2 LITERATURE REVIEW

2.1 Intra-mammary infection (IMI)

In most countries, dairy cattle breeding programs are directed toward milk production traits. Although these traits are of primary economic importance, functional traits such as longevity fertility and udder health are of increased interest to producers to improve herd profitability.

Mastitis is defined as an infection of the udder, caused by bacteria entering the quarter through the teat end (Rodenburg, 1990), and according to the US national mastitis council’s current concepts of bovine mastitis (1996): mastitis is an inflammation of the mammary gland in response to injury for the purpose of destroying and neutralizing the infectious agents and to prepare the way for healing and return to normal function. Inflammation can be caused by many types of injury including infectious agents and their toxins, physical trauma or chemical irritants (Jones and Bailey, 1998). Mastitis is one of the most common dairy diseases (Rajala-Schultz et al. 1999) because of its high incidence (Seegers et al. 1997a and Seegers et al. 1997b). The economic consequences of mastitis either clinical or sub-clinical include loss of milk production, loss of milk sales, increased culling rates, and cost for veterinary treatments, in addition to that high SCC in milk affect the price of milk in many payment systems that are based on milk quality (Schukken et al. 1997). Milk cell count has been used extensively as an indicator of the infection status of the mammary gland (Hillerton, 1999). The German Veterinary Medicine Association (DVG, 1994) categorized the udder health status as shown in table 1.

Table 1: Categorization of udder health status (DVG, 1994)

| Cell count per ml milk | Pathogenic organisms       |  |
|------------------------|-----------------------------|
| < 100x10³               | Normal secretion            | latent infection |
| > 100x10³              | Non-specific mastitis       | mastitis         |

The legal maximum bulk tank SCC is lower in other dairy exporting countries than USA (Smith and Hogan, 1998). Canada has a limit of 500x10³ cells/ml, in the European community, Norway, Switzerland, Australia and New Zealand the maximum bulk tank SCC is 400x10³ cells/ml. In those countries, SCC is calculated as a geometric mean of
successive milk shipments over several weeks, therefore, it is expected to be lower than arithmetic mean (Shook and Ruegg, 1999).

2.2 Classes of mastitis

2.2.1 Clinical mastitis

Clinical mastitis is defined as an infection of the udder that results in visible changes in the udder quarter and milk (Rodenburg, 1990), may it be acute, sub acute or chronic. The development of clinical mastitis in dairy cows can be detected with high sensitivity and specificity in advance of visible changes in foremilk or udder tissue by determining the electrical conductivity of the foremilk (Milner et al. 1997). Weller et al. (1992) and Pösö and Mantysaari (1996) stated that the genetic correlations between clinical mastitis and SCS among different lactations were positive and moderate to high (varied from 0.37 for the first lactation to 0.68 for the third lactation). Whereas Mrode and Swanson (1996) estimated a genetic correlation between SCC and incidence of mastitis of 0.7. Peeler et al. (2000) in a study to assess the level of clinical mastitis and to quantify risk factors associated with the incidence rate of clinical mastitis in U.K, found a mean incidence rate of clinical mastitis of 22.8 cases per 100 cows/year. They also reported that the incidence rate of clinical mastitis increased when farmers reported that they had straw yard housing for milking cows (compared with cubicle housing), mucked out the calving area less frequently than once per month, when they had greater than 50% replacement rate and when always practiced post-milking teat disinfection. Barkema et al. (1999) attributed the increase in the incidence rate of clinical mastitis in herds practicing post-milking teat disinfection to E. coli infections. While Wilson and Kingwill (1975) and Wilesmith et al. (1986) claimed that the incidence rate of clinical mastitis in Great Britain has declined from an estimated 120 cases per 100 cows/year in 1960 to approximately 40 cases per 100 cows/year in 1986 due to a reduction in mastitis caused by contagious pathogens particularly S. aureus, St. agalactia and St. dysgalactia through the introduction of improved control measures. Booth (1988) reported that the reduction in the prevalence of contagious pathogens resulted in a decrease of the average bulk milk SCC from 573x10^3 cells/ml to 352x10^3 cells/ml. But Barkema et al. (1998) showed in a recent study that there was no association between bulk milk SCC and incidence rate of clinical mastitis. Aarestrup and Jensen (1997) found that the presence of bacteria in a quarter before parturition increased the risk of IMI for the lactating cow. And the variability in the prevalence and the duration of intra mammary infection according to the bacterial species
occurred around the first parturition. Lescourret and Coulon (1994) and Schukken et al. (1997) reported that mastitis has many economic consequences among which are loss of milk production, loss of milk sales, increased culling rates and cost for veterinary treatments, in addition to that high SCC in milk affects the price of milk. Rajala-Schultz et al. (1999) studied the effect of clinical mastitis on milk yield in dairy cows, they found that the daily loss during the first 2 weeks after the occurrence of mastitis varied from 1.0 kg to 2.5 kg and the total loss over the entire lactation varied from 110 kg to 352 kg; cows with mastitis did not reach their pre mastitis milk yields during the remainder of the lactation after onset of the disease. Rupp and Boichard (1999) indicated that SCC is a more accurate measure of udder health than records of clinical mastitis. Because SCC are generally routinely recorded in most milk recording systems, in the time that clinical mastitis events are not routinely recorded in most countries except in Scandinavian countries and the field data may not be accurate, complete or standard. In addition to that the heritability of SCC is much greater (0.15) than that of clinical mastitis (0.02-0.03) and SCC also reflects incidence of sub-clinical infections. Trinidad et al. (1990) studied the prevalence of IMI in unbred and primigravid dairy heifers, they found that 97% had IMI and 29% showed clinical symptoms, 75% of the quarters were infected. Presence of mammary inflammation in young dairy animals could be deleterious to the future milk production as the mammary tissue development occurs to the large extent during the first gestation (Anderson, 1985 and Tucker, 1987). Etherington et al. (1996) reported that 6.8% of the culling rate of cows in Ontario-Canada was due to mastitis. Mastitis also found to reduce both milk production (Fetrow et al. 1991) and reproductive performance in a lactating cow (Cullor, 1990; Moore et al. 1991; Moore and O’Connor, 1993). Barker et al. (1998) demonstrated that cows with clinical mastitis during early lactation exhibited a prolonged interval until first service (94 days) compared with animals with no clinical mastitis (71 days). Additionally, cows with clinical mastitis between the first service and the establishment of pregnancy had increased number of days open and a two fold increase in services/conception. Rupp and Boichard (2000) stated that without clinical signs of mastitis during the first month of lactation and with a first test day a SCC lower than 400x10^3 cells/ml. they also claimed that the risk of first clinical mastitis was highest around the second calving in lactation starting in summer and for high-yielding cows. The probability of clinical mastitis occurring increased continuously as initial SCC increased. they also concluded that cows with the lowest initial SCC had the lowest risk for clinical mastitis without any intermediate optimum.
A group of researchers (Emanuelson et al. 1998; Weller et al. 1992; Lund et al. 1994 and Pösö and Mäntysaari, 1996) reported that direct selection against clinical mastitis is difficult because in most countries other than the Nordic ones clinical mastitis event is not widely recorded. And because the corresponding heritability of the trait is very low close to 0.02, while Heringstad et al. (1999) estimated heritability of clinical mastitis in Norwegian cattle to be 0.035.

2.2.2 Subclinical mastitis

Rodenburg (1990) showed that 97% of all cases of mastitis are sub-clinical which do not involve visible changes to the quarter or the milk it produces. While Reneau and Packard (1991) reported that approximately 70 to 80% of the mastitis cases are sub-clinical. Sub-clinical mastitis is found to be associated with decreased milk yield, also a positive relationship clinical mastitis with milk yield has been found (Dohoo and Martin, 1984; Fetrow et al. 1991). Laevens et al. (1997) indicated that the measurement of SCC from dairy herd improvement programs is used worldwide as an indicators of sub-clinical mastitis. Ruffo et al. (1978) and Harmon and Reneau (1993) reported in different studies that IMI have been recognized as major factors that influence SCC. Milk from healthy udder quarters was found to have an average value of SCC between $23 \times 10^3 - 50 \times 10^3$ cells/ml depending on the breed and the physiological status of the animal (Klaas, 2000). The milk yield starts to drop with an increase in SCC over $100 \times 10^3$ cells/ml (Korhonen and Kaartinen, 1995). They also showed that the increase in SCC to a level more than $100 \times 10^3$ cells/ml resulted in 18% reduction in milk yield. De Graaf and Dwinger (1996) estimated the crude milk production losses per cow with sub-clinical mastitis as 1.56 kg/day for daily milk yield, and the milk production loss per affected quarter due to sub-clinical mastitis was estimated to be 17.6% on average. They concluded that the decrease in milk production in heifers with sub-clinical mastitis did not differ significantly from the decrease in production in older cows. Sub-clinical mastitis is also known to affect the reproductive performance of the animals. Schrick et al. (2001) found that cows with sub-clinical mastitis before the first service had an increase of days to first service (74.8±2.7d), days open (107.7±6.9d) and services per conception (2.1±0.2) compared with the control (67.8±2.2d, 85.4±5.8d and 1.6±0.2; p<0.05).

2.3 Etiology and Epidemiology

Mastitis is known to be established as a result of the reaction of three bio-systems namely the causative agent, the animal and the environment in which the animal lives. Sandholm
and Korhonen (1995) reported that the primary and secondary body defense mechanisms prevent the pathogenic microbes from entering the mammary gland through the teat canal orifice. They also indicated that the concentrations of the antibacterial factors in the udder secretion are under genetic control and depend on the lactation stage and udder health. The environmental factors such as management, feeding, hygienic status, bedding, milking and the virulence of the organism contribute to the disease. Lesile (1996) reported that stress factors such as isolation of an individual and mixing groups of cows have been shown to increase somatic cells count in the absence of mastitis, moreover it has been reported that there was no increase in SCC.

2.4 Causative agents

2.4.1 Classes of mastitis pathogens

Several researchers (Bramley, 1985; Wendt et al. 1994; Smith and Hogan 1995) concluded that mastitis causing organisms can be classified into two main groups: Contagious pathogens which spread by means of hands, milking units and include *S. aureus*, *St. agalactiae*, and Mycoplasma. Environmental organisms which live in the cow’s environment and are always present, they include *E. coli*, *St. dysg.*, *St. ubris*. Buzalski and Pyörälä (1995) stated that contagious mastitis is mainly caused by Staphylococci and shows high cell count in bulk milk whereas environmental mastitis results in a high number of clinical cases, but the cell count in the bulk milk is usually not high. Another group of mastitis causing organisms called minor pathogens (Keown, 1997) and include *C. bovis* and CNS. Buzalski and Seuna, (1995) reviewed the results of the microbiological examinations of milk samples that were done in Finish milk inspection laboratories in 1991 and reported the frequency of mastitis causing organisms as given in table 2.
Table 2: Frequency of mastitis causing organisms (FMI, 1991)

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. of samples</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. agalactia</td>
<td>1389</td>
<td>0.63</td>
</tr>
<tr>
<td>St. dysg.</td>
<td>9397</td>
<td>4.29</td>
</tr>
<tr>
<td>St. ubris</td>
<td>10767</td>
<td>4.91</td>
</tr>
<tr>
<td>β-haemolytic streptococci</td>
<td>1553</td>
<td>0.71</td>
</tr>
<tr>
<td>S. aureus</td>
<td>42546</td>
<td>19.42</td>
</tr>
<tr>
<td>CNS</td>
<td>30417</td>
<td>13.88</td>
</tr>
<tr>
<td>E. coli</td>
<td>3178</td>
<td>1.42</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>722</td>
<td>0.33</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>144</td>
<td>0.07</td>
</tr>
<tr>
<td>Actinomyces pyogenes</td>
<td>1272</td>
<td>0.58</td>
</tr>
<tr>
<td>Yeast, moulds and fungi</td>
<td>1224</td>
<td>0.56</td>
</tr>
<tr>
<td>Other</td>
<td>10615</td>
<td>4.84</td>
</tr>
<tr>
<td>Total</td>
<td>113224</td>
<td>51.67</td>
</tr>
<tr>
<td>No growth</td>
<td>105892</td>
<td>48.33</td>
</tr>
<tr>
<td>All samples</td>
<td>219116</td>
<td>100.00</td>
</tr>
</tbody>
</table>

2.4.2 Mode of transmission

Several research studies concluded that the contagious organisms spread during the milking process (Bramley, 1985; Smith and Hogan, 1995; Bray and Shearer, 1996) causing an infection of the udder as a result of entering the teat canal (Rodenburg, 1990). The former authors also showed that scar or connective tissue replacing the destructed milk secreting tissues and result in a permanent loss of the productive ability. Sandholm and Korhonen (1995) reported that the udder becomes infected through the teat canal which represents a physical barrier to the penetration of bacteria. They also added that when the udder is dilated the risk of infection is high. An infected mammary gland can act as a reservoir for mastitis microbes (Davidson, 1961; Barnes et al. 1987). Pre-partum heifer infections have been attributed to the feeding of mastitic milk to heifer calves and allowing heifers to suckle each other (Mc Donald, 1982), however, in another studies it was found that feeding contaminated milk did not increase the prevalence of IMI at parturition over control heifers fed milk free from contagious organisms (Barto et al. 1982; Bushnell,
Kirk (1996) presented that the high risk of contagious organisms can be from the movement of animals onto the dairy herd as they may carry in a pathogen which did not exist or they may themselves not have immunity to pathogens already exist. Chrystal et al. (1999) stated that nearly all IMI occur as a result of microorganisms passing through the teat canal, and that wider teat diameters were associated with higher SCS. On the other hand, David and Shearer (1986) reported that the environmental organisms mainly live in the animal’s environment like rumen and udder. The organism can also be found in feces, polluted water and bedding material. The inflammation results from the cow’s reaction to the bacterial irritation and the progress of the infection depends on the ability of bacteria to adapt to milk environment and on various virulence factors (Ali-Vehmas and Sandholm, 1995).

2.5 Contagious pathogens

2.5.1 *S. aureus*

Bray and Shearer (1986) reported that *S. aureus* Lives in the udder and on the skin surfaces of an infected cow. Ali-Vehmas and Sandholm (1995) showed that the organism can produce capsular material, hemolysin and β-lactamase when incubated in mastitic milk and are transmitted from infected quarters to uninfected quarters during the milking process (Risco et al. 1999). Bray and Shearer (1986) found that *S. aureus* is one of the organisms responsible for about 95% of IMI. Bramley and Dodd (1984) found that *S. aureus* is the most prevalent and costly of the major mastitis pathogens and can result in both clinical and sub-clinical mastitis. Roberson et al. (1994) found that the mean prevalence of *S. aureus* IMI in high prevalence herds (>10%) to be 30% where as the mean prevalence of *S. aureus* IMI in a low prevalence (<5%) herds was 2%. Trinidad et al. (1990) isolated *S. aureus* from 37% of all cases and 14.9% of the quarters. White and McDonald (1961); Oliver and Mitchell (1983) and Pankey et al. (1991) reported that the prevalence of *S. aureus* IMI in primiparous cows at parturition to range from 2-50%. The prevalence of *S. aureus* IMI in pre-partum heifers varied considerably among different regions and herds, Daniel et al. (1986) and Pankey et al. (1991) found a very low prevalence of *S. aureus* IMI. While Aarestrup and Jensen (1991) found no evidence of *S. aureus* infection at all. Other researchers (Trinidad et al. 1990 and Nickerson et al. 1995) reported a relatively high prevalence. Waage et al. (1999) in a study of dairy heifers found that *S. aureus* was most frequently isolated organism from quarters (44.3%). Trinidad et al. (1990) reported 20% of all infected quarters was *S. aureus*. In Latvia a study was conducted by Jemeljanovs et
al. (1999) showed that 55.17% of all cases of udder inflammation of 439 cows' udder secretion were caused by *S. aureus*. Gentilini et al. (1994) discovered that *S. aureus* is considered one of the most etiologic agents in Argentina. Jones and Ward (1989) found that of 20% Staphylococci isolated, 14 were *S. aureus*, and that cows immunization by *S. aureus* experimental vaccine increased their resistance and decreased SCC in comparison with the control groups (Jemeljanovs and Bluzmanis, 2000). (Lucey and Rowlands, 1984; Erb, 1985 and Firat, 1993) reported that *S. aureus* IMI reduced milk yield 230 Kg, while the somatic cells count found to be 900x10^3/ml compared to 200x10^3/ml of non-*S. aureus* infection (Buelow, unpublished thesis, 1993 cited by Zepeda et al. 2000). Barkema et al. (1999) presented that the incidence rate of mastitis caused by *S. aureus* was mostly related to factors associated with bulk milk SCC.

### 2.5.2 *St. agalactiae*

*St. agalactiae* belongs to the group of pyogenic hemolytic streptococci and serologically to Lancefield’s group B (Buzalski and Seuna, 1995). *St. agalactiae* is an obligate organism of the cow’s udder, mastitis caused by it spreads particularly during the milking through the equipment, and is highly contagious, either chronic or recurrent, often the cell count of the milk remains quite low (Pyörrälä, 1995). Morin and Hurley (1999) stated that *St. agalactiae* inhibits ducts and cisterns of the mammary gland. It causes an inflammation which blocks the ducts, leading to decreased milk production and increased SCC. Barkema et al. (1999) reported a 0.004 incidence rate of mastitis of *St. agalactiae* and as was associated with management practices. The US national Mastitis Council (1996) published that *St. agalactiae* as a contagious bacteria is transmitted from infected quarters to uninfected quarters during the milking process. Jemeljanovs and Bluzmanis (2000) showed that 14.85% of the mastitis cases in Latvia was *St. agalactiae*. The organism was reported to have the highest interclass correlation within a cow for natural logarithm SCC (Barkema et al. 1997). In the forties of the last century it was reported that feeding milk containing *St. agalactiae* to heifers calves and subsequent suckling among heifers would result in IMI by this major contagious pathogen at first parturition (Roberson et al. 1994). Ma et al. (2000) found that in milk collected from Holstein cows after IMI with *St. agalactiae*, post infection milk had significantly higher somatic cells count (849x10^3/ml) than pre-infection milk (45x10^3/ml). In a study for mastitis control Bray and Shearer (1986) found that *St. agalactiae* lives in the udder and can not exit outside the gland for a long period, it is
susceptible to penicillin and once eliminated usually does not return to the herd unless infected cows are purchased.

2.6 Environmental pathogens

2.6.1 *St. dysg.*

*St. dysg.* is one of the major pathogens belongs to the Lancefield’s group C, *St. dysg.* is no longer included in the Streptococci group, but retained the name in the mastitis field (Buzalski and Seuna, 1995). The organism lives almost anywhere: in the udder, rumen and feces and in the barn, its spread can be stopped by dipping the whole teat to the base of the udder (Bray and Shearer, 1986). The pathogen is most prevalent in the examined quarter milk samples from 1500 heifers with clinical mastitis before or within 14d after parturition (Jonsson et al. 1991). Pyörälä (1995) stated that the identification of the organism is based primarily on a biochemical reaction and can be isolated from summer mastitis. Sansdholm and Payörälä (1995) found that the incidence of *St. dysg.* increases in herds where teat dipping and dry cow therapy are applied. Whereas, Payörälä and Myllys (1995) reported that the organism is highly susceptible to Penicillin and its derivatives. On the other hand Buzalski and Payörälä (1995) showed that herds infected with *St. dysg.* appears as high cell counts in the bulk milk. Payörälä and Buzalski (1995) found that the organism is found to be associated with teat lesions. In the study conducted by Barkema et al. (1997) it was shown that a lower intra-class correlation within herd (0.03) was detected between the frequency of the organism and SCC (log). Waage et al. (1999) found that the frequency of *St. dysg.* was 18.2% of 1040 heifer’s quarters samples affected with clinical mastitis and that was collected prior or within 14 d after parturition. Aarestrup and Jensen (1997) discovered a strong association between IMI with *St. dysg.* before parturition and IMI with *St. dysg.* after parturition. Whereas Barkema et al.(1999) found a strong positive correlation between the incidence rate of clinical mastitis caused by *St. dysg.* and that caused by *S. aureus*. They also added that the incidence rate of mastitis caused by *St. dysg.* was related to nutrition, milking technique and machine milking. Østerås et al. (1999) stated that a cow had an infection or identification of a major pathogen 45±32 days prior to drying off and a series of composite milk SCC>100x10³/ml before sampling.
2.6.2 *E. coli*

*E. coli* is an environmental polluted organism. It lives in feces, polluted water and bedding materials, it is not susceptible to antibiotics (Bray and Shearer, 1986). The organism belongs to the family Enterobacteriaceae. The injury of the teat canal often leads to acute mastitis caused by *E. coli* (Buzalski and Pyörälä, 1995), and hence it is considered to be an environmental pathogen (Radostits et al.1994). Hogan and Smith (1987) found that the microorganisms may be eliminated before or shortly after onset of clinical symptoms, therefore the host defense system appears to eliminate *E. coli* efficiently (Hill et al. 1978), especially when IMI occurs late in lactation (Hill and Shears, 1979). Recurrent clinical episodes were found in 9.1% of quarters with mastitis caused by *E. coli* (Lam et al. 1996; Lipman et al. 1994), whereas Waage et al. (1999) found the frequency of *E. coli* to be 6.4% from infected quarters. *E. coli* was one of the most prevalent pathogens in the study of Jonsson et al. (1991). Döpfer et al. (1999) discovered that in 4.77% of all episodes of clinical mastitis caused by *E. coli*, persistent IMI caused by the same *E. coli* strain. Jones and Ward (1989) reported that *E. coli* was the predominant cause of mastitis in early and late lactation. Barkema et al. (1999) stated that the incidence rate of clinical mastitis caused by *E. coli* was mostly related to housing, hygienic measures and machine milking.

2.7 Minor pathogens

2.7.1 CNS

CNS were previously called micrococci, species most often isolated from CNS mastitis are *S. hyicus*, *S. simulans*, *S. epidermidis*, *S. warned*, *S.xylosus*, *S. hominis*, *S.haemolyticus* and *S. chromogens* (Buzalski and Seuna, 1995). Mastitis caused by them occurs at all stages of lactation but is most common during drying-off and soon after calving and considered milder than *S. aureus* mastitis because they possess less virulence factors than *S. aureus* (Bramley, 1991). CNS bacteria can often cause teat infection which cause only a slight increase in milk cells count, mastitis occurs particularly in heifers. Jones and Ward (1989) found that of 20 Staphylococci isolated four were CNS, which were seen in cows soon after parturition and caused 14% cases of mastitis. A similar finding was reported by Pankey et al. (1996), they stated that CNS were isolated from 21.8% of the heifers in Waikato. Studies in USA have reported that up to 90% of heifers quarters are infected before parturition and 70% were infected with CNS (Trinidad et al.1990). Aarestrup and
Jensen (1997) found that *S. chromogenes* was the bacterial species isolated most often before parturition (15% of quarters). Whereas Waage et al. (1999) found that of the most prevalent isolates of the CNS were *S. simulans* (53.7%), *S. hyicus* (14.8%) and *S. chromogenes* (14.8%). They also concluded that CNS were the main cause of sub-clinical IMI. Laevens et al. (1997) concluded in a study that a single isolation of CNS was resulted in statistically increase in SCC with least square mean SCC (log_{e}-transformed) as 3.97.

### 2.7.2 *C. bovis*

*C. bovis* is a relatively common causal agent of a mild mastitis, it requires oleic acid present in milk to grow (Buzalski and Seuna ,1995). This organism is considered to be a typical contaminant of milk flowing from the udder (Mantere-Alhonen, 1995). Classified as environmental pathogen that usually causes considerably less somatic cells count elevation (Keown, 1997). Laevens et al.(1997) indicated that a single isolation of *C. bovis* was associated with a numerical increase in somatic cells count. However, Sheldrake et al. (1983) and Rainard et al.(1990) in different studies concluded that a single isolation of *C. bovis* considered to be a false-positive result. Barkema et al.(1997) found in a study that *C. bovis* had the highest intra-class correlation within herd (0.11) with the natural logarithm of SCC.

### 2.8 Factors influencing determinants of IMI

#### 2.8.1 Factors influencing frequency of pathogens and infection rate

Infectious mastitis is present when the pathogen and the inflammatory changes were detected in the secretion, whereas non specific mastitis is present when there were inflammatory changes but no pathogen in the secretion and a latent infection is present when the secretion contained pathogens but had normal cell count (IDF, 1987). Waage et al. (2000) analyzing data of 1122 infected quarters that were clinically affected found that after treatment the reexamination results showed 22% non functional quarters, 14% still affected by clinical mastitis and 12% affected by sub-clinical mastitis. Hogan and Smith (1987) stated that the percentage of quarters infected with environmental streptococci is low and seldom exceeds 10% of quarters. A group of researchers (Linde et al.1980; Brooks and Barnum, 1984; Pankey et al.1985; Watts, 1988; Woodward et al.1988) concluded that in herds in which post-milking teat antisepsis is not practiced, it is not unusual for *C. bovis* to be isolated from more than 60% of quarter milk samples and the new infection rate of such organism was nearly 30 times higher than that of *St. agalactiae* which is attributed to
teat colonization and subsequent contamination of milk samples. Kingwill et al. (1970) (cited after Peeler et al. 2000) stated that the reduction in the incidence rate of mastitis in Great Britain is attributed to the reduction in mastitis caused by contagious pathogens through the introduction of improved control measures. Shoshani and Berman (1998) assessed sub-clinical mastitis by deviation in milk yield and suggested that there are episodic aggravations in mammary health that do not evolve into mastitis but may induce significant losses in milk yield and quality.

2.8.1.1 Herd size

It was earlier suggested that there was a relation between the farm performance and the farm structure (van Asseldonk et al. 1998). Herd size was observed as a risk factor for mastitis with a significant influence (Waage et al. 1998). Although herd size was found to have no significant effect on the occurrence of mastitis in the study of Costa et al. (1998), but Smith et al. (2000) stated that small herds reported more cows leaving for mastitis than high medium and low medium herd size. Wilesmith et al. (1986) claimed that the incidence of mastitis declined with increasing herd size.

2.8.1.2 Year-Season

Waage et al. (1999) in their study of the bacteria associated with mastitis in dairy heifers found that the proportion of *S. aureus* and *Actinomyces pyogenes* were highest and the proportion of CNS were lowest in late autumn and early winter. The proportion of *E. coli* was highest in summer, they concluded that the relative percentage were significantly affected by season. Jonsson et al. (1991) who examined quarter milk samples of 1500 heifers with mastitis before or 14d after parturition, stated that the relative percentages of some organisms were significantly affected by season. Jones and Ward (1989) in their study of the cause of mastitis in dairy cows in Wisconsin, detected mastitis with approximately equal frequency throughout the year. Hogan et al. (1989) in their field survey of clinical mastitis in low SCC herds showed that the rate of infection was different among seasons of the year. Shpigel et al. (1998) reported that the incidence of mastitis in Israeli dairy herds was lower in summer months.

2.8.1.3 Lactation number

The US national mastitis council (1997a) showed that the rate of streptococcal infection increases progressively as the lactation number increases. Schaeffer and Solbu (1987) who investigated the Norwegian red cattle, reported that a first lactation cows had a 10%
probability of having mastitis, which was roughly the same for second, third and fourth lactation, provided that they did not have mastitis in the previous lactations. While cows that had mastitis in the immediately previous lactation, had double this probability of having mastitis again. A fourth lactation cow that had mastitis in the three previous lactations had a 62% probability of having mastitis in the fourth lactation. They also concluded that there does not seem to be an age effect on the probability of mastitis occurrence and any cow that has not had mastitis previously has a 10-11% chance of having mastitis in the current lactation regardless of parity number. Analogous findings were reported by Firat (1993) who analyzed data dealing with susceptibility of clinical mastitis in successive lactations and indicated that cows with mastitis in the preceding lactation were almost twice susceptible to mastitis in the current lactation than those without mastitis in the preceding lactation with probabilities of 0.46 and 0.29, respectively. Fetrow et al. (1991) reported that the carry-over effect of mastitis from one lactation to the next found to be statistically significant but small. Nickerson et al. (1995) found in a Louisiana study of 116 pregnant and unbred Jersey heifers with collected samples from four herds that the bacterial infection were present in 97% of heifers and 75% of quarters, and there were 2.8 infected quarters per animal. Shpigel et al. (1998) observed an increase in the incidence of mastitis as the lactation number increases till the fifth lactation then start to decrease. Hogan et al. (1989) stated that the incidence of mastitis caused by environmental bacteria in the first and second lactation is greater than in older cows. Different from the result that obtained by Zadoks et al. (2001) who found that the rate of infection with *St. uberis* was lower in first and second parity cows than in older cows and was depending on the stage of lactation in one herd. Fleischer et al. (2001) found a significant relationship between the previous 305 days milk yield and the incidence of mastitis.

**2.8.1.4 Stage of lactation**

It is known that the risk of environmental mastitis infection is highest during early lactation and decreases as the lactation advances. The US national mastitis council (1997b) stated that the rate of IMI is higher during the dry period than during lactation, and during the first 75 days postpartum the rate of infection is higher than it is during the remainder of lactation. The percentage of infected quarters with environmental streptococci at any one point is generally low and seldom exceeds 10% of quarters. In an early study, Munch-Petersen (1970) stated that 22% of all quarters in heifers were already infected by the first
day of lactation, and by the end of the first week of the lactation the infection decreased to 9.4%. Trinidad et al. (1990), reporting a US study, found that up to 90% of heifers had quarters infected before parturition, while other researchers in the USA and Europe (Munch-Petersen, 1970; Meaney, 1981; Oliver and Mitchell, 1983; Pankey et al. 1991 and Matthews et al. 1992) claimed that the IMI rate in heifers was moderate (13 to 39%). Jones et al. (1998) stated that the last 7-10 days before calving or early lactation is the time of greatest susceptibility to new environmental streptococci infections.

2.8.1.5 Farm management factors

The US national mastitis council’s fact sheet (1997b) states that housed cows are at greater risk for environmental mastitis compared to cows on pasture. And that post milking teat barrier dips reduce new coliform IMI but their efficacy against the environmental streptococci and contagious pathogens appears to be lower than that of germicidal preparations. They showed also that backflushing of the milking unit does not control environmental mastitis. Additionally, malfunctioning milking machines which result in frequent liner slips and teat impacts can increase cases of environmental mastitis. Washburn et al. (2002) compared seasonally calved Holstein and Jersey cows in confinement or pasture systems and found that cows in confinement had 1.8 times more cases of clinical mastitis and 8 times the culling rate for mastitis than did cows on pasture. Jones and Bailey (1998) reported that purchased heifers from another source could harbor mastitis pathogens and should be sampled for bacteriological culture after calving and should be isolated from the other milking animals until tested negative. In the past decade, hygiene and management practices have been provided as standard program to control IMI (Neave et al. 1969). Radostits et al. (1994) summarized the control measures of mastitis among which pre-milking udder hygiene, post-milking teat dipping and environmental control during the dry and calving periods are to be mentioned. Each of these control measures is aimed at the management of specific pathogen types. Natzke (1981); Pankey (1989); Boddie et al. (1993) and Malinowski (2000) concluded that pre-milking udder hygiene and teat dipping are aimed at reducing infections mainly caused by contagious pathogens and preventing new infections and to a lesser extent at preventing infections that might be caused by environmental pathogens. While Smith et al. (1985) and Todhunter et al. (1995) showed that the environmental management during the transition and calving periods is targeted primarily at preventing new infection with environmental streptococcal species and Coliform bacteria e.g. *E. coli*, *Klebsiella* spp. Over half of the environmental
pathogens acquired during the dry period persist to lactation. Sargeant et al. (2001) claimed that producing high quality milk will require effective udder health programs at the herd level. Management practices at the time of dry-off and during the dry period are essential in this respect. Peeler et al. (2000) in their study of risk factors associated with clinical mastitis in low SCC British dairy herds found that the incidence of mastitis increases when milking cows were housed in straw yard, cows were standing in the yard after milking, which always practiced post-milking teat disinfection and had greater than 50% replacement rate. They discovered also that the incidence of mastitis was lower when the gathering yard used before milking was scraped at least twice a day. Oliver et al. (2001) demonstrated that pre-and post-milking teat disinfections with phenolic combination were significantly more effective in preventing new IMI than was post-milking teat disinfections only. They also added that pre-milking teat disinfections with phenolic combination in association with good udder preparation and post-milking teat disinfections can further reduce the occurrence of new IMI by numerous mastitis pathogens during lactation. A similar conclusion was reported by Saloniemi and Kulkas (2001) who described the mastitis control in Finland. They recommended post-milking teat dipping as control tool in herds with contagious udder pathogen problem. Hogan and Smith (1987) in their practical look at environmental mastitis concluded that no single uniform management procedure effectively prevents environmental mastitis under controlled conditions. Rodenburg (1990) claimed that high energy or high protein diets do not increase or decrease the number of new mastitis infections, however, feeding high producing cows for maximum production does increase stress on the udder and may cause infected cows to flare-up. Rodenburg also showed that too small stalls subjected animals to teat injury. In free-stall barns cows are less likely to lie in the dirt and the lying area is always of adequate size.

2.8.2 Factors influencing levels of SCC

The measurement of SCC from dairy improvement programs is used worldwide as an indicator of sub-clinical mastitis (Ostensson, 1993) because of its relatively high genetic correlation with mastitis which was estimated to be ~0.7 (Mrode and Swanson, 1996) and an important criterion of quality payment systems. As an indicator for the hygienic quality of milk and for the mastitis status in a given herd (DVG, 1989), cow SCC is used to trace sub-clinically infected cows (Laevens et al. 1997), is relatively easy to record and has a higher heritability ($h^2=0.11$) than mastitis incidence ($h^2=0.04$) (Mrode and Swanson, 1996). Philipsson et al. (1995) concluded that it is possible to improve resistance to mastitis by
selecting for a low SCC, due to the higher heritability of the SCC. Philipsson added that selection based on the heritability of the SCC was more efficient than selection directly on mastitis. Results of several studies indicated that SCC is a more accurate measure of the udder health, as it is routinely recorded in most milk recording systems (Rupp and Boichard, 1999). Ma et al. (2000) stated that post-infection milk had a significantly higher SCC (849X10³ cells/ml) than pre-infection milk (45x10³ cells/ml) in experimentally intramammary infected Holstein cows. A high SCC was found to decrease the value of milk intended for manufacturing, has adverse effects in cheese making, reduces curd firmness and decreases cheese yield, and increases fat and casein loss in whey (Politis and Ng-Kwai-Hang, 1988a; Politis and Ng-Kwai-Hang, 1988b; Barbano et al. 1991; Klei et al. 1998).

2.8.2.1 Herd size

Herd size and SCC were declared to be negatively related, and larger herds had lower SCC than smaller herds (Norman et al. 2000; Oleggini et al. 2001; Van Schaik et al. 2002). Lafi et al (1994) found that the mean value of SCC was negatively associated with herd size. Norman et al. (2000) added that herd size and SCC were negatively related and large herds had a lower SCC. Peeler et al. (2000) stated that herds with greater than 50% replacement rate indicate that herd size was increasing culling for some reasons including high individual cow SCC.

2.8.2.2 Year-season

Season of calving is reported to has a significant effect on milk SCC and SCS (Corbett, 1998; Rodriguez et al. 2000). However, Liebe et al. (1996) reported no influence of season on SCC of German brown cows. Leslie (1996) found that SCC were lowest during winter and highest during the summer months of July and August, he attributed the seasonal variations to the effect of housing and temperature changes on infection status. Kelly et al. (2000) found a significant seasonal influence on milk SCC, with cows calving in spring having a SCC>160x10³ cells/ml with higher proportions of polymorphnuclear leukocytes in the total milk SCC than milk from autumn calving cows. Norman et al.(2000) estimated the mean herd SCC to be lower during October through January (280x10³ to 300x10³ cells/ml) than during July and August (340x10³ cells/ml). Rupp et al. (2000) illustrated that regardless of the lactation stage, SCC were higher in summer and lower in autumn of the milk SCC in French dairy breeds. Whereas Allore et al.(1997) found that SCC were significantly higher in spring than in fall. However, Jemeljanovs and Bluzmanis (2000)
determined a seasonal effect on SCC. They claimed that SCC/ml milk was less in summer, a little more in autumn and more high in spring and most SCC encountered in winter. Season was suggested to has no significant influence on SCC in healthy mammary glands (Malinowski, 2001).

2.8.2.3 Lactation number

Several studies revealed a significant effect of the cow age and the lactation number on the level of milk SCC (Corbett, 1998; Kelly et al. 2000; Seker et al. 2000; Haile-Mariam et al. 2001). Kiiman and Saveli (2000) studied the factors affecting milk SCC and reported that milk SCC increased with increasing lactation number, in the first lactation SCC was 285x10^3 whereas in the second, third and fourth lactations were 321x10^3, 461x10^3 and 477x10^3, respectively. Godollo and Tanszek (2000) reviewed 98 scientific publications related to physiological and environmental factors influencing SCC. They reported that the number of lactation significantly affect the SCC in milk. A similar conclusion was realized by Labohm et al. (1998) who found that lactation number influence the SCC in a statistically reliable extent. But attributed the rise in SCC above 100x10^3 to infected quarter. Leslie (1996) reported that higher SCC have been found in the milk of older cows. Hortet and Seegers (1998) investigated the relationship between SCC and variation in milk production at the cow level, they indicated that at the test-day level an average loss of 0.4 kg milk in primiparous cows and 0.6 kg in multiparous by each 2-fold increase of SCC above 50x10^3 cells/ml. At the lactation level, the average trend was a loss of 80 kg of milk in primiparous and 120 kg in multiparous by each 2-fold increase of the geometric mean of SCC above 50x10^3 cells/ml. Similar results were published by Hortet et al. (1999) who found that the reduction in milk yield in kg increased with parity and with days in milk to the extent that the reduction in milk yield was 0.32 kg per 100x10^3 cells/ml increase in SCC, 0.63 kg per 200x10^3 cells/ml SCC and 1.13 kg decrease in milk per 600x10^3 cells/ml increase in SCC. This result is in joint agreement to that of Jemeljanovs and Bluzmanis (2000) in their study of somatic cell and micro-organisms contents in milk. They revealed that SCC in milk increased in clinically healthy cows with the increase in the age. The further interpretation of these findings is that: if 90% of the 2nd lactation cows had up to 200x10^3 cells/ml, then only 63.4% of the older than the 4th lactation cows had such level of SCC and 18.1% had more than 500x10^3 cells/ml SCC. These findings supported the results published earlier by Tyler et al. (1989) who stated that primiparous and multiparous cows were similarly showed production losses due to the increase in SCC. In primiparous cattle
with SCC range $403 \times 10^3$-$665 \times 10^3$ had 5.22 kg decrease in test-day milk yield whereas multiparous cows with the same range had 3.01 kg reduction in milk yield. Koldeweij et al. (1999) found a geometric mean for SCC of 63.1 in the first lactation and 107.2 in the later lactations. They also found an individual milk yield loss of 1.29 kg/day for each unit increase in $\log_{10}(\text{SCC})$ for cows in the first lactation and 2.04 kg/day milk yield decrease per unit $\log_{10}(\text{SCC})$ for cows in the later lactations. Kiiman and Saveli (2000) found a significant ($p<0.001$) effect of lactation number on milk SCC, they found that in the first lactation the milk SCC was $285 \times 10^3$/ml, in the second and third lactation $321 \times 10^3$/ml and $461 \times 10^3$/ml respectively. Laevens et al. (1997) stated no significant effect of lactation number on SCC when cows were bacteriologically negative and the least square mean of SCC for first, second and third lactations were 3.80, 3.93 and 3.97, respectively. Schepers et al. (1997) estimated the variance components for SCC, they illustrated the shape of the SCC curve which was flat for the first lactation cows compared with the shape of the SCC curve for cows in the subsequent lactations.

### 2.8.2.4 Stage of Lactation

A group of researchers reported that SCC and milk yield traits vary the stage of lactation (Vech et al. 1989; Corbett, 1998; Labohm et al. 1998; Kelly et al. 2000; Rupp et al. 2000) and with test-day (Haile-Mariam et al. 2001). Schepers et al. (1997) showed that stage of lactation affected the SCC, since the logarithm SCC was high at the beginning of the lactation, dropped to a minimum between 40 and 80 days postpartum and then steadily increased until the end of lactation. Carnier et al. (1997) stated that from a genetic view point, SCS in early lactation behaves differently from those in later stages of lactation. Williams et al. (1991) claimed that stage of lactation had a pronounced effect on milk SCC, with the level being high in early lactation, low in mid-lactation and high again in late lactation. However, Rodriguez et al. (2000) stated that milk SCS typically reaches a minimum early in lactation and then rises, but lactations starting between October and December had the highest fall of SCS at the beginning of lactation, and smallest increase thereafter. Early results were obtained by Emanuelson et al. (1988) who found a significant effect of the stage of lactation on SCC of morning milk samples from cows over 18 months and concluded that stage of lactation must be taken into account when establishing normal values for ATP as an indicator of mastitis. Seker et al. (2000) found that a positive CMT score increased in Brown-Swiss cows with higher yield and at the 4th and 6th month of lactation. Kirk et al. (1996) indicated that sub-clinical infection with minor pathogens
(primarily CNS.) had no significant effect on average SCC during early and mid lactation. Laevens et al. (1997) obtained least squares mean SCC for first, second and third parity bacteriological negative cows as 3.80, 3.93 and 3.97 respectively, with no significant effect of parity, stage of lactation and parity, stage of lactation interaction, however the effect was significant when including the data of both infected and bacterial free cows.

2.8.2.5 Farm management factors

In the past decade, the standard mastitis control program has provided hygienic and management practices to control IMI (Neave et al. 1969), a decrease in bulk milk SCC is an indicator of the success of the control program (Suriyasathaporn et al. 2000). Yalcin et al.(1999) studied the impact of mastitis control procedures in Scottish dairy herds, and concluded that udder preparation involving washing was associated with higher SCC and had detrimental effects on the efficacy of post-milking teat disinfections. Smith and Ely (1997) reported that free-stall bedding did not significantly affect milk quality, with no difference in linear SCS among the herds studied. They also showed that herds fed inside the free-stall barn or under covered roof had higher milk production and lower SCS than those fed outside. However, Bewley et al. (2001) stated in a comparison of free-stall barns used by modernized Wisconsin dairies that herds with four-row free-stall barns had higher production than herds with six-row barns and that the average linear was SCS significantly (p<0.05) lower in new four-row barns than six-row barns (2.71 vs. 2.95). Omore et al. (1999) assessed the impact of a clinical trial of three mastitis control strategies among which improved udder hygiene in smallholder dairy farms in Kenya, they concluded that the trial had some impact in lowering the prevalence of contagious pathogens by 18%, but found no significant increase in milk yield or lowered SCC. Barkema et al. (1998) reported about post-milking teat disinfections and good milking management as important factors for the prevention of a high bulk milk SCC. Godollo and Tanszek (2000) indicated that technological environment, feeding and milking are known to interfere with changes in SCC. Mazzucchelli et al. (2000) gave an account of the changes in the management of a Spanish herd of cows affected by mastitis by making a dietary adjustment, an improvement of the housing management and improving the design of milking parlors and management of milking. These changes resulted in a reduction of the milk SCC from $380 \times 10^3$ cells/ml to $200 \times 10^3$ cells/ml. Kiiman (2001) indicated that the adequate pre-milking cow preparation was essential to milk SCC as well as over-milking (p<0.001). He
also stated that the effect of milking equipment was not statistically significant for milk SCC.

2.8.3 High milk yield

Gröhn (2000) studied the relationship between disease and milk production, he found that high milk yield predisposed a cow to certain diseases particularly and mastitis. Whitaker et al. (2000) found that there was a positive association between bulk milk SCC and mastitis rate. Haile-Mariam et al. (2001) estimated the correlation between test-day yield and SCC, they stated that genetic correlations between yield and log SCC were positive at the beginning and negative at the end of the first lactation, in the second and third lactations genetic correlations were nearly zero at the beginning of the lactation but negative at the end, however, environmental correlations were always negative. The authors attributed the positive correlations to the fact that high producers are more susceptible to mastitis than cows with average or low production whereas the negative correlations in the second half of the first parity and later parities due to the mastitis cause high SCC and udder damage resulting in reduced milk yield. These findings support results presented by Gröhn et al. (1995) who claimed that cows with mastitis are often higher yielding cows, which produce more milk even having contracted the disease, compared to their healthy and generally lower yielding herd-mates.