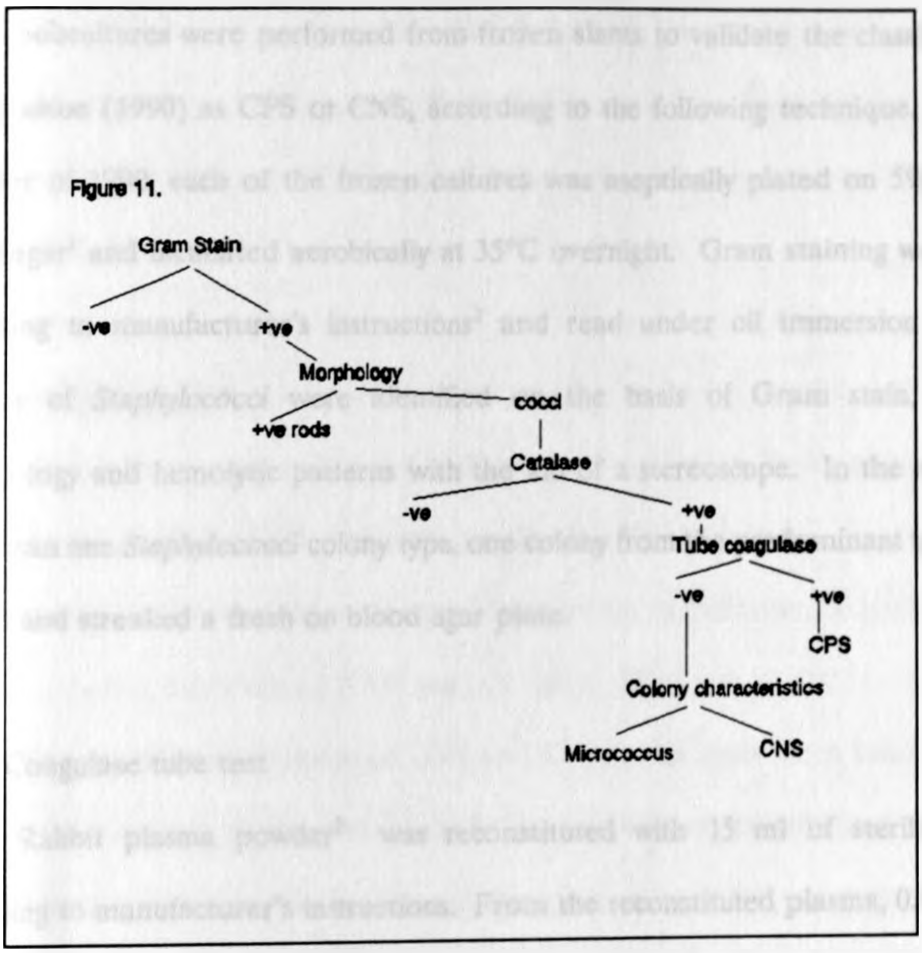

Figure 11. Flow chart used to identify *Staphylococcus*

3.2.2 Pure culture isolation

Subcultures were performed from frozen slants to validate the classification by Davidson (1990) as CPS or CNS, according to the following technique. In the Summer of 1990, each of the frozen cultures was aseptically plated on 5% sheep blood agar[1] and incubated aerobically at 35°C overnight. Gram staining was done according to manufacturer's instructions[2] and read under oil immersion. Pure cultures of *Staphylococci* were identified on the basis of Gram stain, colony morphology and hemolytic patterns with the aid of a stereoscope. In the event of more than one *Staphylococci* colony type, one colony from the predominant type was picked and streaked a fresh on blood agar plate.

3.2.3 Coagulase tube test

Rabbit plasma powder[3] was reconstituted with 15 ml of sterile water according to manufacturer's instructions. From the reconstituted plasma, 0.5ml was drawn and put into test tubes already labelled to correspond with each staphylococcal sample. The tubes were inoculated and incubated aerobically at 35°C for four hours, and coagulase reaction results were read as described by Seeley and VanDemark, (1972). Negative tubes were re-incubated at room temperature overnight and the results read the following morning. For every batch of rabbit plasma reconstituted,

[1] AVC Central Services

[2] Baxter Corporation

[3] Baxter corporation

a test of sterility was run by plating a drop of it on a blood agar plate. American Type Culture Collection (ATCC) 29213 *Staphylococcus aureus* was used as a known positive control, while plasma without inoculum served as the negative control. Cultures were classified as CPS if a clot formed with rabbit plasma or CNS if there was no clot.

3.2.4 Statistical analysis

Data were entered and stored in dBase III Plus (Ashton Tate), and statistical analyses were conducted using Minitab Version 7.1 (Minitab, Inc).

Agreement beyond chance (Kappa) between the classification of *Staphylococci* by this study and Davidson's (1990) was calculated (Martin et al, 1987).

The prevalence estimates of CNS and CPS in this study were based on the classification obtained in the present study for pure cultures isolated and identified on both farm and cow of origin. For the three out of four CNS cultures that were not retained and pure cultures isolated and identified only on farm of origin but not cow, the classification of Davidson (1990) was used. The within herd prevalence estimates were calculated by dividing the number of cows positive for CPS (or CNS) by the number of cows sampled on a given farm. The overall CPS and CNS prevalence estimates for the study sample and 95% confidence interval for the means were calculated according to the methods by Scheaffer et al (1979) for cluster sampling.

Weighted least squares multiple regression analysis was used to relate the herd

prevalence of CNS and CPS to dairy management practices and antibiotic products used at drying off. The arc sine transformation of herd prevalence was used in order to stabilize the variance of these proportions (Snedecor and Cochran, 1989). The weighting factor was the number of cows sampled per farm (variance of a proportion estimate is p(1-p)/n, where n is the number of cows sampled per farm, mean 30, range 8 to 61). This gave more emphasis to farms where more cows were sampled and therefore, with less variation in prevalence estimates (Draper and Smith, 1981). In lieu of evaluating all the potential predictors among management practices in the multivariate analyses, univariate associations with the dependent variables were sought using weighted least squares simple regression. Inclusion level was set at α = 0.50. This relaxed criterion ensured that no potential predictors in the multivariate analyses were omitted.

The following general demographic variables were evaluated: membership in Atlantic Dairy Livestock Improvement Corporation (ADLIC), and years of farming experience. The milking herd size was not considered because it closely approximated the number of cows sampled per farm. Among housing and calf rearing practices, the following were screened: the type of barn for milk cows, the type of barn for dry cows, and heifer source. Milking machine variables included: the type of milking system, the person who checked the milking system, and the number of times the milking system was checked per year. Pre-milking udder hygiene variables assessed for significance included: whether cows were stripped before milking, the teats were washed or dipped before milking, whether disinfectant was

added in wash water, and the use of disposable paper towels. Post-milking hygiene variables included: whether teats were dipped after milking, the dip product used, how the teat dip was applied, the frequency of dip change, and whether the milking cluster was dipped in disinfectant solution between cows.

Among practices that aim to cure infection or eliminate the source of infection from a herd, the following variables were evaluated: the proportion of the milking herd treated for clinical mastitis, whether teats were disinfected prior to antibiotic infusion, the proportion of the herd receiving dry cow therapy, and the proportion of the milking herd culled because of mastitis. Dry cow management practices evaluated included the method of drying off and whether teats were dipped during the pre-partum period.

In the multivariate analyses involving dairy management practices, an *a priori* decision was made to include variables considered more important in the control of mastitis even if the univariate association with prevalence was not significant. Also entered into the multivariate analyses were possible confounders. Interaction terms were not considered in the analyses because of the modest sample size relative to the number of possible predictors (Kleinbaum et al, 1988).

In the regression analysis involving dry cow products, all dry cow products (Table V) were entered in the multivariate analyses. The proportion of the herd receiving dry cow therapy and the model describing prevalence among management practices were entered as a possible confounders. Main dry cow products (Dry Cow Products 1, 2, 3, 4, 6, 7, and 8) were entered in the model before dry cow products

based on active ingredient (Dry Cow Products 9, 10, 11, and 12).

Weighted least squares multiple regression was used to relate prevalence to the independent variables. A forward entry procedure was used in the multivariate analysis. The variable with the highest correlation with the dependent variable was entered first. Subsequent entry depended upon a variable having the highest correlation with the dependent variable at each step of model building (Kleinbaum et al, 1988). Overall model significance was assessed at $p = 0.05$ and inclusion level set at $p = 0.25$. The liberal inclusion criterion would ensure that no bias is introduced in the estimated regression coefficients (Kleinbaum et al, 1988). Plots of residuals on predicted values and probability scores were done for the final model to assess normality and linearity respectively, while collinearity among the predictors was assessed using variance inflation factor (Kleinbaum et al, 1988).

TABLE V. DRY COW PRODUCTS ENTERED IN THE MULTIVARIATE ANALYSIS FOR THE RELATIONSHIP WITH CPS AND CNS PREVALENCE ESTIMATES

Product Code	Active Ingredients
Dry Cow Product 1	Novobiocin sodium
Dry Cow Product 2	Procaine Penicillin G and Novobiocin sodium
Dry Cow Product 3	Procaine Penicillin G, Novobiocin sodium, and Dihydrostreptomycin sulphate
Dry Cow Product 4	Procaine Penicillin G and Dihydrostreptomycin sulphate
Dry Cow Product 6	Cloxacillin Benthazine
Dry Cow Product 7	Cephapirin Benthazine
Dry Cow Product 8	Oxytetracycline hydrochloride
Dry Cow Product 9	Any product containing Novobiocin
Dry Cow Product 10	Any product containing Penicillin G
Dry Cow Product 11	Any product containing a Beta lactam
Dry Cow Product 12	Any product containing Streptomycin

3.3. Results

3.3.1 Results of classification and prevalence estimates

Out of the original 419 cultures, 195 were classified as CPS and 178 as CNS. Nine samples without identification together with another 37 contaminated samples which represented all the samples from 3 farms were discarded. Out of the 373 identified and classified cultures, 324 were matched with milk samples cultured previously (Davidson, 1990) on both farm of origin and cow identity. Both studies (this study and Davidson's, 1990) classified 175 *Staphylococci* as CPS and 118 as CNS (Table VI). The overall agreement beyond chance, kappa on 54 farms was 0.8, with a disagreement of 7% and 13% for CPS and CNS respectively between frozen cultures and milk samples from the previous study (Davidson, 1990).

The mean within herd prevalence of CPS was 14% (SD 15%, median 9%, range 0 to 70) (Figure 12). The distribution was skewed toward high values. The mean within herd prevalence of CNS was 27% (SD 13%, median 26%, range 0 to 72%) (Figure 12). The overall CPS and CNS prevalence estimates for the study were 14.39% and 24.78% respectively. The 95% confidence intervals for the means were 10.44% to 18.34% and 22.24% to 27.32% for CPS and CNS respectively.

TABLE VI. CLASSIFICATION OF STAPHYLOCOCCI BY RAW MILK SAMPLE AND SUBCULTURE

	Raw milk sample	
Subculture	CPS	CNS
CPS	175	13
CNS	18	118

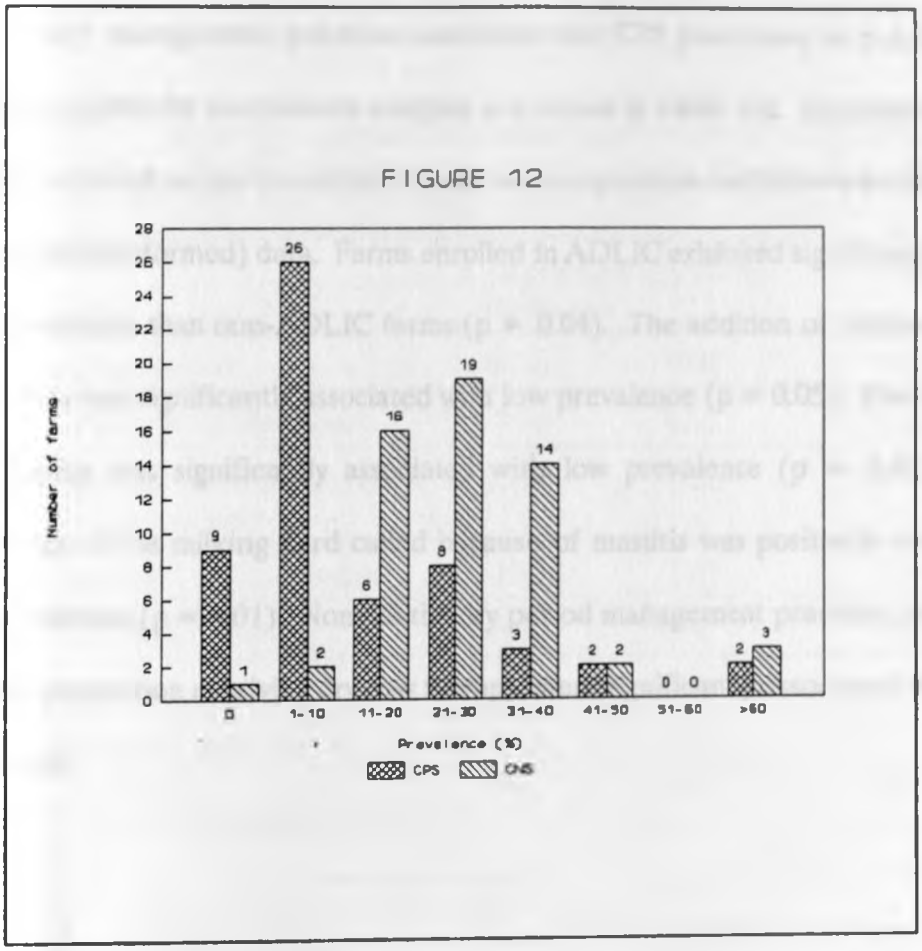

Figure 12. Within herd prevalence of coagulase positive *Staphylococci* (CPS) (mean = 14%, SD = 15%, median = 9%, range = 0-70%) and coagulase negative *Staphylococci* (CNS) (mean = 27%, SD = 13%, median = 26%, range = 0-72) on 54 randomly selected Prince Edward dairy farms.

3.3.2 Results of univariate analysis

Dairy management practices associated with CPS prevalence at $p \leq 0.50$ and therefore eligible for multivariate analysis, are shown in Table VII. Significance tests and R^2 are based on the transformed data, while regression coefficients are based on the raw (untransformed) data. Farms enrolled in ADLIC exhibited significantly lower CPS prevalence than non-ADLIC farms ($p = 0.04$). The addition of disinfectant to wash water was significantly associated with low prevalence ($p = 0.05$). Post-milking teat dipping was significantly associated with low prevalence ($p = 0.01$). The proportion of the milking herd culled because of mastitis was positively correlated with prevalence ($p = 0.01$). None of the dry period management practices, including the herd proportion receiving dry cow therapy, were significantly associated with CPS prevalence.

TABLE VII. SIMPLE LINEAR REGRESSION COEFFICIENTS AND COEFFICIENTS OF DETERMINATION (R^2) FOR DAIRY MANAGEMENT PRACTICES ASSOCIATED WITH CPS PREVALENCE ($p \leq 0.50$)

Variable	Regression coefficient*	p value**	R^2 (%)**
Membership in ADLIC	-8.36	0.04	10.20
High-line pipeline milking system	-3.96	0.31	2.61
Number of times the milking system was checked in one year	1.95	0.22	4.71
Added disinfectant to wash water	-12.50	0.05	9.84
Stripped cows before milking	-3.98	0.43	1.49
Dipped teats after milking	-11.98	0.01	14.93
Used chlorhexidine teat dip Used iodide teat dip Used linear dodecyl benzene sulfonic acid (control)	-1.69 -6.87	0.30***	8.16****
Used a cup to apply teat dip Used a spray to apply teat dip Used a squeeze bottle (control)	0.79 5.54	0.21***	9.72****
The proportion of the milking herd culled because of mastitis	0.72	0.01	14.10

* Regression coefficients are based on the raw (untransformed) data.
** Significance tests and R^2 are based on the transformed data.
*** Overall p value for the 3 dummy variables.
**** Overall R^2 for the 3 dummy variables.

Dairy management practices associated with the prevalence of CNS at $p \leq 0.50$ and therefore eligible for multivariate analysis, are shown in Table VIII. Significance tests and R^2 are based on the transformed data, while regression coefficients are based on the raw (untransformed) data. Freestall and tiestall housing for dry cows were significantly associated with low prevalence ($p = 0.01$ and $p = 0.07$ respectively) versus loose housing. The overall p value for the 3 dummy variables was 0.04. Purchasing of replacement heifers was associated with high prevalence ($p = 0.09$) as opposed to raising them on the farm.

TABLE VIII. SIMPLE LINEAR REGRESSION COEFFICIENTS AND COEFFICIENTS OF DETERMINATION (R^2) FOR DAIRY MANAGEMENT PRACTICES ASSOCIATED WITH CNS PREVALENCE ($p \leq 0.50$)

Variable	Regression coefficient*	p value**	R^2 (%)**
Freestall for dry cows	-11.40		
Tiestall for dry cows	-6.27	0.04***	14.30****
Loose housing for dry cows (control)			
Purchased replacement heifers	6.19	0.09	6.81
High-line pipeline milk system	-3.51	0.32	2.51
Number of times the milking system was checked in a year	1.07	0.29	3.10
Farmer checked the milking system	3.11		
Dealer checked the milking system	-4.67	0.19***	8.62****
Technician checked milking system (control)			
Teats dipped before calving	-3.08	0.35	2.38

* Regression coefficients are based on the raw (untransformed) data.
** Significance tests and R^2 are based on the transformed data.
*** Overall p value for the 3 dummy variables.
**** Overall R^2 for the 3 dummy variables.

3.3.3 Results of multivariate analysis

Significance tests are reported based on the transformed data, while the equations are based on the raw (untransformed) data.

In the CPS prevalence modeling with dairy management practices, all the variables in Table VII were entered for multivariate analysis except membership in ADLIC and the addition of disinfectant to wash water because they were highly correlated with post-milking teat dipping ($r = 0.250$ and $r = 0.315$ respectively). The herd proportion receiving dry cow therapy was entered even though the variable was not significant in the univariate analysis. This was based on the importance of dry cow therapy in the control of CPS. The prevalence of CNS, farming experience, and the proportion of the milking herd treated for clinical mastitis were entered as possible confounders. Post-milking teat dipping (TDM) was significantly associated with low prevalence ($p = 0.02$). The number of times the milking system was checked per year (MSC) was marginally associated with high prevalence ($p = 0.18$). The final model describing CPS prevalence (CPS) ($p = 0.03$) was:

$$CPS = 17.84 - 11.99\ (TDM) + 1.69\ (MSC).$$

Together, these two variables accounted for 19% of the between herd variation in CPS prevalence. Residual plots and variance inflation factor showed no violation of regression assumptions.

In the CNS prevalence modeling with dairy management practices, all variables in Table VIII were entered for multivariate analysis. In addition, the herd proportion receiving dry cow therapy, and all pre- and post-milking udder hygiene

practices were tried although they had insignificant associations at the univariate level. The prevalence of CPS, farming experience, and the proportions of the milking herd treated for clinical mastitis and culled because of mastitis were entered as possible confounders. Freestall (FREEDRY) and tiestall (TIEDRY) housing for dry cows were significantly associated with low prevalence ($p = 0.00$ and 0.03 respectively) as opposed to loose housing of dry cows. High-line pipeline milking systems (HLPMSY) were significantly associated with low prevalence ($p = 0.02$) compared to bucket milking. Teat dipping before calving (TDBC) had a marginal association with low prevalence ($p = 0.06$). The final model describing CNS prevalence (CNS) ($p = 0.01$) was:

CNS = 38.8 - 15.1 (FREEDRY) - 7.92 (TIEDRY) - 8.25 (HLPMSY) - 6.14 (TDBC).

Together, these variables explained 32% of the between herd variation in CNS prevalence. Residual plots and variance inflation factor showed no violation of regression assumptions.

CPS prevalence had no association with any dry cow products used. However, CNS prevalence was associated with certain dry cow products. Dry Cow Product 2 (DCP 2) (Procaine Penicillin G and Novobiocin sodium) was significantly associated with low prevalence ($p = 0.01$), while Dry Cow Product 9 (DCP 9) (any product containing Novobiocin) was associated with high prevalence ($p = 0.09$). The proportion of the herd receiving dry cow therapy (DCT%) and the management factors model describing CNS prevalence did not confound this relationship. These

two variables described 20% of the between herd variation of CNS prevalence (CNS-DCP) ($p = 0.02$). The equation for this relationship was:

$$\text{CNS-DCP} = 22.9 - 12.1\ (\text{DCP 2}) + 5.14\ (\text{DCP 9}).$$

3.4 Discussion

The tube coagulase test was used to differentiate between CPS and CNS. A kappa of 0.8 between the results of classification in this study and the classification by Davidson (1990) was found. One reason for disagreement in the classification could be the result of non-specific reaction between the plasma and some component of the cells and / or the medium which then could give false positive results (Sperber, 1975). This however, was unlikely because this study used negative controls for every batch of rabbit plasma reconstituted. Disagreement could also be due to the fact that some of the cultures were found to be mixed cultures. Freezing has been shown to enhance isolation of intramammary pathogens (Villanueva et al, 1991). One of the mechanisms that was suggested would enhance the isolation is lysing of milk macrophages and neutrophils, releasing phagocytosed bacteria. Another possible reason for disagreement is the interpretation of the degree of clotting. Further, cultures that were coagulase negative after 4 hours of incubation were re-incubated overnight. This could decrease the specificity of the test in the present study for the identification of *Staphylococcus aureus* (CPS) (Hogan et al, 1986). It is not known whether negative cultures were re-incubated overnight in Davidson's (1990) study.

In any case, a kappa value of 0.8 is indicative of a high level of agreement (Martin et al, 1987) and therefore, the classification by the two studies is valid and can be depended upon.

Among the CPS, post-milking teat dipping was significantly associated with low prevalence when all dairy management practices were assessed, and explained most of the variation. However, the type of teat dip product used was not important in predicting the prevalence. It was also observed that farms that teat dipped, also added a disinfectant solution in wash water and were members of ADLIC. ADLIC farms are more likely to be aware of subclinical mastitis through the Somatic Cell Count (SCC) Program. This observation is in agreement with Dargent-Molina et al, (1988), Godkin, (1989), and Hutton et al, (1991) who reported teat dipping as a key management factor in the control of contagious mastitis. The number of times the milking machine was inspected although marginally significant ($p = 0.18$), was positively correlated with CPS prevalence. In PEI, problem herds (bulk tank SCC for 1 month > 200,000 cells/ml or 6-12 months rolling average > 200,000 cells/ml) are visited by the dairy technician or dealer to investigate the underlying cause. Whatever the underlying cause, if the mastitis problem did not resolve quickly enough, milking machines on these farms are bound to have been checked more times. This observation is in agreement with Godkin, (1989) who reported a higher mean prevalence of CPS among farms that had their equipment serviced more often.

The milking herd proportion culled because of mastitis was positively correlated with CPS prevalence in the univariate relationship ($p = 0.01$), but was not

significant in the final model. The significant univariate relationship implies that farms that culled more cows might have been experiencing more relapses. This was expected in this study because it was observed in Chapter Two that an annual culling rate of 8% due to mastitis was above the recommended maximum of 6% on farms with appropriate mastitis control programs (McDonald 1984).

The herd proportion receiving dry cow therapy (DCT%) was not associated with between farm variation in CPS prevalence. Further, no association with dry cow products was found. It is probable that the effect of dry cow therapy may have been nullified by the method of drying off. Only 19% of the farms surveyed dried off cows abruptly, the recommended procedure following infusion with dry cow therapy (Dodd, 1983). However, the method of drying off may not be crucial because a study by Natzke et al (1974) demonstrated that either method (abrupt cessation or intermittent milking) is satisfactory in treated cows. The effect of different dry cow products may have been masked by the fact that most farms used more than one product. This notwithstanding, it may mean that the dry cow products used did not differ in their efficacy against CPS IMI or that they had little or no effect on CPS IMI. Dargent-Molina et al, (1988) Godkin, (1989) and Hutton et al, (1991) in similar surveys found no protective effect with dry cow therapy against CPS. The results of this study contrast with Batra (1988) who reported a microbiologic cure rate of 94.1%. A possible reason for the contrast is the lack of a control group in Batra's (1988) study, in which case, spontaneous recovery can not be ruled out. Secondly, in the present study, the effect of dry cow therapy may have been cancelled out by

new infections during the lactating period. This is because milk samples were taken from all milking cows at different stages of the lactating period unlike in Batra's (1988) study in which milk samples were taken 24 hours after calving. The results of the present study are in partial agreement with the findings of Browning et al (1990) and Buddle et al (1987). Browning et al (1990) did not find any difference in new infection rate during the dry period and early lactation between uninfected cows receiving and not receiving dry cow therapy. The study also reported a significantly lower infection rate during the dry period in cows that had all quarters infused than in cows in which only the infected quarters were infused. This trend was reversed during the lactation period. Buddle et al (1987) reported that although dry cow therapy offered protection, cows infected in 3-4 quarters before drying off had a higher susceptibility to reinfection in subsequent lactation than in cows infected in two or fewer quarters. Cows infected in 1-2 quarters are more likely to undergo spontaneous recovery with less chances of reinfection than cows infected in 3-4 quarters. In the absence of an untreated control group(s), it is hard to tell whether the difference in the reinfection rates observed in the study under comparison was due to spontaneous recovery. Overall, it points to the fact that cows infected in 3-4 quarters are very susceptible to reinfection, and unless such cows are culled, they continue to serve as a source of infection in a herd. These studies indicate that herd dynamics of infection plays the key role and that therapeutic success measured at the herd level rather than quarter basis or individual cow level would be a more useful parameter to evaluate along with other preventative measures.

In the CNS model with dairy management practices, freestall and tiestall housing compared to loose housing for dry cows was associated with low CNS prevalence. The dry period is a high risk period for IMI. The ideal housing that minimizes IMI should be clean, dry, and reduce the chances for teat injury (Jarrett, 1984b). Jarrett (1984b) cites that cows housed in freestalls were cleaner, more gentle, and had fewer teat and udder injuries than cows in loose housing. High pipeline milking system was associated with low prevalence versus bucket milking. The higher prevalence among farms with bucket milking may be a reflection of another aspect of management on these farms that was not directly addressed by this study such as the use of barrier dips in the dry period.

Pre-calving teat dipping was marginally associated with low prevalence in the multivariate model. However, it was not significant in the univariate analysis ($p = 0.35$), an observation that is in agreement with Schultze (1985) and Mathews et al (1988). Thus, the importance of pre-calving teat dipping becomes evident after considering more important factors such as housing for dry cows. The pre-partum period is a high risk time for IMI especially with environmental pathogens but also for CNS which reside on skin surfaces.

Purchasing of replacement heifers in comparison with raising them on the farm had a marginal significant association with high CNS prevalence in the univariate analysis. Purchased heifers may have been infected on the farms of origin.

Post-milking teat dipping did not explain any significant variation in CNS prevalence and this is in agreement with Hogan et al, (1987) who observed that post-

milking teat dipping more effectively reduces IMI by bacteria transmitted to the teat end during milking than bacteria exposed to the teat end primarily during the inter-milking period.

Dry Cow Product 2 (procaine penicillin G and novobiocin sodium) was significantly associated with low prevalence of CNS, while Dry Cow Product 9 (any product containing Novobiocin) had a marginal association with high CNS prevalence. Since most farms used more than one dry cow product plus the fact that DCT% was not a significant predictor, makes it is hard to say whether these associations hold.

The conclusion that can be drawn from this study is that post-milking teat dipping is an efficient method for the control of IMI due to CPS from a management point of view. Good milking hygiene, in particular, the addition of disinfectant in wash water complements the role of teat dipping. Membership in a Somatic Cell Count Program makes farmers aware of subclinical mastitis. The role of dry cow therapy to eliminate existing infections and prevent new IMI due to CPS was not demonstrated. The results of this study confirm that CNS are skin inhabitants with a high risk of infection in the dry period. The prevalence of CNS therefore can be minimized by control methods that include clean and dry environment, pre-partum teat dipping and adequate milking hygiene.

4. A SURVEY OF ANTIMICROBIAL SENSITIVITY

4.1 Introduction

Antimicrobial agents are widely used in the treatment of mastitis. Antimicrobial resistance is one of several reasons for bacteriologic failures of antibiotic therapy of mastitis associated with gram-positive bacteria (Sandholm et al, 1990). Sensitivity data is useful in the design of treatment protocols for mastitis despite the shortcomings of the present *in vitro* methods of sensitivity testing. These shortcomings are due to the fact that the *in vitro* methods are based on extrapolations from serum drug concentrations in human beings, rather than intramammary concentrations in cows (Tyler et al, 1992: Ziv, 1992). Among coagulase positive *Staphylococci* (CPS), host and agent factors add to the uncertainty of *in vitro* antimicrobial sensitivity data in predicting therapeutic response. The host and agent factors include extensive fibrosis, abscessation, and the intracellular location of *S. aureus* during infection. Therefore, the choice of a product should depend on past clinical experience in the herd and the pattern of resistance to antibiotics (Ziv, 1992). It is not known if antimicrobial therapy of mastitis should be directed at CNS in addition to the major pathogens. In any case, information on antimicrobial resistance patterns of CNS can be useful if mastitis due to CNS is to be treated.

Most published reports on antimicrobial sensitivity of staphylococcal mastitis pathogens have dealt with the sensitivity profiles of coagulase positive *Staphylococci* (CPS) without including coagulase negative *Staphylococci* (CNS) (Bishop et al, 1980;

Davidson et al, 1982; Hinckley et al, 1985; Francis and Carroll, 1986; Mackie et al, 1988). However, some workers have published on both CPS and CNS (Owens and Watts, 1988; McDonald and Anderson, 1981; Trinidad et al, 1990).

The primary objective of this part of the study was to survey *in vitro* antimicrobial sensitivities of *Staphylococci* in a random sample of Prince Edward Island (PEI) dairy herds. Secondary objectives were to: 1) determine if some isolates exhibit multiple resistance, 2) determine if sensitivity patterns exist within farms, and 3) compare the proportions sensitive to each antimicrobial between CPS and CNS on the same farms.

4.2 Materials and methods

4.2.1 Sample collection

A random sample of 57 dairy farms out of 520 dairy farms in Prince Edward Island (PEI) was selected. Composite cow milk samples were collected from each milking cow for a total of 1,647 samples. The materials and methods have been described previously (Davidson, 1990). Briefly, all isolates of *Staphylococci* classified as coagulase positive *Staphylococci* (CPS) and a sub-sample representing 25% of other *Staphylococci* isolates classified as coagulase negative *Staphylococci* (CNS) were frozen on slants at -20°C in the Summer of 1989. Each sample was identified by farm, cow, and the date of collection. A total of 419 samples were stored. From the 419 samples, pure cultures were isolated and identified as CPS or CNS according to

the methods described in Chapter Three.

4.2.2 Antimicrobial sensitivity

Antimicrobial sensitivity testing was done on 195 and 178 pure cultures identified as CPS and CNS respectively (Chapter Three). Sensitivity testing was done by the disc diffusion method as described by Bauer et al (1966). The following antimicrobials[4] were used: Erythromycin 15μg, Penicillin G 10 I.U., Gentamicin10μg, Tetracycline 30 μg, Nitrofurans 300 μg, Sulphamethoxazole / Trimethoprim 25 μg, Neomycin 30 μg, Ampicillin 10 μg, Cloxacillin 5 μg, Novobiocin 30 μg, Cephalothin 30 μg, Amoxycillin clavulanic acid 30 μg, and Polymyxin B 300 μg.

A single colony was aseptically inoculated into 3.5 ml peptone water and incubated for four hours at room temperature. Growth at this time was compared to 0.5 McFarland standard. A cotton swab was used to inoculate the entire surface of petri dishes containing Mueller-Hinton agar. Antibiotic impregnated paper discs were placed on the agar using a disc applicator. The plates were incubated at 37°C for 14 hours. For every batch of *Staphylococci* tested, *S. aureus* of known sensitivity, American Type Culture Collection (ATCC) 29213 was tested as a control. Growth inhibition diameters were recorded in millimeters and classified as sensitive or resistant (Bauer et al, 1966).

[4] Oxoid Canada INC.

4.2.3 Statistical analysis

Data were entered and stored in dBase III Plus (Ashton Tate). Descriptive and analytic statistics were performed using Minitab Version 7.1 (Minitab, Inc.) and SAS / STAT™ Version 6 (SAS Institute Inc.,1987) respectively.

The within herd proportions sensitive to each antimicrobial for either CPS or CNS were determined by dividing the number of isolates sensitive by the total number of the respective pure isolates on a given farm.

Counts of isolates showing resistance to one or more antimicrobials were made. The proportions of farms with all the CPS or CNS sensitive to a varying number of antimicrobials were determined.

For each antimicrobial, CPS and CNS proportions sensitive were compared as a ratio using the overall Mantel-Haenszel (MH) chi square test with the farm as stratum and association estimated by the adjusted relative risk for stratified data (Kleinbaum et al, 1982). For each antimicrobial, the decision to use the overall Mantel-Haenszel chi square and risk ratio was determined based on the Breslow and Day chi square test for homogeneity of several risk ratios across strata (Kleinbaum et al, 1982) and the two-tailed sign test of the equality of the stratum specific risk ratios (H_0 median = 1 versus H_A median ≠ 1) (Conover, 1980). The Breslow and Day chi square indicated the presence of significant interaction between farm and the ratio of sensitivities, while the sign test indicated the direction of the interaction where applicable. For each antimicobial, the overall MH chi square test and relative risk were used if the test of homogeneity was not significant at the 0.05 level.

Comparisons of sensitivity ratio whose stratum specific risk ratios had a significant tendency to one direction (as indicated by the sign test) were reported as such. Comparisons of sensitivity ratio not falling in either of the foregoing categories were considered to have significant opposing interaction between farm and proportion sensitive. The MH chi square test and adjusted relative risk were not appropriate for such antimicrobials.

4.3 Results

The within herd proportions sensitive to each antimicrobial among the CPS were greater 91% for all the antimicrobials tested except ampicillin (58%), penicillin G (59%), polymyxin B (8%), and neomycin (86%). Antimicrobial sensitivities with standard deviations within farms greater than 5% were: ampicillin (40%), erythromycin (23%), neomycin (21%), penicillin G (39%), polymyxin B (19%), sulphamethoxazole / trimethoprim (6%), and tetracycline (26%) (Table IX). The within herd proportions sensitive to each antimicrobial among the CNS were greater 91% for all antimicrobials tested except ampicillin (62%), tetracycline (88%), penicillin (60%), sulphamethoxazole / trimethoprim (87%), and polymyxin B (78%). Antimicrobial sensitivities with standard deviations within farms greater than 5% were: ampicillin (33%), tetracycline (22%), penicillin (33%), sulphamethoxazole / trimethoprim (19%), polymyxin B (29%), erythromycin (19%), and nitrofurantoin (10%) (Table X).

TABLE IX. WITHIN HERD ANTIMICROBIAL SENSITIVITY OF 195 CPS ISOLATES ON 44 RANDOMLY SELECTED PEI DAIRY HERDS

Antimicrobial	% sensitive	Standard deviation (SD)	Range
Amoxycillin clavulanic acid	100		
Ampicillin	58	40	0-100
Cephalothin	99	5	67-100
Cloxacillin	100		
Erythromycin	92	23	0-100
Gentamicin	99	4	75-100
Neomycin	86	21	33-100
Nitrofurantoin	99	1	95-100
Novobiocin	100		
Penicillin G	59	39	0-100
Polymyxin B	8	19	0-100
Sulphamethoxazole / Trimethoprim	99	6	67-100
Tetracycline	92	26	0-100

TABLE X. WITHIN HERD ANTIMICROBIAL SENSITIVITY OF 178 CNS ISOLATES ON 50 RANDOMLY SELECTED PEI DAIRY HERDS

Antimicrobial	% sensitive	Standard deviation (SD)	Range
Amoxycillin clavulanic acid	99	3	80-100
Ampicillin	62	33	0-100
Cephalothin	99	3	80-100
Cloxacillin	98	5	80-100
Erythromycin	92	19	0-100
Gentamicin	99	5	68-100
Neomycin	100		
Nitrofurantoin	96	10	50-100
Novobiocin	99	5	67-100
Penicillin G	60	33	0-100
Polymyxin B	78	29	0-100
Sulphamethoxazole / Trimethoprim	87	19	40-100
Tetracycline	88	22	0-100

Figure 13 shows the proportions (%) of *Staphylococcal* isolates, both CPS and CNS, sensitive to a varying number of antimicrobials. Among the CPS, only 8% of the total isolates were sensitive to all the antimicrobials. Forty percent of the isolates were sensitive to 12 antimicrobials, 13% to 11 antimicrobials, 27% to 10 antimicrobials, 10% to 9 antimicrobials, and 2% to 8 antimicrobials.

Among the CNS, 32% of the total isolates were sensitive to all the antimicrobials. Twenty one percent of the isolates were sensitive to 12 antimicrobials, 24% to 11 antimicrobials, 16% to 10 antimicrobials, 5% to 9 antimicrobials, 1% to 8 antimicrobials, and 1% to 7 antimicrobials.

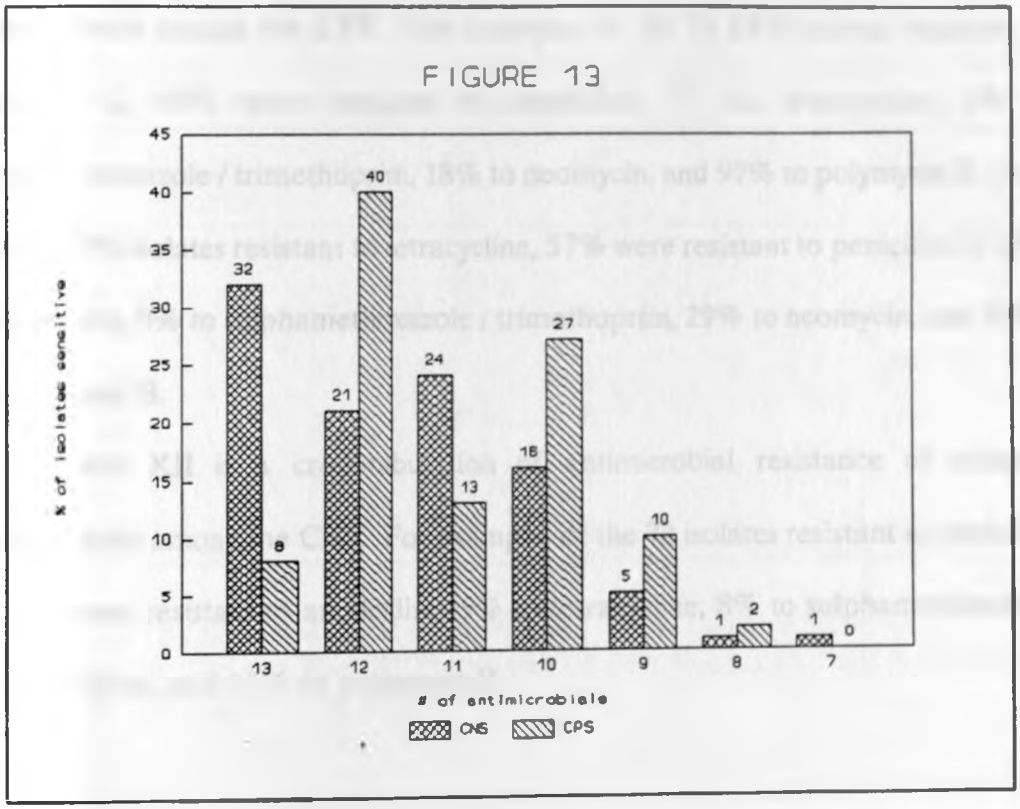

Figure 13. The proportions of CPS and CNS sensitive to a varying number of antimicrobials on a random sample of PEI dairy herds.
Number of herds: CPS = 44, CNS = 50.
Total number of isolates: CPS = 195, CNS = 178.

Table XI is a cross-tabulation of antimicrobial resistance of selected antimicrobials among the CPS. For example, of the 76 CPS isolates resistant to penicillin G, 99% were resistant to ampicillin, 5% to tetracycline, 1% to sulphamethoxazole / trimethoprim, 18% to neomycin, and 97% to polymyxin B. And of the 7 CPS isolates resistant to tetracycline, 57% were resistant to penicillin G, 57% to ampicillin, 0% to sulphamethoxazole / trimethoprim, 29% to neomycin, and 100% to polymyxin B.

Table XII is a cross-tabulation of antimicrobial resistance of selected antimicrobials among the CNS. For example, of the 72 isolates resistant to penicillin G, 89% were resistant to ampicillin, 19% to tetracycline, 8% to sulphamethoxazole / trimethoprim, and 35% to polymyxin B.

TABLE XI. SELECTED ANTIMICROBIALS WITH COMMON RESISTANT ISOLATES AMONG THE CPS FROM THE ORIGINAL 195 ISOLATES ON 44 RANDOMLY SELECTED PEI DAIRY HERDS.

Antimicrobial	Number resistant*	PG**	A**	T**	S/T**	N**	PB**
PG	76	-	99	5	1	18	97
A	77	97	-	5	1	18	97
T	7	57	57	-	0	29	100
S/T	6	17	17	0	-	17	83
N	32	44	44	6	3	-	100
PB	175	42	43	4	3	18	-

* = The total number of CPS isolates resistant to the row antimicrobial
** = Proportion (%) of CPS isolates resistant to the row antimicrobial that were also resistant to the column antimicrobial

Key:
PG = Penicillin G
A = Ampicillin
T = Tetracycline
S/T = Sulphamethoxazole / Trimethoprim
N = Neomycin
PB = Polymyxin B

TABLE XII. SELECTED ANTIMICROBIALS WITH COMMON RESISTANT ISOLATES AMONG THE CNS FROM THE ORIGINAL 178 ISOLATES ON 50 RANDOMLY SELECTED PEI DAIRY HERDS.

Antimicrobial	Number resistant	PG**	A**	T**	S/T**	PB**
PG	72	-	89	19	8	35
A	66	97	-	17	8	36
T	23	48	48	-	9	17
S/T	24	25	21	8	-	17
PB	39	64	62	10	10	-

* = The total number of CNS isolates resistant to the row antimicrobial
** = Proportion (%) of CNS isolates resistant to the row antimicrobial that were also resistant to the column antimicrobial

Key:
PG = Penicillin G
A = Ampicillin
T = Tetracycline
S/T = Sulphamethoxazole / Trimethoprim
PB = Polymyxin B

Figure 14 shows the proportions of farms with all CPS or CNS isolates sensitive to a varying number of antimicrobials. The number of antimicrobials to which all CPS isolates on a farm were sensitive varied from 7 to 13. On 2% of the farms, all the CPS isolates were sensitive to all the 13 antimicrobials tested and, on 23% of the farms all CPS were sensitive to 12 antimicrobials. On 27% of the farms, all the CPS were sensitive to 10 antimicrobials. On 2% of the farms all the CPS isolates were sensitive to only 7 antimicrobials.

There was a wider variation in the number of antimicrobials to which all the CNS on a farm were sensitive (5 to 13). On 10% of the farms all the CNS isolates were sensitive to all 13 antimicrobials tested. On 4% of the farms, all the CNS were sensitive to only 5 of the antimicrobials. On 24% of the farms, all the CNS were sensitive to 11 antimicrobials.

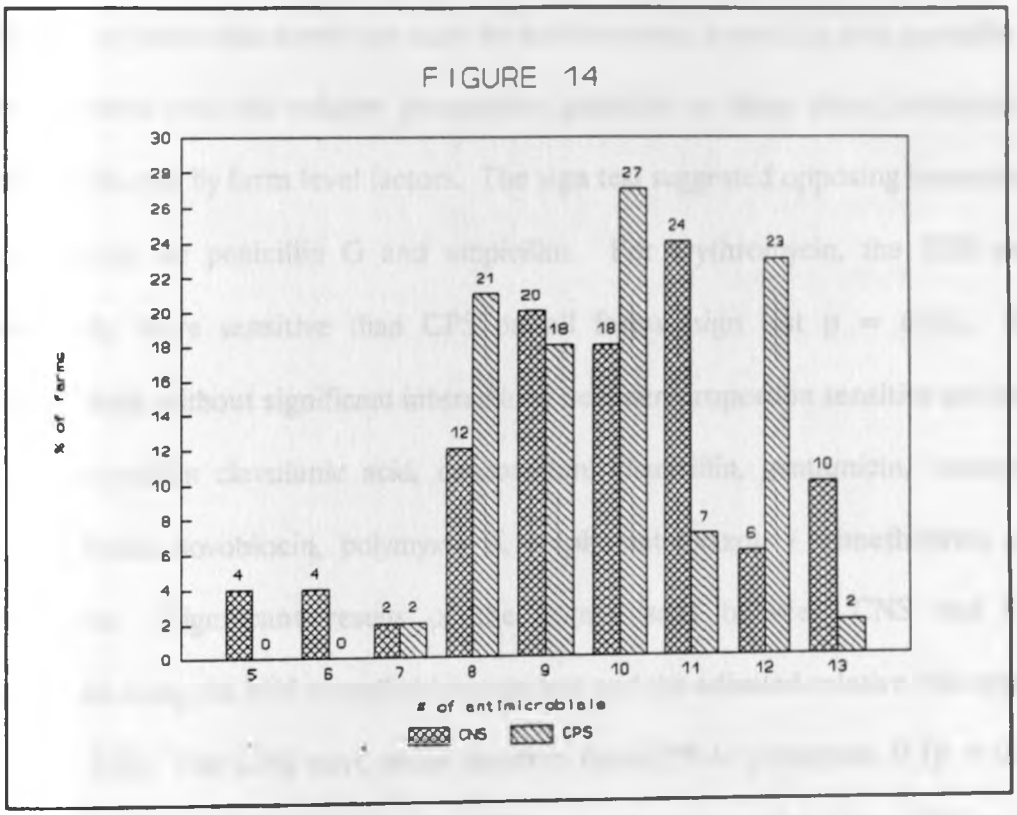

Figure 14. The proportions of farms with all CPS or CNS isolates sensitive to a varying number of antimicrobials on a random sample of PEI dairy herds.

Breslow and Day homogeneity test showed significant interaction between farm and antimicrobial sensitivity ratio for erythromycin, ampicillin, and penicillin G. This indicates that the relative proportions sensitive to these three antibiotics is strongly affected by farm level factors. The sign test suggested opposing interactions across farms for penicillin G and ampicillin. For erythromycin, the CNS were consistently more sensitive than CPS on all farms (sign test $p = 0.00$). The antimicrobials without significant interactions between proportion sensitive and farm were: amoxycillin clavulanic acid, cephalothin, cloxacillin, gentamicin, neomycin, nitrofurantoin, novobiocin, polymyxin B, sulphamethoxazole / trimethoprim, and tetracycline. Significant results of the comparisons between CNS and CPS sensitivities using the MH overall chi square test and the adjusted relative risk appear in Table XIII. The CNS were more sensitive than CPS to polymyxin B ($p = 0.00$) and neomycin ($p = 0.00$), while the CPS were more sensitive than CNS to the following antimicrobials: nitrofurantoin ($p = 0.05$), tetracycline ($p = 0.02$), and sulphamethoxazole / trimethoprim ($p = 0.02$). The overall MH chi square test suggested no significant difference between CNS and CPS sensitivities to gentamicin, cephalothin, amoxycillin clavulanic acid, novobiocin, and cloxacillin.

TABLE XIII. COMPARISONS BETWEEN CNS AND CPS PROPORTIONS SENSITIVE WITHIN FARMS ON A RANDOM SAMPLE OF PEI DAIRY HERDS

Antimicrobial	MH chi square (DF = 1)	Probability	Overall risk ratio CNS/CPS (95% C.I)
Nitrofurantoin	3.91	0.05	0.87 (0.76, 1.00)
Tetracycline	5.60	0.02	0.83 (0.72, 0.97)
Sulphameth-oxazole / Trimethoprim	5.82	0.02	0.83 (0.72, 0.97)
Polymyxin B	109.30	0.00	6.37 (4.50, 9.01)
Neomycin	20.06	0.00	1.45 (1.23, 1.71)

4.4 Discussion

CNS and CPS sensitivity approached 100% for 3 of 5 beta lactams, namely, amoxycillin clavulanic acid, cephalothin, and cloxacillin. The mean CNS and CPS sensitivity to penicillin G and ampicillin was 60%. Sensitivities approaching 100% for both CNS and CPS among the beta lactams other than penicillin G and ampicillin have been reported by other workers (Bishop et al, 1980; McDonald and Anderson, 1981; Schultze, 1983). Sensitivities to penicillin G and ampicillin have been reported to range from 23% to 59.6% (Schultze, 1983; McDonald and Anderson, 1981). The contrast among studies in sensitivity to penicillin G and ampicillin may reflect the variation in beta-lactamase production between geographical locations (Tyler, 1992). Penicillin G and ampicillin are natural penicillins and are therefore susceptible to beta lactamase (Papich, 1987).

Sensitivity to novobiocin among both CPS and CNS approached 100%. Other studies have reported similarly high sensitivities for both CNS and CPS (Owens and Watts, 1988; Carroll and Francis, 1986; Mackie et al, 1988; Schultze, 1983).

Among the aminoglycosides tested in this study, sensitivity to gentamicin approached 100% among both CNS and CPS. Neomycin had 100% sensitivity among CNS but only 83.16% among CPS. Similarly high sensitivities among both CPS and CNS to gentamicin have been reported (McDonald and Anderson, 1981; Owens and Watts, 1988; Schultze, 1983). Low sensitivities to neomycin among the CPS in comparison with CNS have been reported by Schultze, (1983) 86% for CPS versus 97% for CNS.

Sensitivity to tetracycline was 92% and 88% among CPS and CNS respectively. Comparable results (86%-96%) among the CPS have been reported (Schultze, 1983; MacDonald and Anderson, 1981; Francis and Carroll, 1986; Mackie et al 1988). Among the CNS, comparable results, 98% and 82% have been reported by Schultze, (1983) and McDonald and Anderson, (1981) respectively.

CNS sensitivity to polymyxin B was 78% and CPS sensitivity was 8%. Similar differences in sensitivity have been reported by Schultze, (1983) 84% versus 25% and by McDonald and Anderson, (1981) 98.1% versus 0.7% among CNS and CPS respectively.

Erythromycin, the only macrolide tested in this study, had equal sensitivity among the CPS and CNS (92%). Comparable results among both the CPS and CNS (90%-97.7%) have been reported (Schultze, 1983; McDonald and Anderson, 1981; Owens and Watts, 1988).

Sensitivity to sulphamethoxazole/ trimethoprim was 99% and 87% among CPS and CNS respectively. Owens and Watts, (1988) reported 100% sensitivity for both groups. Sensitivity to nitrofurantoin was 99% and 96% among CPS and CNS respectively.

Multiresistance was observed among both CPS and CNS. Resistance to 3-6 antimicrobials is quite common (Timoney et al, 1988). In Tables X and XI it is seen that the probability of either CPS or CNS being resistant to both penicillin G and ampicillin is high ($\geq$ 89%). This suggests penicillinase production to which both the natural penicillins, penicillin G and ampicillin are susceptible (Papich, 1987). The

probability is low between penicillin G or ampicillin and neomycin (Tables X and XI), which suggests different resistance mechanisms. Survey data on the types of dry cow products used (chapter 2) indicate that most farms used more than 2 different dry cow products. This, together with other management practices, may have led to the creation of multiresistance (Levy, 1991).

There were differences in sensitivity between farms among both CPS and CNS (Figure 14). Sensitivity for all isolates on a given farm varied from 7 to 13 antimicrobials among the CPS and 5 to 13 antimicrobials among the CNS. Furthermore, the variation between farms was evident even within groups of farms classified according to the number of antimicrobials all the CPS or CNS isolates were sensitive to. For example, on the 27% of farms where all CPS isolates were sensitive to 10 antimicrobials, the type of antimicrobials varied across farms.

The proportions of CPS sensitive to nitrofurantoin, tetracycline, and sulphamethoxazole / trimethoprim were significantly higher than CNS, while the proportions of CNS sensitive to polymyxin B and neomycin were significantly higher than CPS. The differences in sensitivity between the 2 *Staphylococci* groups are probably due to intrinsic properties inherent in each group. The proportions sensitive to ampicillin, penicillin G, and erythromycin were strongly affected by farm level factors. The proportion of CNS sensitive to erythromycin was consistently higher than CPS on all farms. There were no significant differences between CPS and CNS proportions sensitive to the other antimicrobials (amoxycillin clavulanic acid, cephalothin, cloxacillin, gentamicin, and novobiocin).

In summary, sensitivity among CPS was over 91% to all the antimicrobials tested except Penicillin G (59), ampicillin (58), neomycin (86%), and polymyxin B (8%). These 4 antimicrobials also had wide variations in within farm sensitivity. Multiresistance was evident among the CPS, with 39% of the total isolates being resistant to at least 3 antimicrobials. Among the CNS, sensitivity was over 91% for all the antimicrobials except penicillin G (60%), ampicillin (62%), polymyxin B (78%), tetracycline (88%), and sulphamethoxazole / trimethoprim (87%). These 5 antimicrobials also had wide variations in within herd sensitivity. Twenty three percent of the total CNS isolates were resistant to at least 3 antimicrobials. Between farm variation in sensitivity was evident among both the CPS and CNS. The CPS were significantly more sensitive to nitrofurantoin, sulphamethoxazole/ trimethoprim, and tetracycline than CNS, while CNS were significantly more sensitive to polymyxin B and neomycin. The proportions sensitive to ampicillin, penicillin G, and erythromycin were strongly affected by farm level factors.

In the absence of sensitivity data in a herd, results of this study indicate that none of penicillin G, ampicillin, neomycin, or polymyxin B would be the logical choice for treatment of CPS intramammary infection. Of the remaining 9 antimicrobials (amoxycillin clavulanic acid, cephalothin, cloxacillin, erythromycin, gentamicin, nitrofurantoin, novobiocin, suphamethoxazole / trimethoprim, and tetracycline) the choice would depend on whether the antimicrobial is biologically appropriate in respect of distribution, safety, and residue potential. For example, gentamicin would not be appropriate because of poor distribution in the udder (Ziv, 1992). This

information used together with past clinical experience on a given farm can aid in the selection of an antimicrobial for the treatment of both clinical and subclinical mastitis due to CPS.

If antimicrobial therapy of mastitis should be directed at CNS in addition to CPS, these data indicate that the choice of an ideal antimicrobial for the treatment of staphylococcal mastitis on a given farm should meet the following requirements in order of importance: 1) have a high *in vitro* sensitivity for CPS in a previous study in the geographical location, 2) be biologically appropriate choice in respect of distribution, safety, and residue potential, 3) have an effective past clinical experience with CPS intramammary infections on a given farm, and 4) have a high *in vitro* sensitivity for CNS in a previous study in the geographical location. In this study for example, assuming an effective past clinical experience with CPS, the ideal antimicrobial can be chosen from among antimicrobials whose sensitivity for both CPS and CNS approached 100%. Such antimicrobials include amoxycillin clavulanic acid, erythromycin, novobiocin, cephalothin, and cloxacillin. These antimicrobials also have good to fair potential distribution throughout the udder (Ziv, 1992).

5. THE RELATIONSHIP BETWEEN ANTIMICROBIAL SENSITIVITY AND DAIRY MANAGEMENT PRACTICES AND THE CORRELATION WITH PREVALENCE OF STAPHYLOCOCCAL MASTITIS PATHOGENS

5.1 Introduction

Resistance, and in particular multiresistance, propagates where antibiotics are overused and poor hygiene is practised (Levy, 1991). It is therefore reasonable to speculate that antimicrobial sensitivity or resistance among staphylococcal mastitis pathogens, both coagulase positive *Staphylococci* (CPS) and coagulase negative *Staphylococci* (CNS) depend on the dairy management practices peculiar to a given farm. Further, it is speculated that antimicrobial sensitivity or resistance and prevalence are correlated after adjusting for dairy management factors.

The objectives of this part of the study were: 1) to assess which dairy management factors affect the sensitivity of staphylococcal mastitis pathogens (CPS and CNS); and 2) to determine if significant correlations exist between the prevalence of staphylococcal mastitis pathogens (CPS and CNS) and their sensitivity to different antimicrobials after controlling for dairy management factors.

5.2 Materials and methods

The frequency of adoption of management factors that control mastitis were described in Chapter 2, prevalence and antimicrobial sensitivities were determined in Chapters 3 and 4, respectively.

5.2.1 Statistical analysis

Statistical analyses were performed using Minitab Version 7.1 (Minitab, Inc.) and SAS / STAT™ Version 6 (SAS Institute Inc., 1987).

For the first objective, weighted least squares multiple regression was used to relate sensitivities to farm management factors. The proportions of CPS or CNS sensitive to different antimicrobials were the dependent variables. Only antimicrobials with within herd sensitivity SD greater than 5% (Chapter 4) that were used on the study farms (Chapter 2) were candidates. These were penicillin G and tetracycline. The arcsine transformations of the proportions of CPS and CNS sensitive were used to stabilize the variance of these proportions (Snedecor and Cochran, 1989). The weighting factor was the number of CPS or CNS samples tested per farm (variance of a proportion estimate is p(1-p)/n, where n is the number of samples tested per farm that were either CPS, mean 4, range 1 to 21 or CNS, mean 4, range 1 to 10). This gave more emphasis to farms with more samples and therefore, with less variation in sensitivity estimates (Draper and Smith, 1981). The independent variables were management factors and, separately, the dry cow products used at drying off. Management factors and dry cow products that were entered in the multivariate modeling in the relationship with prevalence (Chapter 3) were candidates for the relationship with antimicrobial sensitivity. In the relationship involving dry cow products and antimicrobial sensitivity, the herd proportion receiving dry cow therapy and the model describing sensitivity among management factors were

entered as possible confounders. A forward entry procedure was used in the multivariate analysis. The variable with the highest correlation with the dependent variable was entered first. Subsequent entry depended upon a variable having the highest correlation with the dependent variable at each step of model building ($p = 0.25$) (Kleinbaum et al, 1988). Overall model significance was assessed at $p = 0.05$. A variable was dropped from the equation if it exhibited a significance level greater than 0.25. No interactions were considered in the analyses because of small sample size. Plots of residuals on predicted values and probability scores were done for the final model to assess normality and linearity respectively, while collinearity was assessed using variance inflation factor (Kleinbaum et al, 1988).

The correlation between the prevalence of staphylococcal mastitis pathogens and antimicrobial sensitivity after controlling for dairy management factors was measured by the Pearson partial correlation coefficient, the estimate of the population partial correlation coefficient (Kleinbaum et al, 1988). The partial correlation coefficient was determined using CORR procedure in SAS / STAT™. CPS and CNS sensitivities to penicillin G and tetracycline were the assumed predictor candidates. The two antimicrobials were chosen for the same reasons as given in the first objective, above. The arcsine transformations of CPS and CNS prevalence estimates were the assumed dependent variables. The transformation was used to stabilize the variance of these proportions (Snedecor and Cochran, 1989). Dairy management factors controlled for were those that predicted prevalence (Chapter 3) and or antimicrobial sensitivity (objective one above).

5.3 Results

Significance tests were based on the transformed data, while the coefficients reprinted in the equations are based on the raw (untransformed) data.

Management factors associated with CPS sensitivity to penicillin G were: post-milking teat dipping (TDM) ($p = 0.02$), the milking herd proportion culled because of mastitis (CULL%) ($p = 0.15$), and years of farming experience (YEARS) ($p = 0.14$). Together, these variables explained 36% ($p = 0.01$) of the between herd variation in the arcsine CPS sensitivity to penicillin G (CPS-PEN) according to the equation:

CPS-PEN = 47.66 + 38.13 (TDM) - 1.23 (CULL%) - 0.85 (YEARS).

Management factors associated with CPS sensitivity to tetracycline were: post-milking teat dipping (TDM) ($p = 0.01$), the milking herd proportion treated for clinical mastitis (TREAT%) ($p = 0.01$), years of farming experience (YEARS) ($p = 0.07$), and the milking herd proportion culled because of mastitis (CULL%) ($p = 0.08$). Together, these variables explained 43% ($p = 0.01$) of the between herd variation in the arcsine CPS sensitivity to tetracycline (CPS-TETRA) according to the equation:

CPS-TETRA = 106.13 + 15.56 (TDM) - 0.94 (TREAT%) - 0.45 (YEARS) - 0.99 (CULL%).

Management factors associated with CNS sensitivity to penicillin G were: post-milking teat dipping (TDM) ($p = 0.09$), the herd proportion receiving dry cow therapy (DCT%) ($p = 0.03$), and dipping the milking cluster between cows

(CLUSTER) ($p = 0.10$). Together, these variables explained 29% ($p = 0.02$) of the between herd variation in the arcsine CNS sensitivity to penicillin G (CNS-PEN) according to the equation:

$$\text{CNS-PEN} = 24.85 + 21.73\ (\text{TDM}) + 0.21\ (\text{DCT\%}) + 11.73\ (\text{CLUSTER}).$$

Management factors associated with CNS sensitivity to tetracycline were: the milking herd proportion treated for clinical mastitis (TREAT%) ($p = 0.01$), the herd proportion receiving dry cow therapy (DCT%) ($p = 0.09$), and the milking herd proportion culled because of mastitis (CULL%) ($p = 0.08$). Together, these variables explained 30% ($p = 0.01$) of the between herd variation in the arcsine CNS sensitivity to tetracycline (CNS-TETRA) according to the equation:

$$\text{CNS-TETRA} = 102.90 - 0.71\ (\text{TREAT\%}) - 0.11\ (\text{DCT\%}) + 0.70\ (\text{CULL\%}).$$

There were significant associations between dry cow products used and CNS sensitivity to penicillin G and tetracycline but not CPS sensitivity. Herds using DCP3 (procaine penicillin G, novobiocin sodium, and dihydrostreptomycin sulphate) had lower CNS sensitivity to penicillin G ($p = 0.02$). The herd proportion receiving dry cow therapy (DCT%) and the model describing CNS sensitivity to penicillin G among management practices were not confounders for the relationship. Thus, DCP3 solely explained 18% of the between herd variation in the arcsine CNS sensitivity to penicillin G (CNS-PEN-DCP) according to the equation:

$$\text{CNS-PEN-DCP} = 67.5 - 24.6\ (\text{DCP3})$$

Herds using DCP2 (procaine penicillin G and novobiocin sodium) and DCP9 (any product containing novobiocin) had lower CNS sensitivity to tetracycline, $p =$

0.03 and p = 0.04, respectively. The herd proportion receiving dry cow therapy and the model describing CNS sensitivity to tetracycline among management practices were not confounders for the relationship. The two dry cow products explained 35% of the between herd variation in the arcsine CNS sensitivity to tetracycline (CNS-TETRA-DCP) (p = 0.00) according to the equation:

CNS-TETRA-DCP = 94.23 - 22.22 (DCP2) - 10.90 (DCP9).

The Pearson partial correlation between the proportion of CPS sensitive to tetracycline and prevalence of CPS (0.49) was significant (p = 0.02). Thus, after controlling for management factors, 24% of the variation in CPS prevalence was explained by the variation in CPS sensitivity to tetracycline. The variables controlled for were: post-milking teat dipping, the number of times the milking machine was checked, years of farming experience, the milking herd proportion treated for clinical mastitis, and the milking herd proportion culled because of mastitis. A plot of CPS prevalence (arcsine transformed) on the proportion of CPS sensitive to tetracycline indicated that a few extreme data points may have exerted undue influence on the correlation coefficient (Cody and Smith, 1991). Therefore, the Spearman's partial rank-order correlation, a nonparametric test was applied. The Spearman's partial correlation (0.07) was not significant (p = 0.77).

The Pearson partial correlation between CPS sensitivity to penicillin G and CPS prevalence (0.06) was not significant (p = 0.77). The variables controlled for were: post-milking teat dipping, the number of times the milking machine was checked, years of farming experience, and the milking herd proportion culled because

of mastitis.

The Pearson partial correlation between CNS sensitivity to penicillin G and CNS prevalence (-0.21) was not significant ($p = 0.35$). The variables controlled for were: post-milking teat dipping, dipping the milking cluster between cows, high-line pipeline milking system versus bucket milking system, pre-calving teat dipping, free or tie stalls versus loose housing for dry cows, and the herd proportion receiving dry cow therapy.

The Pearson partial correlation between CNS sensitivity to tetracycline and CNS prevalence (0.15) was not significant ($p = 0.50$). The variables controlled for were: high-line pipeline milking system versus bucket milking system, pre-calving teat dipping, free or tie stalls versus loose housing for dry cows, the herd proportion receiving dry cow therapy, the milking herd proportion treated for clinical mastitis, and the milking herd proportion culled because of mastitis.

5.4 Discussion

Post-milking teat dipping was significantly associated with high CPS sensitivity to both penicillin G and tetracycline. This, in effect, means that post-milking teat dipping is effective in preventing the emergence and spread of resistance among CPS. Post-milking teat dipping was marginally associated with higher CNS sensitivity to penicillin G, but was not associated with CNS sensitivity to tetracycline. The marginal and lack of significant relationships for the two antibiotics respectively and post-

milking teat dipping can be explained in that post-milking teat dipping more effectively reduces bacteria transmitted to the teat end during milking (CPS) than bacteria exposed to the teat end primarily during the inter-milking period (CNS) (Hogan et al, 1987).

The herd proportion receiving dry cow therapy did not explain the between farm variation in CPS sensitivity to penicillin G or tetracycline. Further, the dry cow products used did not explain the variation in CPS sensitivity to penicillin G or tetracycline. This implies that dry cow therapy is not responsible for the creation of resistance to these antibiotics among the CPS. The herd proportion receiving dry cow therapy had a significant positive correlation with CNS sensitivity to penicillin G. The significant positive correlation may mean that other dry cow products that are used on the farms readily clear strains that are resistant to penicillin G. The herd proportion receiving dry cow therapy had a weak negative correlation with CNS sensitivity to tetracycline. A possible explanation is that dry cow therapy is responsible for the creation of resistance and that other dry cow products used on the farms do not clear strains of CNS that are resistant to tetracycline.

The milking herd proportion treated for clinical mastitis had significant negative correlations with CPS and CNS sensitivities to tetracycline. This means that organisms more frequently exposed to antibiotics are more likely to develop resistance.

The milking herd proportion culled because of mastitis had a weak negative correlation with CPS sensitivity to penicillin G and, weak positive correlations with

CPS and CNS sensitivities to tetracycline. This may be explained in that the number of cows culled on these farms was determined by treatment failure, probably due to resistance to these antibiotics among others. The sign of the correlation depends on other predictor management factors peculiar to penicillin G or tetracycline sensitivity respectively.

In the relationship between CNS sensitivity and dry cow products used, the use of DCP3 (procaine penicillin G, novobiocin sodium, and dihydrostreptomycin sulphate) was significantly associated with low sensitivity to penicillin G. The use of DCP2 (procaine penicillin G and novobiocin sodium) or DCP9 (any product containing novobiocin) was significantly associated with low CNS sensitivity to tetracycline. The exact nature of these relationships cannot be speculated upon. This is because most farms used more than one dry cow product. Also the use of dry cow products is aimed at intramammary infections (IMI) caused by CPS and not CNS. It is not reasonable therefore to argue that farms turned to products containing novobiocin (sensitivity to novobiocin was 100%) after they experienced therapeutic failure using either penicillin G or tetracycline. This follows from the fact that CPS sensitivity to these antimicrobials (penicillin G and tetracycline) was not explained by the use of either DCP2, DCP3 or DCP9.

There was no significant correlation between either CPS or CNS sensitivity and the prevalence of *Staphylococcal* mastitis pathogens after controlling for management factors. Thus, after accounting for the variation in prevalence and sensitivity due to management factors, antimicrobial sensitivity and prevalence for these 2 antibiotics

are independent of each other.

In summary, it was found that post-milking teat dipping, a practice that is conducive to low prevalence of CPS, prevents the emergence and spread of resistance among CPS. CPS and CNS organisms more frequently exposed to antibiotics through clinical treatment are more likely to develop resistance. The study did not demonstrate the role of dry cow therapy in the creation of resistance among CPS. It is probable that CNS resistance to penicillin G and tetracycline is in part the result of some dry cow products used. Yet, other dry cow products used readily clear the CNS that are resistant to penicillin G but not to tetracycline.

When resistance was present, neither CPS nor CNS sensitivities had any correlation with prevalence. In effect, this means that antimicrobial resistance has not compromised the control of mastitis due to staphylococcal pathogens.

6. SUMMARY AND CONCLUSIONS

This study undertook to describe the frequency of adoption of management factors that control mastitis, to survey *in vitro* antimicrobial sensitivity of staphylococcal mastitis pathogens (both coagulase positive *Staphylococcus* (CPS) and coagulase negative *Staphylococcus* (CNS)), to assess the effect of management factors on the prevalence of CPS and CNS and, on their *in vitro* antimicrobial sensitivity in a random sample of Prince Edward Island (PEI) dairy herds. The study also undertook to determine if significant correlations exist between the prevalence of CPS or CNS and their *in vitro* antimicrobial sensitivity in the same herds.

Information on the frequency of adoption of management factors that control mastitis was sought through a mailout questionnaire which realized a response rate of 77%. Descriptive statistics demonstrated that the study population was representative of the provincial dairy industry.

The majority of the farms surveyed practised good pre-milking hygiene. Thus, the desire to work in a clean environment and yield an unadulterated product is shared by many. However, the Provincial Dairy Act Regulations in PEI that make udder washing and the use of disposable paper towels mandatory may have biased this observation.

The adoption rates for post-milking teat dipping and dry cow therapy for all cows were only 79% and 43% respectively despite their importance in the control of contagious mastitis. These modest adoption rates indicate unawareness about the actual role of these two practices or may imply more varied perceptions.

Farmers who are not fully aware of the importance of mastitis and how each specific control procedure helps mitigate this disease are not likely to achieve maximum benefits.

Among dry cow products used, 78% of the farms used products containing beta lactams, and 43% of these contained penicillin G. Among products used singly, cloxacillin benthazine was the most widely used (48%) followed by oxytetracycline (32%).

Post-milking teat dipping, a practice that minimizes residual contamination and colonization of teats was significantly associated with low prevalence of CPS. This practice explained most of the between herd variation in CPS prevalence when all management factors were simultaneously assessed. Good milking hygiene, in particular, the addition of disinfectant to wash water, probably complemented the role of teat dipping. Membership in a somatic cell program may have made farmers aware of subclinical mastitis due to CPS. Farms that had their milking machines checked more often had a marginal association with high prevalence of CPS. It is probable that high bulk tank SCC led to more frequent checks of milking machines on these farms. The milking herd proportion culled because of mastitis was directly proportional to the prevalence of CPS. The study demonstrated the importance of control measures that minimize residual contamination and colonization of teats and measures that prevent cow-to-cow and quarter-to-quarter spread in the control of contagious mastitis caused by CPS. Culling as a measure to eliminate the source of infection in a herd may play a supporting role if done on time. The study did not demonstrate the role of dry cow therapy, a measure that cures existing infections and, additionally,

prevents new infections during the early part of the dry period. The failure to demonstrate the role of dry cow therapy may in part be due to the use of gradual drying off procedure, as opposed to abrupt on a majority of farms.

Dry period management practices were associated with prevalence of CNS. Freestall and tiestall compared to loose housing for dry cows were associated with low prevalence of CNS. This means that tiestalls and freestalls are cleaner, drier, and with less chances of teat injury in comparison with loose housing. Pre-calving teat dipping was associated with low prevalence of CNS. This supports the observation that the dry period is a high risk time for intramammary infections (IMI) with environmental mastitis pathogens including the CNS which reside on skin surfaces. Post-milking teat dipping had no association with CNS prevalence. Earlier research (Hogan et al, 1987) has demonstrated that post-milking teat dipping more effectively reduces IMI by bacteria transmitted to the teat end during milking than bacteria exposed to the teat end primarily during the inter-milking period. Although the herd proportion receiving dry cow therapy did not explain the between herd variation in CNS prevalence, the dry cow products used did.

CNS are skin inhabitants with a high risk of infection during the dry period. Control of CNS is aided by control measures that include clean and dry environment for dry cows, pre-partum teat dipping, and proper milking hygiene.

A survey of *in vitro* antimicrobial sensitivity in the study herds showed that sensitivity among the CPS was greater than 91% to amoxycillin clavulanic acid, cephalothin, cloxacillin, erythromycin, gentamicin, nitrofurantoin, novobiocin, sulphamethoxazole / trimethoprim, and tetracycline. Sensitivities below 91% were

recorded to neomycin (86%), penicillin G (59%), ampicillin (58%), and polymyxin B (8%). Sensitivity among CNS was greater than 91% to amoxycillin clavulanic acid, cephalothin, cloxacillin, erythromycin, gentamicin, neomycin, nitrofurantoin, and novobiocin. Sensitivities below 91% were recorded to tetracycline (88%), sulphamethoxazole / trimethoprim (87%), polymyxin B (78%), ampicillin (62%), and penicillin G (60%).

Between farm variation in sensitivity was evident among both CPS and CNS. Multiresistance was also evident in both groups. After controlling for farm, the proportions of CPS sensitive to nitrofurantoin, sulphamethoxazole / trimethoprim, and tetracycline were significantly higher than CNS. The proportions of CNS sensitive to polymyxin B and neomycin were significantly higher than CPS. These differences probably reflect inherent properties peculiar to either of CPS or CNS. The proportions sensitive to ampicillin, penicillin G and erythromycin were strongly affected by farm level factors. There were no differences in the proportions of CPS and CNS sensitive to amoxycillin clavulanic acid, cephalothin, cloxacillin, gentamicin, and novobiocin.

Thus, in the absence of sensitivity data in a herd the choice of an ideal antimicrobial for the treatment of staphylococcal mastitis on a given farm should meet the following requirements in order of importance: 1) have a high *in vitro* sensitivity for CPS in a previous study in the geographical location, 2) be biologically appropriate in respect of distribution, safety, and residue potential, 3) have an effective past clinical experience with CPS IMI in the herd, and 4) have a high *in vitro* sensitivity for CNS in a previous study in the geographical location. In this study for example, assuming an effective past clinical experience with CPS,

the ideal antimicrobial can be chosen from among those whose sensitivity for both CPS and CNS approached 100%. Such antimicrobials include amoxycillin clavulanic acid, erythromycin, novobiocin, cephalothin, and cloxacillin. These antimicrobials have a good to fair potential distribution throughout the udder.

In order to determine a valid relationship between antimicrobial sensitivity and management factors, only antimicrobials with within herd sensitivity SD greater than 5% (Chapter 4) that were used on the study farms (Chapter 2) were candidates. These were penicillin G and tetracycline.

The study found that post-milking teat dipping, a practice that is conducive to low prevalence of CPS, prevents the emergence and spread of resistance among CPS. CPS and CNS organisms more frequently exposed to antibiotics through clinical treatment are more likely to develop resistance. The study did not demonstrate any association between dry cow therapy and resistance among CPS. It is probable that resistance to penicillin G and tetracycline is in part the result of some dry cow products used. Yet, other dry cow products used readily clear the CNS that are resistant to penicillin G but not to tetracycline.

When resistance was present, neither CPS nor CNS sensitivities had any correlation with prevalence. Thus, after accounting for the variation in prevalence and sensitivity due to management factors, antimicrobial sensitivity and prevalence are independent of each other. In effect, this means that antimicrobial resistance has not compromised the control of mastitis due to staphylococcal pathogens.

Areas for future research include:

1. Studies to determine whether the timing of dry cow therapy contributes to its inadequacy. Thus, the pathophysiology of the udder during the first week following drying off may be a contributing factor to failure of dry cow therapy. At the beginning of the second week post-drying off, the fluid volume of the udder is greatly reduced. This could be an opportune time to infuse antibiotics into the udder. Such studies should have all the essential features of a clinical trial.
2. More studies are needed to validate the role of pre-calving teat dipping in the control of CNS, since this study is at variance with earlier studies.
3. Research is needed to come up with post-milking teat dips that have good residual action. Such dips would be ideal in preventing IMI caused by environmental mastitis pathogens including CNS.
4. More research is needed to devise new methods of *in vitro* sensitivity testing that will mimic the udder environment.
5. Other studies are needed to validate the associations reported in this study between antimicrobial sensitivity and management factors.

APPENDIX A SURVEY #_____

THE RELATIONSHIP BETWEEN STAPHYLOCOCCAL MASTITIS, MANAGEMENT PRACTICES AND ANTIBIOTIC USE

The questions that follow pertain to 1989, the year when milk samples were taken. Please fill in the blank or circle the choice which best fits your farm.

SECTION A: QUESTIONS IN THIS SECTION PERTAIN TO GENERAL DAIRY MANAGEMENT PRACTICES.

1. How long have you been in the dairy industry?
   _____YEARS

2. What was the average production per milking cow per day in 1989?
   _____KG

3. How many cows were you milking in the summer of 1989?
   _____COWS

4. Were you on DHAS in 1989?
   1. YES
   2. NO (Skip question 5)

5. What was your rolling herd milk BCA?
   _____KG

6. What type of barn did you keep milking cows in 1989?
   1. FREESTALL
   2. TIESTALL
   3. OTHER (Please specify)_____

7. What was the main type of bedding used for milking cows in 1989? Circle one only.
   1. STRAW
   2. SHAVINGS
   3. SAND
   4. OTHER (Please specify)_____

8. What outdoor area did milking cows have access to in the summer of 1989?
   1. PASTURE
   2. YARD/DRYLOT(SMALL FENCED AREA)
   3. OTHER (Please specify)_____.

9. What type of barn did you keep the dry cows in last year?
   1. FREESTALL
   2. TIESTALL
   3. OTHER (Please specify)_____

10. What was the main type of bedding used for dry cows last year? Circle only one.
   1. STRAW
   2. SHAVINGS
   3. SAND
   4. OTHER (Please specify)_____

11. What outdoor area did dry cows have access to in the summer of 1989?
   1. PASTURE
   2. YARD/DRYLOT(SMALL FENCED AREA)
   3. OTHER (Please specify)_____

12. Where do cows calve?

   _____

13. How many maternity pens are on your farm?

   _____

14. What was the main type of bedding used for maternity pens in 1989? Circle only one.
   1. STRAW
   2. SHAVINGS
   3. SAND
   4. OTHER (Please specify)_____

15. (Question was excluded)

16. How do you get replacement heifers?
   1. RAISE THEM MYSELF
   2. PURCHASE
   3. OTHER (Please specify)_____

17. What type of milking system do you have?
    1. BUCKET/DUMP STATION
    2. HIGH PIPELINE
    3. PARLOUR
    4. OTHER (Please specify)_____

18. How many times was your milking equipment checked in 1989?
    _____TIMES

19. Who checked the equipment?
    1. SELF
    2. DEALER
    3. VETERINARIAN
    4. OTHER (Please specify)_____

20. Do you use automatic take off units?
    1. YES
    2. NO

21. How many times did you change milk liners last year?
    _____TIMES

22. How many cows were treated for clinical mastitis last year?
    _____COWS

23. How many cows were culled because of mastitis last year?
    _____COWS

SECTION B: THIS SECTION ASKS YOU QUESTIONS CONCERNING UDDER WASH/COW PREPARATION. PLEASE ANSWER FOR 1989.

24. Do you strip milk your cows before milking?
    1. NO
    2. YES, BEFORE WASHING
    3. YES, AFTER WASHING

25. Which of the following methods of udder preparation do you use?
    1. TEAT WASH
    2. PREDIPPING
    3. TEAT WASH PLUS PREDIPPING
    4. OTHER (please specify)_____

26. What do you use to wash cows' udders?
    1. DISPOSABLE PAPER TOWELS
    2. REUSABLE CLOTH/SPONGE/PAPER
    3. NOTHING

27. Do you use disinfectant in wash water?
    1. YES
    2. NO

28. What was the main product you used in wash water in 1989?

    _____

29. Do you dip units between cows?
    1. YES
    2. NO

30. Do you dip teats after milking?
    1. YES
    2. NO

31. What was the main teat dip product you used in 1989?

    _____

32. What type of applicator do you use for teat dip?
    1. CUP
    2. SPRAY
    3. SQUEEZE BOTTLE
    4. NONE
    5. OTHER (Please specify)_____

33. How often do you change dip in applicator?
    1. I DO NOT CHANGE DIP
    2. EACH MILKING
    3. DAILY
    4. 2-3 TIMES A WEEK
    5. LESS OFTEN

34. Are cows' teats dipped prior to calving?
    1. YES
    2. NO

SECTION C: THE FOLLOWING PERTAIN TO DRY COW MANAGEMENT AND PRODUCTS. IF YOUR DRY COW MANAGEMENT HAS CHANGED RECENTLY, PLEASE FILL OUT HOW YOU DID IT IN 1989.

35. How do you dry your cows off?
    1. ABRUPTLY
    2. GRADUALLY

36. What percentage of your cows do you treat with dry cow therapy?
    _____%

37. How do you prepare teat ends before treatment?
    1. WASH ONLY
    2. DISINFECTANT (ALCOHOL/TEAT DIP)
    3. I APPLY TREATMENT STRAIGHT AWAY

38. For each of the following dry cow products:

Put a check in column A if you have used product in the past two years.

Put a check in column B if you are still using the product.

PRODUCT	COLUMN A (USED PRODUCT IN PAST TWO YEARS)	COLUMN B (STILL USING PRODUCT)
Albadry Plus Suspension		
Albadry Suspension		
Antimast		
Biodry		
Biotef		
Cefa-Dri		
Combisec Dry Cow		
COOP Dry Cow Mastitis		
Dry-Clox		
Erythro-36		
Erythro-Dry Cow		
Gallimycin-36		
Liquamast		
Mastitis Care		
Neospan		
Novodry Suspension		
Novodry Plus Suspension		
Orbenin		
Quartermaster		
Special Formula 17900		
Terramycin Liquid		

39. For each of the following lactating cow products:
Put a check in column A if you have used product in the past two years.
Put a check in column B if you are still using the product.

PRODUCT	COLUMN A (USED PRODUCT IN PAST TWO YEARS)	COLUMN B (STILL USING PRODUCT)
Albacillin		
Cefa-Lak		
COOP Mastitis Formula A		
Coopamast		
Erythro-36		
Gallimycin-36		
K-25		
Liquamast		
Mastex Plus		
Mastitis Care		
Neospan		
Orbenin Quick Release		
quarter Cure		
Shur-Gain Mastitis		
Special Formula 17900		
Special Formula 17900 NP		
Terramycin Liquid		

APPENDIX B. DEFINITION OF LACTATION COW PRODUCTS AS ENTERED IN DATA BASE

Variables	Active ingredients	Product brand name
PRODUCT 1	Procaine Pen. G Novobiocin Sodium	Albacillin, Co-op Mastitis Formula A, Coopmast
PRODUCT 2	Pen. G Potassium Streptomycin sulphate Bacitracin	Mastex Plus, Mastitis Care, Quarter Cure, Shurgain Mastitis
PRODUCT 3	Pen. G Potassium Streptomycin sulphate Bacitracin Polymyxin B	Neospan
PRODUCT 4	Procaine Pen. G Novobiocin Sodium Dihydrostreptomycin sulphate	Special Formula 17900
PRODUCT 5	Cephapirin Sodium Base	Cefa-Lak
PRODUCT 6	Cloxacillin Sodium	Orbenin Quick Release
PRODUCT 7	Oxytetracycline hydrochloride	Liquamst, Terramycin Liquid
PRODUCT 8	Erythromycin	Erythro-36, Gallamycin-36
PRODUCT 9	Any Product containing Novobiocin	PRD1, PRD4
PRODUCT 10	Any Product containing Penicillin G	PRD1, PRD2, PRD3, PRD4
PRODUCT 11	Any Product containing Beta lactams	PRD1, PRD2, PRD3, PRD4, PRD5, PRD6
PRODUCT 12	Any Product containing Streptomycin	PRD2, PRD3, PRD4

APPENDIX C. DEFINITION OF DRY COW PRODUCTS AS ENTERED IN DATA BASE

Variables	Active ingredients	Product brand name
Dry Cow Product 1	Novobiocin Sodium	Albadry Plus Suspension, Biodry, Novodry Suspension, Coop Dry Mastitis
Dry Cow Product 2	Procaine Pen. G Novobiocin Sodium	Novodry Plus Suspension, Albadry Suspension
Dry Cow Product 3	Procaine Pen. G Novobiocin Sodium Dihydrostreptomycin sulphate	Special Formula 17900
Dry Cow Product 4	Procaine Pen. G Dihydrostreptomycin sulphate	Combisec Dry Cow, Quarter Master
Dry Cow Product 5	Procaine Pen. G Streptomycin sulphate Neomycin Polymyxin B	Neospan
Dry Cow Product 6	Cloxacillin Benthazine	Orbenin, Dry-Clox
Dry Cow Product 7	Cephapirin Benthazine	Cefa-Dri
Dry Cow Product 8	Oxytetracycline hydrochloride	Terramycin Liquid, Liquamast
Dry Cow Product 9	Any Product containing Novobiocin	DCP1, DCP2, DCP3
Dry Cow Product 10	Any Product containing Procaine Pen. G	DCP2, DCP3, DCP4, DCP5
Dry Cow Product 11	Any Product containing Beta lactams	DCP2, DCP3, DCP4, DCP5, DCP6, DCP7
Dry Cow Product 12	Any Product containing Streptomycin	DCP3, DCP4, DCP5

BIBLIOGRAPHY

ANDERSON JC. Progressive pathology of *Staphylococcal* mastitis with a note on control immunization and therapy. Vet Rec 1982; 110: 372-376.

ATLANTIC DAIRY LIVESTOCK CORPORATION (ADLIC), Annual summary 1989.

BATRA TR. Effect of complete dry cow treatment on mastitis control in dairy cattle. Can J Anim Sci 1988; 68: 553-556.

BAUER AW, KIRBY WMM, SHERIS JC, TURCK M. Antibiotic susceptibility by a standardized single disk method. Am J Clin Pathol 1966; 45:493-496.

BISHOP JR, BODINE AB, JANZEN JJ. Sensitivities to antibiotics and seasonal occurrence of mastitis pathogens. J Dairy Sci 1980; 63: 1134-1137.

BRAMLEY AJ. The Effect of subclinical *Staphylococcus epidermidis* infection of the lactating bovine udder on its susceptibility to infection with *Streptococcus agalactiae* or *Escherichia coli*. Br Vet J 1978; 184: 146-151.

BROWN RW, SCHERER RK. Classification of *Staphylococcus epidermidis* and *Micrococcus* strains isolated from Bovine Milk. Am J Vet Res 1978; 39: 767-772.

BROWNING JW, MEIN GA, BARTON M, NICHOLS TJ, BRIGHTLING P. Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. Aust Vet J 1990; 67: 440-442.

BUDDLE BM, HERCEG M, RALSTON MJ, PULFORD HD. Reinfection of bovine mammary glands following dry-cow antibiotic therapy. J Vet Microbiol 1987; 15: 191-199.

BURTON MJ, WILLIAMSON NB, BROWN WB, BAUMANN LE. Mastitis control measures used on Some Minnesota dairy farms. Prev Vet Med 1988; 5: 225-232.

BUSHNELL RB. The importance of hygienic procedures in controlling mastitis: Symposium on bovine mastitis. Vet Clinics of North America: Large Animal Practice 1984; 6: 361-370.

CODY RP, SMITH JK. Applied statistics and the SAS programming language. 3rd ed. New York: Elsevier Science Publishing Co., Inc., 1991.

CONOVER WJ. Practical nonparametric statistics. 2nd ed. Toronto: John Wiley and Sons, 1980.

CRAVEN N. A review: Efficacy and financial value of antibiotic treatment of bovine clinical mastitis during lactation. Br Vet J 1987; 143: 410-421.

CRAVEN N, ANDERSON JC. The location of *S. aureus* in experimental mastitis in the mouse and the effect on the action of sodium cloxacillin. Br J Exp Pathol 1979; 60: 453-459.

CRAVEN N, ANDERSON JC, JONES TO. Antimicrobial drug susceptibility of *Staphylococcus aureus* isolated from bovine mastitis. Vet Rec 1986; 118: 290-291.

CRAVEN N, ANDERSON JC. Phagocytosis of *S. aureus* by bovine mammary gland and intracellular protection from antibiotic action *in vitro* and *in vivo*. J Dairy Res 1984; 51: 513-523.

DAIRY PROFILE, PRINCE EDWARD ISLAND AGRICULTURAL PROFILE, AGRICULTURE CANADA March 1991.

DARGENT-MOLINA P, SCARLETT J, POLLOCK RVH, ERB HN, SEARS P. Herd-level risk factors for *Staphylococcus aureus* and *Streptococcus agalactiae* intramammary infections. Prev Vet Med 1988; 6: 127-142.

DAVIDSON TJ. The effects and control of coagulase negative *Staphylococci* mastitis in dairy cattle in P.E.I. Master of science thesis, University of Prince Edward Island, Canada, 1990.

DAVIDSON JN, BABISH JG, DUNNY GM. Bovine mastitis: Antimicrobial resistance patterns. J Am Vet Med 1982; 180: 153-155.

DEVRIESE LA. Staphylococci in healthy and diseased animals. J Appl Bacteriol 1990; Symposium suppl: 71s-80s.

DEVRIESE LA, DE KEYSER H. Prevalence of different species of coagulase-negative *Staphylococci* on teats and milk samples from dairy cows. J Dairy Res 1980; 47: 155-158.

DODD FH. Symposium: Advances in understanding mastitis: Mastitis-progress on control. J Dairy Sci 1983; 66: 1773-1780.

DODD FH. Progress in mastitis control. Proceedings of National Mastitis Council Inc, 25th Annual Meeting 1986; 5-10.

DRAPER NR, SMITH H. Applied regression analysis. 2nd ed. Toronto: John Wiley and Sons, 1980.

ETGEN WA, JAMES RE, REAVES PM. Dairy cattle feeding and management. 7th ed. Toronto: John Wiley and Sons, 1987.

FOX LK, HANCOCK DD. Effect of segregation on prevention of intramammary infections by *Staphylococcus aureus*. J Dairy Sci 1989; 72: 540-544.

FOX LK, NAGY JA, HILLERS JK, CRONRATH JD, RATKOWSKY DA. Effects of postmilking teat treatment on the colonization of *Staphylococcus aureus* on chapped teat skin. Am J Vet Res 1991; 52: 799-802.

FRANCIS PG, CARROLL PJ. Antibiotic resistance patterns of *Staphylococcus aureus* strains isolated from clinical bovine mastitis. Vet Rec 1986; 118: 361-363.

GALTON DM, PETERSON LG, MERILL WG. Effects of pre-milking udder preparation practices on bacterial counts in milk and on teats. J Dairy Sci 1986; 69: 260-266.

GALTON DM, PETERSON LG, MERILL WG, BANDLER DK, SHUSTER DE. Effects of pre-milking udder preparation on bacterial population, sediments, and iodine residue in milk. J Dairy Sci 1984; 67: 2580-2589.

GODKIN A. The relationship between bulk tank cultures, management factors used in mastitis control and the herd prevalence of mastitis. DVSc thesis, University of Guelph, Canada, 1989.

GUTERBOCK WM, BLACKMER PE, DUFFY J. Cleaning the milking system: Symposium on bovine mastitis. Vet Clinics of North America: Large Animal Practice 1984; 6: 377-390.

HINCKLEY LS, BENSON RH, POST JE, DeCLOUX JC. Antibiotic susceptibility profiles for mastitis treatment. J Am Vet Med 1985; 187: 709-711.

HODGES RT, JONES YS, HOLLAND JTS. Characterisation of *Staphylococci* associated with clinical and subclinical bovine mastitis. N.Z. Vet J 1984; 32:141-145.

HOGAN JS, CORNETTA A, PANKEY JW. Comparison of four test procedures to identify *Staphylococcus aureus* isolated from bovine intramammary infections. Am J Vet Res 1986; 47: 2017-2019.

HOGAN JS, PANKEY JW, SMITH KL. *Staphylococcus* species other than *Staphylococcus aureus*. Proceedings of National Mastitis Council Inc, 26th Annual Meeting 1987; 21-27.

HOGAN JS, SMITH KL, TODHUNTER DA, SCHOENBERGER PS. Rate of environmental mastitis in quarters infected with *Corynebacterium bovis* and *Staphylococcus* species. J Dairy Sci 1988; 71: 2520-2525.

HUTTON CT, FOX LK, HANCOCK DD. Risk factors associated with herd-group milk somatic cell count and prevalence of coagulase - positive *Staphylococcal* intramammary infections. Prev Vet Med 1991; 11: 25-35.

JARP J. Classification of coagulase-negative *Staphylococcus* isolated from bovine subclinical mastitis. J Vet Microbiol 1991; 27: 151-158.

JARP J, BUGGE HP, LARSEN S. Clinical trial of three therapeutic regimens for bovine mastitis. Vet Rec 1989; 124: 630-634.

JARRETT JA. Mechanical milking and its relationship to mastitis: Symposium on bovine mastitis. Vet Clinics of North America: Large Animal Practice 1984a; 6: 349-360.

JARRETT JA. Environmental effects on mastitis and milk quality: Symposium on bovine mastitis. Vet Clinics of North America: Large Animal Practice 1984b; 6: 371-375.

KLEINBAUM DG, KUPPER LL, MORGENSTERN H. Epidemiologic research: Principles and quantitative methods. 1st ed. Ontario: Macmillan of Canada, 1982.

KLEINBAUM DG, KUPPER LL, MULLER KE. Applied regression analysis and other multivariable methods. 2nd ed. Boston: PWS-KENT Publishing Company, 1988.

LEVY SB. Antibiotic availability and use: Consequences to man and his environment. J Clin Epidemiol 1991; 44(suppl.II): 83s-87s.

LINDE C. The effect of coagulase-negative *Staphylococci* in the cow's udder on experimental induction of mastitis and on milk production. PhD thesis, Swedish University of Agricultural Sciences, Uppsala. 1982.

MACKIE DP, LOGAN EF, POLLOCK DA, RODGERS SP. Antibiotic sensitivity of bovine *Staphylococcal* and coliform mastitis isolates over four years. Vet Rec 1988; 123: 515-517.

MARTIN SW, MEEK AH, WILLEBERG P. Veterinary epidemiology: Principles and methods. Iowa State University press, 1987.

MATTHEWS KR, HARMON RJ, LANGLOIS BE, CRIST WL, HEMKEN RW. Use of latex teat dip with germicide during the preparation period. J Dairy Sci 1988; 71: 1940-1946.

MATTHEWS KR, HARMON RJ, SMITH BA. Protective effect of *Staphylococcus chromogenes* infection against *Staphylococcus aureus* Infection in the lactating bovine mammary gland. J Dairy Sci 1990; 73: 3457-3462.

McDONALD JS. *Streptococcal* and *Staphylococcal* Mastitis: Symposium on bovine mastitis. Vet Clinics of North America: Large Animal Practice 1984; 6: 269-285.

McDONALD JS, ANDERSON AJ. Antibiotic sensitivity of *Staphylococcus aureus* and coagulase negative *Staphylococci* isolated from infected bovine mammary glands. Cornell Vet 1981; 71: 391-396.

MEEK AH, GOODHOPE RG, BARNUM DA. Bovine Mastitis: A survey of Ontario dairy producers. Can Vet J 1981; 22:46.

MERCER HD. Viewpoint and current concepts regarding accepted and tried products for control of bovine mastitis. J Am Vet Med 1976; 169: 1104-1114.

MERCER HD, TESKE RH. Special considerations for the development of drugs for acute clinical mastitis. J Am Vet Med 1977; 170: 1190-1193.

MOORE GA, HEIDER LE. Treatment of mastitis: Symposium on bovine mastitis. Vet Clinics of North America: Large Animal Practice 1984; 6: 323-332.

NATZKE RP, EVERETT RW, BRAY DR. Effect of drying off practices on mastitis infection. J Dairy Sci 1974; 58: 1828-1835.

NATZKE RP, EVERETT RW, GUTHRIE RS, KEOWN JF, MEEK AM, MERRILL WG, ROBERTS SJ, SCHMIDT GH. Mastitis control program: Effect on milk production. J Dairy Sci 1972; 55: 1256-1260.

NICKERSON SC, OWENS WE. New treatment strategies. Proceedings of National Mastitis Council Inc, 29th Annual Meeting 1990; 114-123.

NICKERSON SG, PAAPE MJ, HARMON RJ, ZIV G. Mammary leukocyte response to drug therapy. J Dairy Sci 1986; 69: 1733-1742.

NICKERSON SC, WATTS JL, BODDIE RL, RAY CH. Effect of postmilking teat antisepsis on teat canal infections in lactating dairy cows. J Dairy Sci 1990; 73: 373-380.

OLIVER SP, MITCHELL BA. Intramammary infections in primigravid heifers near parturition. J Dairy Sci 1983; 66: 1180-1183.

O'SHEA J, O'CALLAGHAN E. Milking machine effects on new infection rate. Proceedings of National Mastitis Council Inc, 17th Annual Meeting 1978; 262-268.

OSTERAS O, LUND A. Epidemiological analyses of the associations between bovine udder health and housing. Prev Vet Med 1988b; 6: 79-80.

OSTERAS O, LUND A. Epidemiological analyses of the associations between bovine udder health and milking machine and milking management. Prev Vet Med 1988a; 6: 91-108.

OWENS WE. Evaluation of various antibiotics for induction of L-forms from Staphylococcus aureus strains isolated from bovine mastitis. J Clin Microbiol 1988; 26: 2187-2190.

OWENS WE, NICKERSON SC. Morphologic study of *Staphylococcus aureus* L-form, reverting, and intermediate colonies *in situ*. J Clin Microbiol 1989; 1382-1386.

OWENS WE, NICKERSON SC. Treatment of *Staphylococcus aureus* with penicillin and novobiocin: Antibiotic concentrations and bacteriologic status in milk and mammary tissue. J Dairy Sci 1990; 73: 115-124.

OWENS WE, NICKERSON SC, WASHBURN PJ, RAY CH. Efficacy of a cephapirin dry cow product for treatment of experimentally induced *Staphylococcus aureus* mastitis in heifers. J Dairy Sci 1991; 74: 3376-3382.

OWENS WE, WATTS JL. Effects of milk on activity of antimicrobics against *Staphylococcus aureus* isolated from bovine udders. J Dairy Sci 1987; 70: 1946-1951.

OWENS WE, WATTS JL. Antimicrobial susceptibility and beta-lactamase testing of *Staphylococci* isolated from dairy herds. J Dairy Sci 1988; 71: 1934-1939.

OWENS WE, WATTS JL, BODDIE RL, NICKERSON SC. Antibiotic treatment of mastitis: Comparison of intramammary and intramammary plus intramuscular therapies. J Dairy Sci 1988; 71: 3143-3147.

PAAPE MJ, NICKERSON SC, ZIV G. *In vivo* effects of chloramphenicol, tetracycline and gentamicin on bovine neutrophil function and morphologic features. Am J Vet Res 1990; 51: 1055-1061.

PANKEY JW. Postmilking teat antisepsis: Symposium on bovine mastitis. Vet Clinics of North America: Large Animal Practice 1984; 6: 335-348.

PANKEY JW. Teat dipping: A tool for control of environmental pathogens. Proceedings of National Mastitis Council Inc, 26th Annual Meeting 1987; 156-159.

PANKEY JW, WILDMAN EE, HUESTIS PD, HOGAN JS. Efficacy studies on pre-milking teat sanitation. Proceedings of National Mastitis Council Inc, 25th Annual Meeting 1986; 81-107.

PANKEY JW, HOGAN JS, MURDOUGH PA. Bacteriological monitor for mastitis. Proceedings of National Mastitis Council Inc, 26th Annual Meeting 1987; 10-16.

PAPICH MG. The beta lactam antibiotics: Clinical pharmacology and recent developments. Compendium on Continuing Education 1987; 9: 68-74.

PHILPOT WN. Economics of Mastitis Control: Symposium on bovine mastitis. Vet Clinics of North America 1984; 6: 233-245.

RAINHARD P, POUTREL B. Effect of naturally occurring intramammary infections by minor pathogens on new infections by major pathogens in cattle. Am J Vet. Res 1988; 49: 327-329.

SANDHOLM M, KAARTINEN L, PYORALA S. Bovine Mastitis- why does antibiotic therapy not always work? An overview. J Vet Pharmacol Therap 1990; 13: 248-260.

SCHEAFFER, MENDENHALL, OTT. Elementary sampling. 2nd ed. Massachusetts: Duxbury Press North Scituate, 1979.

SCHULTZE WD. Control of new intramammary infection at calving by preparation teat dipping. J Dairy Sci 1985; 68: 2094-2099.

SEARS PM, FETTINGER M, MARSH-SALIN J. Isolation of L-forms after antibiotic treatment in *S. aureus* bovine mastitis. J Am Vet Med 1987; 191: 681-683.

SEELEY and VANDEMARK. Microbes in action: A manual for microbiology. 2nd

ed. San Francisco: WH Freeman and company, 1972.

SMITH KL. Practical measures of mastitis control. Proceedings of National Mastitis Council Inc, 25th Annual Meeting 1986; 36-47.

SNEDECOR GW, COCHRAN WG. Statistical methods. 8th ed. Iowa State University Press / Ames, 1989.

SOBACK S. Therapeutic success or failure in mastitis therapy: A pharmakinetic approach. Proceedings of National Mastitis Council Inc, 26th Annual Meeting 1987; 1b-10b.

SOBACK S, ZIV G, WINKLER M, SARAN A. Systemic dry cow therapy : A preliminary report. J Dairy Sci 1990; 73: 661-666.

SPERBER WH, TATINI SR. Interpretation of the tube coagulase test for identification of *Staphylococcus aureus*. J Appl Microbiol 1975; 29: 502-505.

TIMONEY JF, GILLESPIE JF, SCOTT FW, BARLOUGH JE. Hagan and Bruner's microbiology and infectious diseases of domestic animals. 8th ed. Cornell University Press, 1988.

TIMMS LL, SCHULTZ LH. Dynamics and significance of coagulase-negative *Staphylococcal* intramammary infections. J Dairy Sci 1987; 70: 2648-2657.

TRINIDAD P, NICKERSON SC, LUTHER DG. Antimicrobial susceptibilities of *Staphylococcal* species isolated from mammary glands of unbred and primigravid dairy heifers. J Dairy Sci 1990; 73: 357-362.

TYLER JW, WILSON RC, DOWLING P. Treatment of subclinical mastitis. Vet Clinics of North America: Food Animal Practice 1992; 8: 17-28.

VILLANUEVA MR, TYLER JW, THURMOND MC. Recovery of *Streptococcus agalactiae* and *Staphylococcus aureus* from fresh and frozen bovine milk. JAVMA 1991; 198: 1398-1400

WRIGHT CL. Mastitis control hindered. Vet Rec 1977; 101: 160-161.

ZIV G. Availability and usage of new antibacterial drugs in Europe. J Am Vet Med 1980; 176: 1122-1128.

ZIV G. Treatment of peracute and acute mastitis. Vet Clinics of North America: Food Animal Practice 1992; 8: 1-15.

ZIV G, PAAPE MJ, DULIN AM. Influence of antibiotics and intramammary antibiotic products on phagocytosis of *S. aureus* by bovine leukocytes. Am J Vet Res 1983; 44: 385-388.