**Sand And Sawdust Bedding Affect Populations Of Coliforms, Klebsiella Spp. And Streptococcus Spp. On Teat Ends Of Dairy Cows Housed In Freestalls**

by

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**ABSTRACT**

The main objectives of the study were: 1) to compare bacterial populations of mastitis causing organisms on the outside of teats of lactating cows housed on sand and on sawdust bedding and, 2) to examine the relationship between bacterial counts in the two bedding types with those on teat ends. Sixteen cows were housed on either sand or sawdust-bedded freestalls using a crossover design with 3 week per bedding type. Bedding samples were collected on d 0 (prior to cows lying on the bedding), 1, 2 and 6. Teat ends were sampled prior to the morning milking on d 1, 2 and 6. All samples were analyzed to determine coliform, *Klebsiella* spp., and *Streptococcus* spp. populations. For teat end samples, there was two times more coliform and six times more *Klebsiella* spp. on teat ends of cows housed on sawdust compared to those housed on sand. In contrast, there was a 10-fold increase in the number of *Streptococcus* spp. associated with sand than with sawdust. In both sawdust and sand bedding, coliform, *Klebsiella* and *Streptococcus* counts increased over each experimental week, although patterns varied with bedding and bacteria type. Bacterial counts on teat ends were correlated to bacterial counts in sawdust: r = 0.47, r = 0.69 and r = 0.60 for coliforms, *Klebsiella* spp. and streptococci, respectively. Bacterial counts on teat ends were correlated to bacterial counts in sand: r = 0.35 for coliforms and r = 0.40 for *Klebsiella* spp. Streptococcal counts on teat ends were not correlated to bacterial counts in sand. In conclusion, coliform and *Klebsiella* spp. on teat ends were more numerous when using sawdust bedding, but *Streptococcus* spp. were more numerous on teat ends of cows housed on sand.

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**In Memory of Dr. James Shelford**

**(1944 – 2002)**

Your ever-present optimism and encouragement has been pivotal in continuing my studies of Animal Science at the graduate level. Your enthusiasm for work and research will be an inspiration in my future endeavors.

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**Chapter I**

**1.1 General Introduction**

Mastitis is one of the most complex and costly diseases of dairy cows (Harmon,

1994; Andrews, 2000). This disease has an enormous economic impact for a producer

due to lowered milk yield, reduced milk quality and poor animal health (Andrews, 2000).

Economic losses in the United States due to mastitis are estimated to be approximately

US$ 185 per cow annually which amounts to US$ 1.8 billion annually (National Mastitis

Council, 1996). Approximately 33% of that cost is due to the loss in milk production

caused by intramammary infections (IMI) (National Mastitis Council, 1996). Mastitic

cows are treated with antibiotics and if the mastitis problems persist, they are culled from

the herd.

Mastitis is caused by pathogens that can be characterized as contagious or

environmental (Table 1.1). Contagious mastitis can often be successfully controlled;

however, environmental mastitis is more difficult to control since these pathogens are

always present in the cows' environment.

Bedding, water, and soil are thought to be the main source of environmental

pathogenic infections in most dairy housing systems. Studies exploring the relationship

between bedding and more specifically, bacterial populations found in different types of

bedding and the incidence of mastitis have identified preventive and control measures to

minimize environmental infections (Bishop et al., 1981; Hogan et al., 1989; Neave and

Oliver, 1962; Rendos et al., 1975; Smith et al., 1985). Bedding for dairy cattle can be

broadly classified into two groups: organic (such as sawdust, recycled dairy waste and

straw) and inorganic (such as sand and limestone). The common consensus in the dairy

industry, particularly in North America, is that use of sand bedding minimizes the rates of mastitis infection in dairy herds. A recent survey study of 302 Wisconsin dairy farms revealed that farmers perceive sand bedding provides some advantages for udder health and cow comfort over mattresses (Bewley et al., 2001). These perceptions have resulted in a move towards the use of sand as the preferred bedding choice for dairy producers in North America, including the lower Fraser Valley region of British Columbia. However, it will become clear from this literature review provided that the relationship between bedding types and mastitis has not been satisfactorily explored.

The main objectives of this literature review are to provide the reader with: 1) the definition and pathogenesis of mastitis, 2) an assessment of differences in bacterial counts associated with various beddings, and 3) the relationship between mastitis and bacterial counts in bedding.

## 1.2 Mastitis

### 1.2.1 Definition

Mastitis is an inflammation of the mammary gland that usually occurs as a result of injury or bacterial infection (Andrews, 2000). The degree of inflammation and the severity of disease manifest into either clinical or subclinical mastitis. Clinical mastitis is a condition characterized by abnormalities of the udder (swollen, hard and hot quarters) and milk (flakes, clots and watery appearance), which nearly always results in a decrease in total milk production as well as a change in milk composition (Harmon, 1994). Subclinical mastitis occurs when there is no observable abnormality of the udder or milk, but milk production decreases and bacteria are present in the milk (National Mastitis

Council, 1996). Infections can also be classified as acute and chronic. Acute mastitis is characterized by the sudden onset of redness, swelling, hardness, pain, and grossly abnormal milk and reduced milk yield (National Mastitis Council, 1996). Fever, loss of appetite, rapid pulse and dehydration can also be present in cows suffering from acute infections. Chronic mastitis cases are those that remain in the subclinical phase indefinitely, or alternate between subclinical and clinical phases (National Mastitis Council, 1996).

Intramammary infections begin when bacteria invade the mammary gland through the teat orifice and multiply in the mammary tissues causing inflammation (Figure 1.1) (National Mastitis Council, 1996). The magnitude of the inflammatory response can be influenced by environmental factors (climate, housing, bedding, milking machines, nutrition), host factors (stage of lactation, milk yield, age, immune status, genetics, presence of lesions on the teats, and mammary gland structure), and pathogen factors (bacteria type and strain, numbers of organisms and susceptibility to antibiotics) (Poutrel, 1982).

### 1.2.1.1 Contagious mastitis

Contagious mastitis is caused by pathogens that are found in mammary glands of cows; therefore, the infection can be spread by cows, teat cups, and human contact when milking. The two main contagious pathogens are *Staphylococcus aureus* and *Streptococcus agalactiae*. *S. aureus* can easily colonize the teat canal and infect teat lesions. These microorganisms can also be found in other parts of the cow, such as the vulva and lips, and are implicated in IMI in calves and heifers (Jain, 1979). *S. aureus*

mainly produces subclinical and chronic mastitis that can persist throughout the lactation and into subsequent lactations. Occasionally, it may also cause acute mastitis which progresses to gangrene of the quarters (Jain, 1979).

*S. agalactiae* is a gram-positive streptococcus that grows in liquid media such as milk. It is an obligate parasite of the udder, which means that it can only live and reproduce in the gland, and usually causes subclinical and chronic infections (Jain, 1979).

The principle reservoirs for contagious pathogens are infected mammary glands. Thus, the eradication of these microorganisms from dairy herds can be achieved by the implementation of proper prevention and control programs such as teat dipping, proper maintenance of milking equipment, total dry cow therapy (the use of intramammary antibiotic therapy immediately prior to dry-off), culling and appropriate treatment of clinical cases.

### 1.2.1.2 Environmental mastitis

The most common pathogens causing environmental mastitis are coliforms and streptococci (other than *S. agalactiae*). Environmental mastitis is most commonly observed during early lactation and dry cow periods (Smith et al., 1985). The infection rate peaks in the summer and is most common among intensively housed cows (Hogan and Smith, 1987). There is no single preventive or control treatment against infections caused by environmental bacteria as cows are exposed to those pathogens, not only during milking but also between milkings (Rendos et al., 1975; Smith et al., 1985). Bedding is often the most obvious source of environmental infections because udders

come into direct contact with bacterial populations in the bedding. Minimizing teat end exposure to pathogenic microorganisms by maintaining clean and dry housing and maximizing the cows' resistance to infection are two possible methods to decrease the rates of environmental infections in dairy herds (Smith et al., 1985).

## 1.3  Bacterial counts in bedding

### 1.3.1 Bedding

The lying surface used by dairy cattle is an important area of investigation, as they spend between 50 and 60% of their time lying down in stalls (Dechamps et al., 1989; Haley et al., 2001). It has been well established that the type of bedding material can influence lying time (Haley et al., 2001) and susceptibility to intramammary infections, since mastitis causing pathogens found in the bedding come in the direct contact with the udder (National Mastitis Council, 1996).

Dairy producers select bedding on the basis of which is most appropriate for their production system. In general, factors such as availability, cost, housing system, waste disposal equipment used on the dairy farm, as well as the beddings' qualities in terms of udder health and cow comfort are taken into consideration. Each type of bedding has its advantages and disadvantages. In the Fraser Valley of British Columbia the most common types of bedding are sawdust and sand.

Sawdust is an organic type of bedding originating from either softwood or hardwood depending upon the geographic region of North America. For example, softwoods (such as pine, spruce and fir) are most common in the Pacific North West region of North

America and hardwoods (such as alder, maple and oak) are most common in Northeast region of Canada and the United States.

Sand is gaining popularity as a bedding material for dairy cows in the lower Fraser Valley. The main advantage of sand bedding over sawdust is that it does not contain carbon and nitrogen; thereby, lacking nutrients essential for bacterial growth. Unlike sawdust, which has a dry matter (DM) content of approximately 73 % (Fairchild et al., 1982), sand does not retain moisture and even in wet conditions has a DM content of approximately 90% (Hogan et al., 1989).

### 1.3.2 Coliforms

Coliform is a general term for a group of bacteria that includes gram-negative rods from genera *Escherichia*, *Klebsiella*, and *Enterobacter* (Fairchild et al., 1982). Coliforms are found in soil, water and manure and they also inhabit the intestinal tract of cows (Eberhart et al; 1979; Fairchild et al., 1982)*. Klebsiella pneumoniae* and *Escherichia coli* are the most common species associated with mastitis (International Dairy Federation, 1999) in dairy cattle.

Rate of coliform infection is higher during the dry period than during lactation; and dry cow therapy (treating cows with antibiotics at the end of lactating period) has been shown to have no effect on reducing the coliform infection rate (Smith et al., 1985). Coliform populations exceeding $10^6$ colony-forming units (cfu) per gram of wet bedding have been reported to increase the probability of IMI occurring (Bramley and Neave, 1975). In addition, bedding temperature and moisture influence coliform growth. It has

been shown in *in vitro* studies that bedding temperatures between 30 and 44$^{\circ}$ C are most favorable for coliform multiplication (Bramley and Neave, 1975).

The presence of coliforms in different types of bedding materials has been thoroughly researched (Carroll and Jasper, 1978; Fairchild et al., 1982; Hogan et al., 1989; Hogan et al., 1990; Natzke and LeClair, 1976; Smith et al., 1985; Zehner et al., 1986). Hogan and his colleagues (1990) measured bacterial counts associated with recycled newspaper, pelleted corn and wood shavings. They found that coliform counts were similar across all three materials. Scientists comparing bacterial counts in sawdust, lime, recycled newspaper and sand found that coliform counts were highest in sawdust and paper, with negligible counts in lime and sand (Fairchild et al., 1982). Zehner et al., (1986) compared bacterial growth in fine hardwood chips, recycled dried manure, chopped newspaper, softwood sawdust and chopped straw, and reported that coliforms grew more rapidly and declined less rapidly than environmental streptococci on all types of bedding (Zehner et al., 1986).

*Klebsiella* spp. are most numerous in sawdust bedding as reported by Bramley and Neave (1975) and Fairchild et al. (1982), who both reported *Klebsiella* spp. outbreaks when cows were bedded using fresh sawdust. Zehner et al. (1986) studied the growth of environmental pathogens across different bedding types without addition of feces and urine and reported that the growth of *Klebsiella* spp. and *E. coli* were highest in recycled manure, second highest in straw, third highest in hardwood, and lowest in paper and softwood.

### 1.3.3 Environmental Streptococci

The genus *Streptococcus* is found in a wide variety of human, animal, and plant habitats, and not surprising the environmental streptococci responsible for IMI in cows can be found throughout the dairy barn. The most common environmental mastitis causing species is *Streptococcus uberis*, a gram-positive, spherical shaped bacterium (International Dairy Federation, 1999). *S. uberis* is responsible for clinical and subclinical cases of mastitis in lactating and dry cows. It has been isolated from the lips, tonsils, teat, vulva, rectum, rumen, and coat of the cow, as well as from used bedding and pasture. The failure of teat dipping procedures and dry cow therapy programs in controlling this pathogen is not surprising given the numerous sites of origin for *S. uberis* infections (Bramley et al., 1979; Hill, 1988).

The incidence of mastitis caused by environmental streptococci appears to be increasing in the dairy industry (Erskine et al., 1988; Hill, 1988). This may be attributed to the difficulty in controlling environmental streptococci, particularly during the dry cow period and in the summer when the highest infection rates are seen (Smith et al., 1985). In one study, *S. uberis* accounted for nearly 90% of environmental mastitis cases in heifers within the first 5days of lactation (Pankey et al., 1996). McDonald and McDonald (1976) reported that *S. uberis* accounted for 56.5% of all streptococcal infections.

*S. uberis* is frequently found in straw bedding (Bramley, 1982; Bramley et al., 1979) and streptococcal counts in chopped straw are usually higher than in sawdust (Hogan et al., 1989; Rendos et al., 1975). Hogan et al. (1990), reported that chopped newspaper, pelleted corn and wood shaving had similar streptococcal counts. However, Hogan et al.

(1989) also compared inorganic (sand and crushed limestone) and organic (sawdust and straw) bedding types and found that organic bedding had higher streptococcal counts than inorganic bedding. Another study compared streptococcal counts in dairy waste solids, crushed limestone and in a 50:50 mixture of dairy waste solids and limestone and found that counts were minimal in the crushed limestone bedding and higher in the other two beddings (Janzen et al., 1982).

## 1.4  Relationship Between Bedding And Mastitis

### 1.4.1 Association between bacterial counts on teat ends and mastitis

Neave and Oliver (1962) recorded the rates of IMI after exposing the teats of dry cows to mastitis pathogens. These authors used three different cultures of *Staphylococcus aureus* to infect three quarters of each experimental animal and found that only contamination of teats with 15 x $10^6$ cfu/ml resulted in IMI. In another study, Schultze and Thompson (1980) examined the rates of IMI after exposing teats of lactating cows to a culture of *E. coli* (after contamination of teats with coliform bacteria between milkings, cows tended to more likely experience IMI). It is clear from these findings that experimentally exposing cows' teats to pathogens can lead to IMI. Correspondingly, other work has shown that removing contamination by pre- and post-milking teat dipping and washing hands with disinfectants, reduced the rates of mastitis (Neave et al., 1966).

### 1.4.2 Association between bacterial counts on teat ends and bedding

Clearly, there is evidence that a relationship between bacterial counts on teat ends and mastitis exists; therefore, the next logical step is to determine whether there is an

association between bacterial types and bacterial counts on teat ends and in bedding. Rendos et al, (1975) found that the differences in coliform, *Klebsiella* spp. and streptococci populations on teat ends of cows housed on various types of bedding appeared to be related to the differences in the bacterial populations present in the bedding. In contrast, Bishop et al, (1981) explored the relationships between the bedding, teat, and milk micro flora, and concluded that there was no correlation between bacterial counts (*E. coli, Enterobacter, Staphylococcus aureus, Staphylococcus epidermis, and Streptococcus* spp.) in bedding and bacterial counts on teats and milk. However, the latter study included only six animals, which were observed for only 28 days.

More recent work has found that bacterial counts in sawdust and recycled manure bedding are correlated with counts on teat ends. The highest correlations between bacteria and bedding type were found for staphylococci (r = 0.85), streptococci (r = 0.61), *Klebsiella spp.*(r = 0.45) and coliforms (r = 0.45) (Hogan et al., 1990).

### 1.4.3 Association between bacterial counts in bedding and mastitis

If as discussed above, the type of bacteria present in the bedding affect the type of bacteria present on teats, and bacteria on teat ends increase the rates of IMI, it stands to reason, that there must be a relationship between bacterial counts in bedding and IMI. Natzke and LeClair (1976) determined whether artificial contamination of sawdust bedding with *E. coli* would increase the IMI rate in dairy cattle herds. They found that elevated bacterial counts in the bedding resulted in higher teat ends counts of cows housed on that bedding in comparison to control animals. However, no new coliform infections occurred even though bacterial counts in sawdust bedding were consistently

above $10^6$ cfu/g of wet bedding, a level sufficient to cause IMI in a previous study (Bramley and Neave, 1975).

Hogan et al. (1989) studied the association between bacterial counts in organic (sawdust and straw) and inorganic (sand and limestone) bedding and rates of clinical mastitis cases in nine commercial dairy farms. They found a weak positive relationship ($r^2 \leq 0.16$) between total rates of clinical mastitis and counts of gram-negative bacteria and *Klebsiella spp.* on bedding. However, they did not find differences in mastitis rates among herds using various bedding materials. These authors suggested that inability to identify individual species in the microbiological procedures used might explain the failure to detect any differences between the commercial herds since not all counted bacteria were necessarily pathogenic (Hogan et al., 1989). Measured bacterial counts may not be reflective of the mastitis pathogens present in bedding. The lack of differences in mastitis rates among cows housed on different beddings suggests that there are many factors contributing to overall udder health of dairy cows.

## 1.5  Conclusion

Scientific research over the last 40 years has shown the importance of bedding in the prevention and control of mastitis. The ease of bacterial transfer from bedding to teat ends depended on the type of bedding since various bedding types support the growth of different bacteria species. Overall, bacterial counts were reported to be lower in inorganic bedding than in organic bedding, suggesting that sand and limestone are better choices for dairy cow bedding. However, only a few studies specifically addressed the issue of bacterial counts in sand bedding and much of the literature cites anecdotal evidence.

Further work is also needed to understand the association between bacterial counts in bedding, and counts on teat ends and in teat canals, as correlations are likely to differ among various bedding materials. Chapter II, outlines an experiment designed to compare bacterial populations of mastitis-causing organisms on the outside of teats of cows housed on sand and sawdust – two popular types of bedding in BC's Fraser Valley. The hypothesis is that bacterial counts on teat ends of dairy cows housed on sawdust will be lower than bacterial counts on teat ends of cows housed on sand. I also examine the relationship between numbers of bacteria in bedding and those found on teat ends. Improved knowledge of bacterial population dynamics in bedding should enable dairy producers to make better-educated decisions about their housing management.

**Table 1.1**. Major mastitis pathogens found in dairy cattle (National Mastitis Council, 1996).

| Bacteria | Type of Mastitis | Primary Source | Means of Spread |
|---|---|---|---|
| *Staphylococcus aureus* | Contagious | Infected udder | Quarter to quarter; cow to cow during milking |
| *Streptococcus agalactiae* | Contagious | Infected udder | Quarter to quarter; cow to cow during milking |
| Coliforms:<br>• *Escherichia coli*<br>• *Klebsiella* spp<br>• *Enterobacter* | Environmental | Bedding, manure, water, soil | Environment to cow |
| Environmental streptococci*:*<br>• *Streptococcus uberis*<br>• *Streptococcus dysgalactiae* | Environmental | Bedding, manure | Environment to cow |
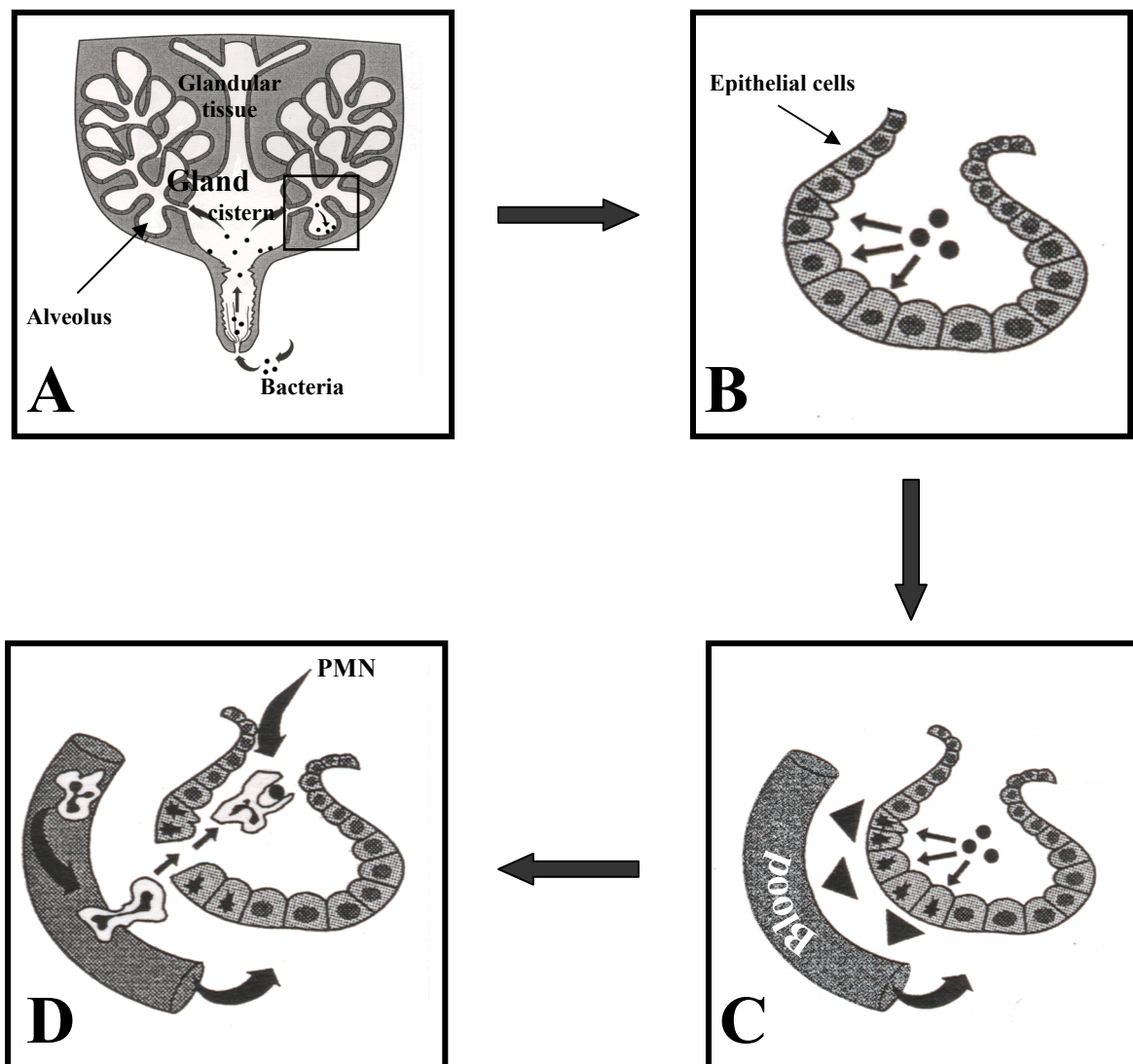
**Figure 1.1**. The process of intramammary infection. Bacteria (•) pass through the teat canal and enter the gland cistern (A). Bacteria adhere to the tissue lining and enter glandular portion where they affect alveolar cells (B). Toxins produced by bacteria (small arrows) cause death or damage to the milk producing epithelial cells, and these cells release substances (large arrows) to the blood stream that increase blood vessel permeability (C). This allows polymorphonuclear neutrophil (PMN) leukocytes to move from the blood into the alveolus where they function by engulfing bacteria (D). Adapted from National Mastitis Council (1996).

**1.6 References**

Andrews, A. H., ed. 2000. The health of dairy cattle. Blackwell Science. Oxford. UK.

Bewley, J., R. W. Palmer, and D. B. Jackson-Smith. 2001. A comparison of free-stall barns used by modernized Wisconsin dairies. J. Dairy Sci. 84:528-541.

Bishop, J. R., J. J. Janzen, A. B. Bodine, C. A. Caldwell, and D. W. Johnson. 1981. Dairy waste solids as a possible source of bedding. J. Dairy Sci. 64:706-711.

Bramley, A. J. and F. K. Neave. 1975. Studies on the control of coliform mastitis in dairy cows. Br. Vet. J. 131:160-169.

Bramley, A. J., J. S. King, and T. M. Higgs. 1979. The isolation of *Streptococcus uberis* from cows in two dairy herds. Br. Vet. J. 135:262-270.

Bramley, A. J. 1982. Sources of *Streptococcus uberis* in the dairy herd. I. Isolation from bovine faeces and from straw bedding of cattle. J. Dairy Res. 49:369-373.

Carroll, E. J. and D. E. Jasper. 1978. Distribution of *Enterobacteriaceae* in recycled manure. J. Dairy Sci. 61:1498-1508.

Dechamps, P., B. Nicks, B. Canart, M. Gielen, and L. Istasse, 1989. A note on resting behaviour of cows before and after calving in two different housing systems. Appl. Anim. Behav. Sci. 23:99-105.

Eberhart, R. J., R. P. Natzke, F. H. S. Newbould, B. Nonnecke, and P. D. Thompson. 1979. Coliform mastitis – a review. J. Dairy Sci. 62:1-22.

Erskine, R. J., R. J. Eberhart, L. J. Hutchinson, S. B. Spencer and M. A. Campbell. 1988. Incidence and types of clinical mastitis in dairy herds with high and low somatic cell count. JAVMA. 192: 761-765.

Fairchild, T. P., B. J. McArthur, J. H. Moore, and W. E. Hylton. 1982. Coliform counts

in various bedding material. J. Dairy Sci. 65:1029-1035.

Harmon, R. J. 1994. Physiology of mastitis and factors affecting somatic cell count. J. Dairy Sci. 77:2103-2112.

Haley, D. B., A. M. de Passillé, and J. Rushen. 2001. Assessing cow comfort: effects of two floor types and two tie stall designs on the behaviour of lactating dairy cows. Appl. Anim. Behav. Sci. 71:105-117.

Hill, A. W. 1988. Protective effect of previous intramammary infection with *Streptococcus uberis* against subsequent clinical mastitis in the cow. Res. Vet. Sci. 44:386-387.

Hogan, J. S. and K. L. Smith. 1987. A practical look at environmental mastitis. Compend. Contin. Educ. Pract. Vet. 9:F341-346.

Hogan, J. S., K. L. Smith, K. H. Hoblet, D. A. Todhunter, P. S. Shoenberger, W. D. Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider, B. L. Brockett, and H. R. Conrad. 1989. Bacterial counts in bedding materials used on nine commercial dairies. J. Dairy. Sci., 72:250-258.

Hogan, J. S., K. L. Smith, D. A. Todhunter, and P. S. Shoenberger. 1990. Bacterial counts associated with recycled newspaper bedding. J. Dairy Sci. 73:1756-1761.

Hogan, J. S. and K. L. Smith. 1997. Bacteria counts in sawdust bedding. J. Dairy Sci. 80:1600-1605.

International Dairy Federation. 1999. Suggested interpretation of mastitis terminology. International Dairy Federation. 338:3-26.

Jain, N. C. 1979. Common mammary pathogens and factors in infection and mastitis. J. Dairy Sci. 62:128-134.

Janzen, J. J., J. R. Bishop, A. B. Bodine, C. A. Caldwell, and D. W. Johnson. 1982. Composted dairy waste solids and crushed limestone as a bedding in free stalls. J. Dairy Sci. 65:1025-1028.

McDonald, T. J. and J. S. McDonald. 1976. Streptococci isolated from bovine intramammary infections. Am. J. Vet. Res. 37:377-381.

National Mastitis Council. 1996. Current concepts of bovine mastitis. National Mastitis Council. Madison. USA.

Natzke, R. P. and B. L. LeClair. 1976. Coliform contaminated bedding and new infections. J. Dairy Sci. 59:2152-2154.

Neave, F. K. and J. Oliver. 1962. The relationship between the number of mastitis pathogens placed on the teats of dry cows, their survival, and the amount of intramammary infection caused. Dairy Res. 29:79-93.

Neave, F. K., F. H. Dodd, and R. A. Kingwill. 1966. A method of controlling udder disease. Vet. Res. 78:521.

Pankey, J. W., R. P. Pankey, B. M. Barker, J. H. Williamson, and M. W. Woolford. 1996. The prevalence of mastitis in primiparous heifers in eleven Waikato dairy herds. N. Z. Vet. J. 44: 41-44.

Poutrel, B. 1982. Susceptibility to mastitis: a review of factors related to the cow. Ann. Rech. Vet. 13:85-99.

Rendos, J. J., R. J. Eberhart, and E. M. Kesler. 1975. Microbial populations of teat ends of dairy cows, and bedding materials. J. Dairy Sci. 58:1492-1500.

Schultze, W. D. and P. D. Thompson. 1980. Intramammary coliform infection after heavy external contamination of teats. Am. J. Vet. Res. 41:1397-1401.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental mastitis: causes, prevalence, prevention. J. Dairy Sci. 68:1531-1553.

Zehner, M. M., R. J. Farnsworth, R. D. Appleman, K. Larntz, and J. A. Springer. 1986. Growth of environmental mastitis pathogens in various bedding materials. J. Dairy Sci. 69:1932-1941.

**Chapter II**

**Sand and sawdust bedding affect populations of coliforms, *Klebsiella* spp. and *Streptococcus* spp. on teat ends of dairy cows housed in freestalls**

**2.1 Introduction**

Cases of clinical mastitis result in significant economic losses to the dairy producer as well as impair the health and welfare of affected cows (Andrews, 2000). Mastitis is one of the major causes of involuntary culling of lactating dairy cattle (Smith et al., 1985). Dairy producers are able to minimize the incidence of clinical mastitis caused by contagious pathogens through the implementation of dry cow therapy programs and use of germicidal teat dips (Smith et al., 1985). However, the incidence of environmental mastitis has proven to be much more difficult to control because sources of environmental pathogens include soil, feces and bedding, all of which are components found within dairy cattle housing systems. No single control treatment has been shown to be effective in eliminating the bacteria responsible for infection arising from these environmental sources (Smith et al., 1985). Thus, it has been proposed that by minimizing the exposure of teat ends to pathogenic microorganisms and by maximizing the cows' resistance to infection, producers will be able to decrease the rates of environmental intramammary infections in dairy herds (Smith et al., 1985).

Dairy cattle spend between 50 and 60% of their time lying down (e.g. Dechamps et al., 1989; Haley et al., 2001), and bacteria can be transferred between the lying surface and the teats (Hogan et al., 1999; Hogan and Smith, 1997). Thus, in an effort to minimize teat end exposure to pathogenic microorganisms, it is important to understand the extent

to which the lying surface contributes to the proliferation of bacteria. Previous work in this area focused on the differences in bacterial counts between inorganic and organic bedding (Fairchild et al., 1982; Janzen et al., 1982). Counts of bacteria in inorganic bedding are usually lower than those in organic bedding, depending on the bacterial strain and the type of material (Fairchild et al., 1982). Availability of nutrients, amount of moisture available, pH and stall cleanliness are the main factors affecting bacterial growth in bedding (National Mastitis Council, 1996). Sand bedding is becoming increasingly popular on North American dairy farms in part because farmers perceive an improvement in udder health and cow comfort when cows are housed on sand (Bewley et al., 2001). However, there has been little work to date examining the bacterial strains prevalent in sand bedding (Fairchild et al., 1982; Hogan et al., 1989).

This study was designed to compare bacterial populations of mastitis-causing organisms on teats of cows housed on sand and on sawdust bedding. In addition, this study examined the relationship between the numbers of bacteria in bedding and found on teat ends. The associations between bacterial counts in bedding and the DM of the bedding and stall cleanliness were also explored.

## 2.2 Materials and Methods

The study was conducted at The University of British Columbia Dairy Education and Research Center in Agassiz, BC. The animals were cared for according to standards set by the Canadian Council on Animal Care (1993) and approved by The University of British Columbia Animal Care and Use Committee.

**2.2.1 Experimental Design**

The experiment was a crossover design with two groups of Holstein cows. Each group

rotated between two treatments: sand bedding and sawdust bedding. Groups were

subjected to each treatment for 5 weeks, but data were collected only during the last 3

weeks of each period.

**2.2.2 Animals**

Sixteen Holstein cows were selected using the following criteria: 1) animals had

previously been housed in stalls with sand and sawdust bedding; 2) cows had no history

of mastitis and somatic cell counts below 200,000 cells/ml on their last six Dairy Herd

Improvement reports; and 3) had calved within 60 d from the start of the study.

All animals were scored prior to treatment allocation for the presence of teat damage

following Britt and Farnsworth (1996) (Appendix 1). Cows were then assigned into two

experimental groups (eight cows per group) balanced according to parity (average =

2.25), stage of lactation (average = 40 d in milk), and teat end score (average = 1.3).

**2.2.3 Housing**

Selected cows were housed in two different pens within a freestall barn. Each pen had

two rows of four deep – bedded stalls each (eight stalls per pen), facing one another

('head-to-head'). Each stall had a bed length of 240 cm and the width of 117.5 cm and

was approximately 40 cm deep. The sand bedding consisted of river sand obtained from

Armstrong Sand and Gravel Ltd. (Rosedale, BC), which had been previously sieved over

a 2-mm screen with water to wash out any silt. Kiln-dried softwood sawdust was

obtained from Friesen Bros. Inc. (Burnaby, BC). Newly purchased sand was stored outside in a pile whereas new sawdust was stored in a shed. Fresh bedding was added every 7d. Visible fecal matter was removed twice daily to keep stalls visibly clean.

**2.2.4 Stall cleanliness**

The amount of fecal matter in each stall was recorded 4 d per week (at the time of bedding sampling but before daily removal of any visible fecal material) to assess stall cleanliness. A 1-$m^2$ metal grid, containing 100 equal sized squares, was placed along the back of the stall and centered between the stall partitions and the total number of squares containing any visible fecal matter recorded.

**2.2.5 Behavioral observations**

Each pen was video taped using a single camera attached to a video multiplexer (Panasonic WJ-FS 216) and a time-lapse videocassette recorder (Panasonic AG-6540). Pens were recorded for 12 h before sampling on d 1, 2 and 6 of each experimental week. To enable recording during the dark period a red light (100 W, < 5 lx) was suspended over each pen. All animals were uniquely identified with a symbol marked with color (black or blonde) dye on their coats. Videotapes were scored using scan sampling at 10 min intervals. At each scan, we recorded which cows were lying down in each stall. This sampling regime allowed us to estimate the amount of time each cow spent lying in each stall between evening and morning milking, and thus determine the potential exposure to microorganisms found in the bedding.

**2.2.6 Bedding samples**

Bedding samples were taken on d 0 (immediately after fresh bedding was added to stalls), 1, 2, and 6 of every week, during the morning milking. A 118 ml sterile plastic scoop (Bel-Art Products) was used to collect bedding samples from 16 randomly selected locations within the previously described metal grid in the back one-third of each stall. Bedding samples were collected from the same locations at each sampling. The depth of sampling (10 cm below the surface) and the total amount of material (approximately 0.5 kg) collected was consistent throughout the study. Samples from a single stall were combined in sterile 710 ml Whirl-Pak plastic bags (Nasco, Inc.). These composite samples were immediately taken to the laboratory where they were analyzed for bacterial counts.

Wet bedding samples were thoroughly mixed by shaking the plastic bag with its contents for 5 min. Sample DM was determined by placing a 25 g composite sample in a convection oven at $100^{o}C$ for 24 h or until constant weight was achieved. Samples for microbiological analyses were prepared by adding 10 g of the mixed wet bedding sample plus 90 ml of sterile 0.1 % peptone solution to a 118-ml Qorpak square glass bottle (All-Pak, Inc.). Bottles were then mixed thoroughly by manual agitation by swinging the bottles from side to side in $30^{o}$ arc for 5 min. Bottle contents were allowed to settle for 2-3 min and appropriate dilutions ($10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$) of the liquid phase were plated for enumeration on the surface of MacConkey agar (MC) (Beckman Dickinson Microbiology Systems, Canada), MacConkey-inositiol-carbenicillin agar (MCIC) (Beckman Dickinson Microbiology Systems) and Streptosel agar (Beckman Dickinson Microbiology Systems). Prior to plating each Petri dish was divided into two equal parts

by drawing a line on the plastic lid of the dish using a black felt marker. This was done so that each Petri dish could be used for plating two dilutions. Inositol (10mg/l BBB) and carbenicillin (BBB) were added to MC agar for MCIC as described by Bagley and Sheidler (1978). Inoculum (0.1 ml) was spread on the agar plates with a sterilized steel spreader. Inoculated agar plates were incubated for 24 h at $37^{o}$C and bacteria were counted using standard enumeration methods (Tortora et al., 1998). Only plates containing 20 to 200 colonies were used to estimate bacterial counts and all plates showing visible signs of cross contamination were discarded. Bacterial counts were expressed as $\log_{10}$ cfu per g of sample. Bacterial groups were identified as coliforms (lactose-positive colonies on MC agar), *Klebsiella* spp. (pink to red colonies on MCIC) and streptococci (total growth on Streptosel agar).

### 2.2.7 Teat samples

Teat samples were taken on d 1, 2 and 6 of each experimental week immediately before morning milking using BBL Collection and Transport Culture Swab (BD Microbiology Systems, Canada). Teat swabs were collected individually from all four teats by rotating a swab around the exterior of the teat orifice. Teat swabs were analyzed immediately for coliform, *Klebsiella* spp., and *Streptococcus* spp. counts. The four swabs from each cow were pooled by placing them in a single test tube containing 4 ml of peptone solution and shaken vigorously in a circular motion for 60 s. Rinse solution and its dilutions ($10^2$, $10^3$, $10^4$) were plated on the three types of agar media and processed as described previously for the bedding samples.

**2.2.8 Statistical analyses**

All potential dependent variables were screened for normality and the presence of outliers by visual assessment of the distributions and by calculation of kurtosis and skewness (Proc Univariate in SAS, version 8.2). Bacteria counts were normalized by logarithmic transformations. Observations across all three weeks and two three-week periods were averaged for each cow. The effect of bedding treatment on bacterial counts of teat ends was tested using a general linear model that accounted for treatment, cow, day and group (Proc GLM in SAS, version 8.2) to analyze bacterial counts. The least-square means test was used to compare differences between treatments (SAS version 8.2). Bacterial bedding counts were not tested for treatment differences but the averages over all weeks and phases were tested for differences within a treatment. The effect of day on bacterial counts in the bedding was tested using a general linear model that accounted for day and pen (Proc GLM in SAS, version 8.2). Correlations among bacterial counts in bedding, bacterial count on teat ends, DM and stall cleanliness were determined by Spearman correlation coefficients on the averages over all weeks and phases. Correlations between bedding counts and teat counts were determined with the aid of behavioral observations using two different methods.

*Cow- bedding count (1)*: For each cow, the time spent lying in each stall following the previous evening milking was recorded. This value (s) was then multiplied by the bacterial count for that particular stall. In cases where cows used multiple stalls this procedure was repeated for each stall and summed for all stalls. This summed value resulted in a 'cow - bedding count 1' for each individual cow. The 'cow - bedding count

1' was then correlated to the bacterial count found on the teat ends of a given cow for each experimental day.

*Cow- bedding count (2)*: For each cow, the individual stalls used were identified and the corresponding bacterial counts in the bedding noted and then averaged across stalls used and labeled as 'cow - bedding count (2)'. The relationship between the 'cow - bedding count (2)' and the bacterial count found on the teat ends of a given cow for each experimental day was calculated.

For the purposes of this thesis the correlations derived using 'cow -bedding count (1)' will be presented in the main body of the thesis. The correlations derived using 'cow - bedding count (2)' are given in Appendix 2.

## 2.3 Results

### 2.3.1 Bedding samples

#### 2.3.1.1 Coliforms

There were significantly more coliforms in sand bedding on d 1, 2, and 6 than on d 0 (P < 0.0001; Figure 2.1 A). Counts on d 0 were almost 1.1 log units lower than on d 1, 2 or 6. During the course of the week, coliform counts in sand reached their peak on d 2 and were 0.4 log units lower by d 6 (P < 0.05).

In sawdust, coliforms reached maximum population by d 2 (Figure 2.1 B). Coliform counts in sawdust were lower on d 0 and 1 than on d 2 and 6 (P < 0.001) with 1.2 log units fewer coliforms on d 0 than on d 2. Of particular interest was that the highest coliform count for sand (on d 2) was similar to the lowest count observed for sawdust (on d 0).

**2.3.1.2 *Klebsiella.***

In sand, there were significantly more *Klebsiella* spp. on d 1, 2, and 6 than on d 0 (P < 0.001; Figure 2.1 C). Over the seven-day period, *Klebsiella* spp. populations in sand reached their peak on d 2. Bacterial counts were 1.1 log units lower on d 0 than on d 2. There was no significant difference between d 2 and 6 in *Klebsiella* spp. counts in the sand bedding.

In sawdust, *Klebsiella* spp. counts were lowest on d 0, and slowly increased reaching maximum levels by d 2 (P < 0.001; Figure 2.1 D). There was 1.3 log units more *Klebsiella* spp. on d 2 than on d 0. The highest *Klebsiella* spp. counts in sand (on d 2) were lower than the lowest counts in sawdust (on d 0).

**2.3.1.3 Streptococci**

In sand, there were more streptococci on d 1, 2, and 6 than on d 0 (P < 0.0001; Figure 2.1 E). Counts increased by a 0.6 log units from d 0 to d 2 and then plateauedbetween d 2 and 6.

In sawdust bedding, streptococci counts increased continually from d 0 to d 6 (P < 0.01; Figure 2.1 F).

**2.3.1.4 Dry matter**

The mean values for DM over the study were 94.7 % and 79.5 % for sand and sawdust bedding, respectively (Table 2.1). There were no differences in DM content of the sand bedding over the week. However, the DM in sawdust bedding decreased

significantly throughout the week  (P < 0.0001) with the lowest DM (71.7 %) occurring

on d 6 (P < 0.001).


**2.3.1.5 Stall cleanliness**

For both types of bedding, the stalls became increasingly contaminated with feces

over the course of the week (Table 2.2). The mean grid count for sand was 7, and 14 for

sawdust bedding.


**2.3.2 Teat End Swabs**

**2.3.2.1 Coliforms**

Coliform counts on teat ends of cows housed on sand were higher on d 2 when

compared to d 1 and 6 (P < 0.05, Figure 2.2 A). In contrast, the coliform counts of cows

housed on sawdust increased during the week with the lowest count occurring on d 1 and

the highest count on d 6 (P < 0.01; Figure 2.2 A). On d 1 and 2, coliform counts on teat

swabs were the same for the two bedding treatment groups; however, by d 6, cows

bedded on sand had 1 log unit fewer bacteria than cows bedded on sawdust (P < 0.0001).


**2.3.2.2 *Klebsiella***

*Klebsiella* spp. counts on teat ends of cows housed on sand increased on d 2 and then

returned to their original levels on d 6 (P < 0.01)  (Figure 2.2 B). However, it should be

noted that in the present study, *Klebsiella* spp. counts from teat swabs taken from cows

housed on sand on d 0 were often below the sensitivity of the plating procedures ($< 10^{-2}$

cfu/g). *Klebsiella* spp. counts on teat ends of cows housed on sawdust increased steadily

during the week reaching a maximum on d 6 (P < 0.01). Overall, there were 0.8 log unit more *Klebsiella* spp. on the teat swabs collected from cows when bedded on sawdust compared to when they were housed on sand (P < 0.0001).

### 2.3.2.3 Streptococci

Streptococcal counts on teat swabs for cows bedded on sand were the same on d 1 and 2, but were greater on d 6 (P < 0.05) (Figure 2.2 C). However, when cows were bedded on sawdust, teat swabs counts increased progressively during the course of the week. Cows bedded on sand had on average 1 log unit higher streptococcal counts than cows bedded on sawdust (P < 0.0001). The lowest count for cows bedded on sand was the same as the highest count for cows bedded on sawdust.

### 2.3.3 Correlation between bacterial counts in bedding and on teat ends

There was a significant correlation between 'cow - bedding count 1' and the bacterial counts on teat swabs for cows housed on sand: r = 0.35 (P < 0.05) for coliforms and r = 0.40 (P < 0.05) for *Klebsiella* spp. There was no significant correlation for 'cow - bedding counts 1' and streptococci counts r = 0.28 (P = 0.06). For cows exposed to the sawdust treatment, correlation coefficients were r = 0.47 (P < 0.001), r = 0.69 (P < 0.001), and r = 0.60 (P < 0.001) for coliforms, *Klebsiella* spp., and streptococci, respectively. Correlations for each treatment by day are presented in Appendix 3.

**2.3.4 Correlation between bedding bacterial counts and bedding DM**

For sand bedding, only coliform counts were correlated to bedding DM ($r = 0.31$; $P <$ 0.01). For sawdust treatments, all types of bacteria were negatively correlated to bedding DM with correlation coefficients of $r = -0.57$ ($P < 0.0001$) for coliforms, $r = -0.47$ ($P <$ 0.0001) for *Klebsiella* spp., and $r = -0.66$ ($P < 0.0001$) for streptococci. Results for a correlation between bedding bacterial counts and bedding DM by three-week periods are presented in Appendix 4.

**2.3.5 Correlation between bedding counts and stall cleanliness**

Correlation coefficients between bacterial counts in sand and stall cleanliness were $r =$ 0.46 ($P < 0.001$) for coliforms, $r = 0.50$ ($P < 0.001$) for *Klebsiella* spp., and $r = 0.48$ ($P <$ 0.0001) for streptococci. Correlation coefficients for bacterial counts in sawdust were $r =$ 0.43 ($P < 0.0001$) for coliforms, $r = 0.30$ ($P < 0.0001$) for *Klebsiella* spp., and $r = 0.35$ ($P < 0.0001$) for streptococci. Results for correlations between bedding bacterial counts and stall cleanliness by three-week periods are presented in Appendix 5.

**2.4 Discussion**

In the present study, it was determined that bacterial counts on teat ends are strongly correlated with bacterial counts in sawdust bedding which is consistent with previous reports in literature (Rendos et al., 1975; Hogan et al., 1989; Hogan et al., 1999; Hogan and Smith, 1997). The results for sawdust fell well within the range of other experiments. For example, Hogan and Smith (1997) reported correlations coefficients for bacterial counts in sawdust and teat end swabs: $r = 0.62$ for coliforms, $r = 0.78$ for *Klebsiella* spp.,

and r = 0.90 for streptococci. However, their study was done with cows housed in tie stalls; whereas, the present study was using dairy cows housed in freestalls. The use of the freestall housing equipped with video cameras allowed us to estimate the exposure to the bacterial populations present in the lying material by each cow by correlating the 'cow – bedding count 1' to bacterial counts on teat ends of cows housed. The weak relationship between bacterial counts in sand bedding and those on teat ends suggests that the mechanism of bacterial contamination teat ends of cows housed on sand is different than for sawdust. The physical properties of sand, namely its adhesion to skin surfaces, possibly due to high DM content, and abrasiveness, may make bacterial exposure occur in a more sporadic manner for this type of bedding.

Counts of both coliforms and *Klebsiella* spp. were higher in bedding and on teat ends when using sawdust compared to sand as bedding. These findings are in agreement with other studies (Fairchild et al., 1982; Janzen et al., 1982; Bramley and Neave, 1975). In the current study, we found between three and six times more coliforms and *Klebsiella* spp. in sawdust than in sand. Fairchild et al. (1982) reported lower coliform and *Klebsiella* spp. counts in sand than in either sawdust or newspaper bedding. The National Mastitis Council (1996) reported more coliforms and *Klebsiella* spp. in sawdust bedding than in sand bedding and their results were well within the range of our results. Increased coliforms and *Klebsiella* spp. in sawdust can be attributed to the presence of moisture and availability of nutrients. Over the course of a week, stalls bedded with sawdust were more likely to become contaminated with manure and exhibited decreasing DM levels. In contrast, the DM content of the sand bedding remained constant over the period of the study regardless of the extent of manure contamination. The apparent differences in grid

count between sand and sawdust can be accounted for by difficulties in distinguishing between fecal matter and sand. Manure contamination was visually harder to identify in gray sand than in yellow sawdust. Not surprisingly, coliforms, *Klebsiella* spp., and streptococci counts in sawdust were positively correlated to bedding DM. Therefore, it is clear that moisture plays an important role in the susceptibility for sawdust to harbor bacterial growth. We found *Klebsiella* spp. were most often associated with sawdust bedding, and previous work has documented outbreaks of this infection in cows bedded on fresh sawdust (Bramley and Neave, 1975; Fairchild et al., 1982). Contamination by this pathogen can vary depending on the type of wood, the kiln-drying process and the handling and storage of sawdust on the farm (Zehner et al., 1986). In the present experiment, sawdust was stored indoors protected from potential environmental contamination.

In the present study, teat ends of cows housed on sand had at least 10 times higher streptococci counts than on teat ends of cows housed on sawdust bedding. These results do not correspond with Janzen et al, (1982) who reported all bacterial counts being lower (*Escherichia coli*, *Enterobacter*, *Staphylococcus aureus*, *Staphylococcus epidermis*, and streptococci) in inorganic material (limestone) than in organic material (dairy waste solids, and the mixture of limestone and dairy waste solids). However, in contrast to sand, limestone is an alkaline material and its high pH may not be suitable for the growth of many microorganisms. The reasons for the elevated levels of *Streptococcus* spp. in sand in the current study might include the ability of these bacteria to proliferate in anaerobic environments. Sawdust is lighter in weight in comparison to sand and therefore there may be more oxygen available between sawdust particles. Consequently,

immediately below the sand surface, the oxygen supply may be limited, promoting the growth of anaerobes such as *Streptococcus* spp. In addition, water activity in the sand at the sampled depth may have been limiting and may not have been conducive to proliferation of some bacterial strains. It may be that there was a lot of bacterial growth at the interface of the sand but most of the liquid and nutrients would have accumulated on the concrete floor underneath the sand layer; therefore, conditions for multiplication of some strains of bacteria may not be favourable.

Coliforms are complex organisms that can metabolize a variety of organic compounds and they can be found in many bedding materials (Madigan et al., 2000). In sawdust, there were a variety of nutrients available for bacterial growth resulting in ideal conditions for coliform species growth (Zehner et al., 1986); whereas, sand was somewhat limited in nutrient and since streptococci are more robust organisms than coliforms, they were more abundant in sand. In dairy bedding, there are many strains of bacteria competing for the same resources; therefore, compounds broken down by one group of bacteria can be utilized by others. Zehner et al. (1986) reported that when chopped straw was inoculated with a mixed culture of *S. uberis* and *K. pneumoniae, S. uberis* showed greater growth than in any of the other treatments using the *S. uberis* and straw combination. Our own findings showed elevated *Streptococcus* spp. with corresponding low levels of coliforms and *Klebsiella* spp. in sand bedding.

Actual bacterial counts varied during the course of the week for both sand and sawdust bedding. Bacterial counts in sawdust increased at the beginning of the week, reaching their maximum population numbers by d 2. The initial bacterial populations might be due to the availability of nutrients in fresh sawdust. As the week progressed, the

sawdust bedding became more contaminated with manure, possibly resulting in differences in potential nutrient availability for bacteria. The competition between bacterial populations increased, causing their total numbers to decline. Hogan and Smith (1997) and Hogan et al. (1999) reported similar time trends for bacterial growth within sawdust bedding.

Bedding counts were positively correlated to stall cleanliness. It has been shown in previous studies that feces and urine contamination of bedding plays an important role in bacterial multiplication (Carroll and Jasper, 1978; National Mastitis Council, 1996; Zehner et al., 1986). The present study was not able to address the relationship between bacterial counts on teat ends and the rates of IMI.

## 2.5 Conclusion

Bacterial counts in sawdust bedding are correlated to bacterial counts on teat ends of dairy cows housed on these surfaces. Overall, coliform and *Klebsiella* spp. were more numerous on teat ends of cows housed on sawdust bedding but *Streptococcus* spp. were more numerous on teat ends of cows housed on sand.

**Table 2.1**. Mean ± least squares S. E. dry matter (%) of freestall bedding over each week of observations in sand and sawdust bedding (n = 16).

| Bedding type | Day | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 1 | | 2 | | 6 | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Sand | 93.7 | ± 0.8 | 95.1 | ± 0.8 | 95.3 | ± 0.8 | 94.9 | ± 0.8 |
| Sawdust | 86.9$^a$ | ± 0.8 | 80.5$^b$ | ± 0.8 | 79.1$^b$ | ± 0.8 | 71.7$^c$ | ± 0.8 |

$^{a-b}$ Means with different superscripts differ within the row ($P < 0.0001$)

**Table 2.2**. Mean ± least squares S. E. stall cleanliness (grid count) over each week of observations in sand and sawdust bedding (n = 16).

| Bedding type | Day | | | | | |
| | 1 | | 2 | | 6 | |
| | Mean | SE | Mean | SE | Mean | SE |
|---|---|---|---|---|---|---|
| Sand | 5 [a] | ± 1 | 7 [a] | ± 1 | 11 [b] | ± 1 |
| Sawdust | 12 [a] | ± 1 | 14 [ab] | ± 1 | 16 [b] | ± 1 |

[a-b] Means with different superscripts differ within the row (P < 0.01)

**Sand**                                    **Sawdust**
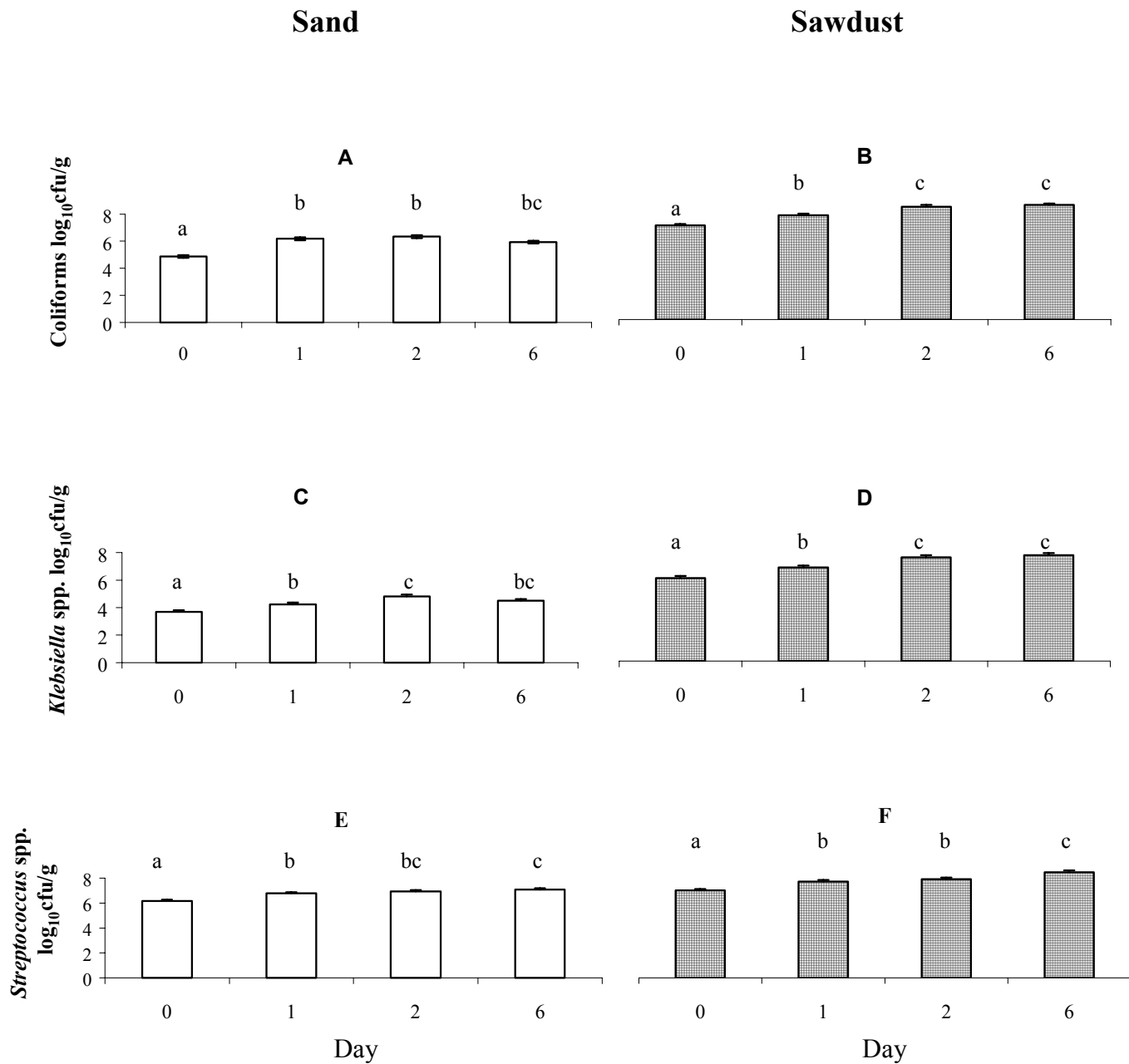


**Figure 2.1**. Mean + standard error counts of coliforms in sand (A) and sawdust (B), *Klebsiella* spp. in sand (C) and sawdust (D), and streptococci in sand (E) and sawdust (F) on d 0, 1, 2, and 6 after the addition of fresh bedding in 16 freestalls (a, b, c - means with different letters are significant within a figure at P < 0.05).

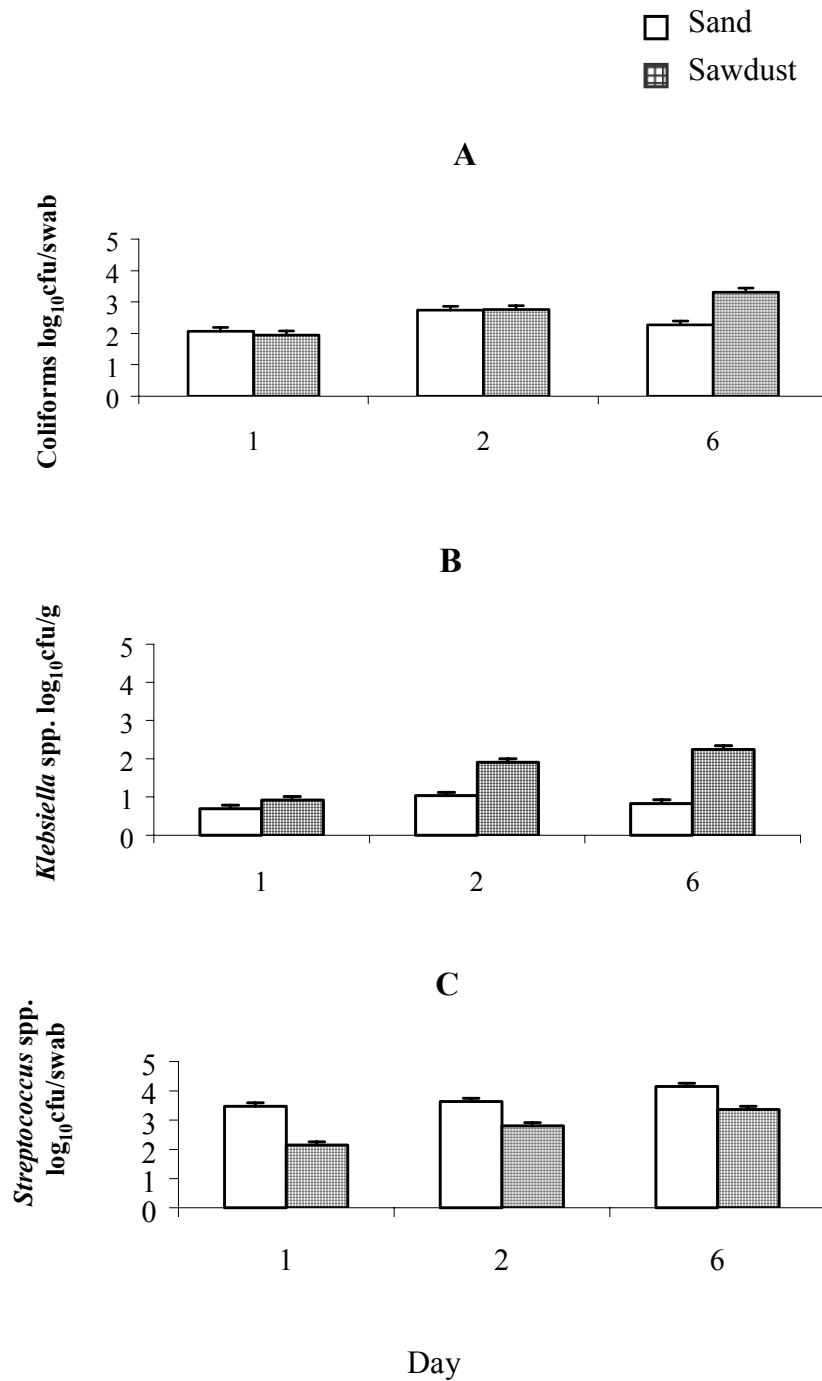**Figure 2.2**. Mean + standard error counts of coliforms (A), *Klebsiella spp.* (B), and *Streptococcus spp.* (C) from teat swabs of 16 cows bedded on sand and sawdust on d 1, 2, and 6 after the addition of fresh bedding to the freestalls.

## 2.6 References

Andrews, A. H., ed. 2000. The health of dairy cattle. Blackwell Science. Oxford. UK.

Bagley, S. T. and R. J. Sheidler. 1978. Primary *Klebsiella* identification with
MacConkey–inositol-carbenicillin agar. Appl. Environ. Microbiol. 6:536-539.

Bewley, J., R. W. Palmer, and D. B. Jackson-Smith. 2001. A comparison of free–stall
barns used by modernized Wisconsin dairies. J. Dairy Sci. 84:528-541.

Bramley, A. J. and F. K. Neave. 1975. Studies on the control of coliform mastitis in
dairy cows. Br. Vet. J. 131:160-169.

Britt, J. S. and R. Farnsworth. 1996. Here's a system for evaluating teat condition.
Hoard's Dairyman. August 25. page 570.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental
animals. Volume 1. E. D. Olfert, B. M. Cross, and A. A. McWilliam, eds. 2nd.
CCAC, Ottawa, Canada.

Carroll, E. J. and D. E. Jasper. 1978. Distribution of Enterobacteriaceae in recycled
manure bedding on California dairies. J. Dairy Sci. 61:1498-1508.

Dechamps, P., B. Nicks, B. Canart, M. Gielen, and L. Istasse, 1989. A note on resting
behaviour of cows before and after calving in two different housing systems.
Appl. Anim. Behav. Sci. 23:99-105.

Fairchild, T. P., B. J. McArthur, J. H. Moore, and W. E. Hylton. 1982. Coliform counts
in various bedding material. J. Dairy Sci. 65:1029-1035.

Haley, D. B., A. M. de Passillé, and J. Rushen. 2001. Assessing cow comfort: effects of
two floor type sand two tie stall designs on the behaviour of lactating dairy cows.
Appl. Anim. Behav. Sci. 71:105-117.

Hogan, J. S. and K. L. Smith. 1987. A practical look at environmental mastitis.

Compend. Contin. Educ. Pract. Vet. 9:341-346.

Hogan, J. S., K. L. Smith, K. H. Hoblet, D. A. Todhunter, P. S. Shoenberger, W. D.

Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider, B. L. Brockett, and H. R.

Conrad. 1989. Bacterial counts in bedding materials used on nine commercial

dairies. J. Dairy. Sci., 72:250-258.

Hogan, J. S. and K. L. Smith. 1997. Bacteria counts in sawdust bedding. J. Dairy Sci.

80:1600-1605.

Hogan, J. S., V. L. Bogacz, L. M. Thompson, S. Romig, P. S. Schoenberger, W. P.

Weiss, and K. L. Smith. 1999. Bacterial counts associated with sawdust and

recycled manure bedding treated with commercial conditioners. J. Dairy Sci.

82:1690-1695.

Janzen, J. J., J. R. Bishop, A. B. Bodine, C. A. Caldwell, and D. W. Johnson. 1982.

Composted dairy waste solids and crushed limestone as a bedding in freestalls. J.

Dairy Sci. 65:1025-1028.

Madigan, M. T., J. M. Martinko, and J. Parker. 2000. Brock Biology of Microorganisms.

9th ed. Prentice Hall. Upper Saddle River, USA.

National Mastitis Council. 1996. Current concepts of bovine mastitis. National Mastitis

Council. Madison. USA.

Rendos, J. J., R. J. Eberhart, and E. M. Kesler. 1975. Microbial populations of teat ends

of dairy cows, and bedding materials. J. Dairy Sci. 58:1492-1500.

SAS User's Guide: Statistics, Version 8.2 Edition. 2002. SAS Inst., Inc., Cary. USA.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental mastitis:

causes, prevalence, prevention. J. Dairy Sci. 68:1531-1553.

Tortora, G.J., B.R. Funke, and C.L. Case. 1998. Microbiology: an introduction. 6th ed. Benjamin/Cummings Publishing Co. Inc., Menlo Park, USA.

Zehner, M. M., R. J. Farnsworth, R. D. Appleman, K. Larntz, and J. A. Springer. 1986. Growth of environmental mastitis pathogens in various bedding materials. J. Dairy Sci. 69:1932-1941.

**CHAPTER III**

**3.1 General conclusion**

In the lower Fraser Valley region of British Columbia many dairy farmers have begun to adopt sand as their preferred choice of free-stall bedding. The literature reviewed in Chapter I established that there is a relationship between bacterial counts in bedding and on the teat ends of cows. However, there are reasons to suspect that this relationship may not hold for sand bedding. There has been little research published on the use of sand as a bedding material for dairy cows.

The findings presented in Chapter II show a significant correlation between bacterial counts in sand and sawdust bedding and those on teat ends from cows housed on sand and sawdust, in agreement with previous research.

As reported in Chapter II, and in agreement with previous work, the present results showed that there were more coliform and *Klebsiella* spp. associated with sawdust than with sand bedding. However, I also found that there were more *Streptococcus* spp. on teats of cows housed on sand than on sawdust, the first such published report in the scientific literature. Mastitis cases arising from *Streptococcus* spp. are easier to treat than the coliform cases which are more typical of sawdust and other organic bedding materials.

The experiment described in Chapter II was not designed to address the effects of this bedding treatment on rates of clinical mastitis. This would be of great interest for future work, but given the relatively low prevalence of such infections, a study similar to the present one would require far greater animal numbers and a longer period of study. Another research project focusing on a comparison between the rates and the types of

intramammary infections across dairy farms using either sand or sawdust to bed dairy

cows would provide a better insight for understanding the role of sand in mastitis control

and prevention.

**Appendix 1**

Teat end classification system (Britt and Farnsworth, 1996).

| Teat end score | Description |
| --- | --- |
| 1 | Smooth bottom, no or smooth callous, no lesions |
| 2 | Raised callous ring with slight roughness |
| 3 | Rough callous with hyperkeratosis |
| 4 | Very rough callous with hyperkeratosis and radial cracking |
| 5 | Lesions: open skin, hemorrhage, trauma or any abnormal condition |

**Appendix 2**

Correlations between 'cow – bedding count 2' and bacterial counts on teat ends of cows presented as means by day across all experimental weeks.

| Bedding type | Bacteria | Correlation coefficients (r) for cow-bedding count and bacterial counts on teat ends of cows | | | |
|---|---|---|---|---|---|
| | | Overall | Day 1 | Day 2 | Day 6 |
| | Coliforms | NS | NS | NS | 0.62* |
| Sand | *Klebsiella* spp. | 0.32* | - 0.53* | NS | NS |
| | *Streptococcus* spp. | NS | NS | NS | NS |
| | Coliforms | 0.46* | - 0.63* | NS | NS |
| Sawdust | *Klebsiella* spp. | 0.62** | NS | NS | NS |
| | *Streptococcus* spp. | 0.50** | - 0.50* | NS | NS |

\* P < 0.05
\*\* P < 0.001

## Appendix 3

Correlations between 'cow – bedding count 1' and bacterial counts on teat ends of cows presented as means by day across all experimental weeks.

| Bedding type | Bacteria | Correlation coefficients (r) for cow-bedding count and bacterial counts on teat ends of cows | | | |
|---|---|---|---|---|---|
| | | Mean | Day 1 | Day 2 | Day 6 |
| Sand | Coliforms | 0.35* | NS | NS | 0.56* |
| | *Klebsiella* spp. | 0.40* | NS | NS | 0.53* |
| | *Streptococcus* spp. | NS | NS | NS | NS |
| Sawdust | Coliforms | 0.47** | NS | NS | NS |
| | *Klebsiella* spp. | 0.69** | NS | 0.60* | NS |
| | *Streptococcus* spp. | 0.60** | NS | NS | NS |

* P < 0.05
** P < 0.001

**Appendix 4**

Correlation between bacterial counts in the bedding and DM of the bedding presented as means across all experimental weeks and days by two three-week periods.

| Bedding type | Bacteria | Correlation coefficients (r) for bedding bacterial count and DM of bedding | | |
|---|---|---|---|---|
| | | Mean | Period 1 | Period 2 |
| Sand | Coliforms | 0.31* | NS | 0.75* |
| | *Klebsiella* spp. | NS | NS | 0.47* |
| | *Streptococcus* spp. | NS | NS | 0.57* |
| Sawdust | Coliforms | -0.57* | -0.73* | -0.52* |
| | *Klebsiella* spp. | -0.47* | -0.77* | -0.31* |
| | *Streptococcus* spp. | -0.66* | -0.86* | -0.69* |

* significant at P < 0.05

# Appendix 5

Correlation between bacterial counts in the bedding and stall cleanliness presented as means across all experimental weeks and days by two three-week periods.

| Bedding type | Bacteria | Correlation coefficients (r) for bedding bacterial count and stall cleanliness | | |
| --- | --- | --- | --- | --- |
| | | Mean | Period 1 | Period 2 |
| Sand | Coliforms | 0.46* | 0.46* | 0.60* |
| | *Klebsiella* spp. | 0.50* | 0.39* | 0.66* |
| | *Streptococcus* spp. | 0.48* | 0.53* | 0.64* |
| Sawdust | Coliforms | 0.43* | 0.69* | 0.62* |
| | *Klebsiella* spp. | 0.30* | 0.72* | 0.41* |
| | *Streptococcus* spp. | 0.35* | 0.66* | 0.66* |

* significant at $P < 0.05$