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CROSS SECTIONAL & LONGITUDINAL STUDY OF BOVINE MASTITIS IN URBAN & PERI-URBAN DAIRY SYSTEMS IN THE ADDIS ABABA REGION, ETHIOPIA

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A thesis submitted in partial fulfilment for the degree of Master of Science in Tropical Veterinary Epidemiology at the Freie Universität Berlin and Addis Ababa University

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CROSS SECTIONAL AND LONGITUDINAL STUDY OF BOVINE MASTITIS IN URBAN AND PERI-URBAN DAIRY SYSTEMS IN THE ADDIS ABABA REGION, ETHIOPIA

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TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AACM	Australian Agricultural Consulting and Management Company
AADPA	Addis Ababa Dairy Producers Association
CI	Cumulative Incidence rates
CNS	Coagulase Negative Staphylococci
CMT	California Mastitis Test
°c	Degree celcius
DMSCC	Direct Method of Somatic Cell Count
ID	Incidence Density
IMI	Intra Mammary Infection
LF	Left Front
LR	Left Rear
m^2	meter square
MOA	Ministry Of Agriculture
NGO	Non Government Organization
RF	Right Front
RR	Right Rear
SCC	Somatic Cell Count
SSA	Sub-Saharan Africa

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SUMMARY

Prevalence and incidence of bovine mastitis was investigated in detailed in 114 farms organized under the Addis Ababa Dairy Producers Association in 1999 for 7 months. Udder infection was one of the frequently recorded clinical cases in the study area. A detailed study of mastitis was required for the formulation of possible control measures. In the 114 urban and peri-urban dairy farms 479 lactating cows were examined for mastitis using the California Mastitis Test, Somatic Cell Count and bacteriological diagnostic methods in a prevalence and follow-up incidence study. The prevalence of clinical mastitis was 5% and 1.8% at the cow and quarter levels respectively. The prevalence of subclinical mastitis was 32.2% and 13.6% at cow and quarter levels respectively. The prevalence of subclinical mastitis was significantly different ($p < 0.001$) between different size categories at the cow as well as quarter levels.

The incidence density rate in the 3 investigation months and the incidence per cow-month of clinical mastitis were 4% and 1.33 %, respectively. The incidence rate of clinical mastitis was highest in the intermediate farm size category followed by the large and small farm categories. Mean farm incidence rates of clinical mastitis between different farm sizes were significantly different ($p < 0.001$). The number of new cases decreased from the first to the last interval in all farm categories. The incidence density rate in the 3 months and the incidence per cow-month of subclinical mastitis were 33.6 % and 11.2 %, respectively. The mean farm incidence rates between the different farm sizes were also significantly different ($p < 0.001$) for subclinical mastitis. The incidence in the small farms (42.5%) was higher than in the intermediate (30.2%) and the large (25.6 %) farms, taken separately. As with clinical mastitis the number of new cases decreased from the first to the last interval in all farm categories. At the end of the longitudinal study, a final cross-sectional study again was carried out to assess the intensity and direction of mastitis. The prevalence of clinical mastitis was 2.2% and 0.7% at the cow and quarter level, respectively. The prevalence of subclinical mastitis was 24% and 10.3% at the cow and quarter level, respectively. There was a clear drop of mastitis prevalence in the farms investigated when compared with the initial prevalence study.

Potential risk factors identified included general hygiene of cows, workers and cubicles; ventilation of barns, drainage of barns, parity, stage of lactation and herd size. The prevalence ratios for hygiene, ventilation, parity, stage of lactation and drainage were 5.2, 5, 2.57, 1.23 and 1.9, respectively. Multivariate analysis, using logistic regression of SPSS program,

indicated that when ventilation, stage of lactation, herd size and parity were kept constant, the Odds Ratio for hygiene was 2.0. For parity, the other three variables kept constant, the Odds Ratio was 3.5, for ventilation 2.1, stage of lactation 0.74 and herd size 0.95 (at $p < 0.05$). In this adjusted analysis, drainage and location were found to have no statistical significance on mastitis occurrence ($p > 0.05$).

A total of 683 agent isolations were done in the initial prevalence study, 249 for major pathogens and 434 for minor pathogens. Emphasis was given to the major pathogens. *Staphylococcus aureus* and *Streptococcus* species were major mastitis pathogens most frequently encountered, comprising 58% of the total major pathogenic microorganisms isolated. The most commonly isolated agents were coagulase negative staphylococci which were 41.9% of total isolates. The profiles of the etiological agents identified in the incidence and final prevalence studies were similar with those recovered in the initial study, with the exception that yeasts were not found. A total of 246 isolates of major and minor pathogens obtained in the study were tested for their *in vitro* susceptibility to 11 antimicrobial drugs. Generally, erythromycin, polymyxin B, sulphamethoxazole, gentamycin and oxytetracycline were found to be effective drugs. Ampicillin, tetracycline and streptomycin, in contrast, were found to be the weakest of all the drugs used in the antibiogram. Streptomycin, however, was effective against *Streptococci*. *Staphylococcus aureus* showed the widest spectrum of resistance next to yeasts.

For evaluation of a linear association between Somatic Cell Count and California Mastitis Test, regression analysis was carried out. The results showed a fitting linear model to describe the relationship between the two screening tests for the 4 teats (RF, RR, LF and LR). There was a statistically significant ($p < 0.01$) relationship between CMT and SCC at the 99% confidence level. The R–squared statistic indicated that the model fitted explained 40.6%, 44.3%, 39.0% and 52.7% for RF, RR, LF and LR quarters; respectively; of the variability in the corresponding CMTs. The correlation coefficients were 0.64, 0.67, 0.63 and 0.73 for RF, RR, LF and LR quarters; respectively, indicating a moderately strong relationship between the variables. The mean somatic cell counts increased with the increase in CMT grades. The CMT grades of ++, +++ and somatic cell counts of more than 500,000 were found to be good predictors of positive bacterial culture of milk samples.

Mastitis was found to be one of the major constraints to the dairy farms studied. The prevalence and incidence of infection was related to management factors.

1. INTRODUCTION AND OBJECTIVES

Dairying as a component of livestock production is an important economic activity in Sub-Saharan Africa (SSA). For instance, the share of the locally produced milk in the value of all locally produced livestock food products in SSA has averaged well over 50 per cent since the beginning of the 1970s (Mbogoh, 1984). However, SSA as a region has not performed satisfactorily in terms of achieving self-sufficiency in dairy products over the last two or so decades. As a result, the level of dairy imports into the region, either on commercial terms or as food aid, has continued to increase relatively fast since the 1960s (Mbogoh, 1984; von Massow, 1984). Given this situation, there is a dire need to intensify efforts and improve dairy production in SSA.

Meeting the food needs of urban populations in particular is of growing concern in developing countries. The case of dairy products in SSA is illustrative: demand for milk and dairy products exceeds supply in most parts of SSA (Brokken and Seyoum, 1992). Over the last decades, the population growth in SSA, combined with rising per capita income has caused rapid growth in food consumption, in particular of dairy products. The World Bank (1992) has estimated that demand for milk and dairy products in SSA will increase by 5.5 million tones by the year 2025, an annual growth rate of 4%, questioning the supply side. Much of this increased demand will be concentrated in urban areas. The population in SSA is expected to increase by 2.75% a year between 1990 and 2025, resulting in an additional 800 million people to feed. Of these, over 500 million will live in cities and large towns. Meeting the food needs of these people will present an enormous challenge to African farmers and their governments for whom welfare of urban consumers is becoming a political concern.

Increased domestic dairy production by small holders has the potential in much of Africa to generate income and employment on a wide scale, and thus to improve the welfare of people on an economically sustainable basis (Walshe *et al.*, 1991, Winrock International, 1992). In urban and peri-urban areas around the world, dairy typically has been part of the
adjustment of production patterns when small holder farmers have been faced with shrinking arable land, higher human population density, and rising wage rates. Under such conditions, dairy farming may be one of the few activities that can provide enough income to maintain the

economic viability of small holder farming, and in any event is likely to be part of viable farming systems (AACM, 1984). Nevertheless, it is striking that small holder peri-urban dairy development has not been widespread in SSA outside of Kenya. The favorable highland climate, institutional and other policy factors played key roles in the development of small holder dairying in that country. Unfavorable national and international policies have probably done much to discourage dairy in other African countries (Delgado, 1991; Walshe *et al.*, 1991; Winrock International, 1992).

Ethiopia has the largest livestock population of any country in Africa, comprising about 27 million cattle, 24 million sheep, 18 million goats, 7 million equines, 1 million camels and 52 million poultry (FAO, 1993). Over 60% of the cattle and sheep are found in the highlands. This significant resource provides agricultural traction and transportation, meat and milk for households while remaining a major source of cash income. Total annual national milk production is assessed at 780,000 to 830,000 tones of raw milk equivalents (FAO, 1986). Of total national production, between 85 and 89% is derived from cattle. Per capita availability is estimated at 17 to 18 kg per annum (FAO, 1993), a low figure compared to neighbouring countries. Milk remains, however, a by-product of the meat and draught capabilities of the national cattle herd. As a result, production per local cow is low and subject to seasonal fluctuations. Local breeds produce 400 to 680 kg of milk per cow per lactation period of less than seven months (Gryseels and Anderson, 1983; Nicholson, 1983). In contrast, the modern, intensive system, which comprises state and privately owned dairy farms, uses exotic breeds and their crosses. The Ethiopian Ministry of Agriculture (MOA) estimates that only 0.3% of the national herd are upgraded animals. Production from such improved animals varies from six to ten liters per day over a 270 to 300 day lactation period. Such production is oriented towards supplying milk and milk products to the urban populations of Addis Ababa and other towns.

The human population in Ethiopia, currently estimated at 60 million is growing by about 3.5% per annum. This figure will increase to about 139 million by the year 2020, making Ethiopia the third most populous country in Africa hence the demand for animal products, both in terms of quantity and quality, is estimated to increase substantially. Market-oriented dairy production systems with improved genotypes are found mainly in urban and peri-urban areas. These fast growing peri-urban dairy production systems operating at different levels of intensification are becoming one of the most important and dominant systems, particularly around big urban centers (Azage and Alemu, 1998). Almost all the fluid milk supply to major urban centers comes from these urban and peri-urban dairy producers. Urban dairy farms, although currently being important players in the business, are limited in terms of land and other resources and environmental and social concerns are putting a lot of pressure on these farms. Azage and Alemu (1998) noted that the expansion, further development and sustainability of the peri-urban dairy production systems has to be carefully examined, as dairy production requires a relatively large initial investment and a long term commitment. They further emphasized the need for alleviation of problems related to technical and non-technical problems associated with these systems such as policy issues, land rights and ownership, availability and cost of dairy genotypes, feed resources and feeding systems, trained personnel, veterinary services, product quality, marketing, processing and formation of strong dairy associations.

Milk serves as an excellent culture and protective medium for certain microorganisms, particularly bacterial pathogens, whose multiplication depends mainly on temperature and competing microorganisms and their metabolic products. As regards their disease-producing capacity, these pathogens depend upon the initial load of infection of the milk and on the subsequent dilution, processing, time lapse before the milk is consumed, and other factors (Heesche, 1994). Pathogenic organisms in milk are derived from the cow itself, the human handler or the environment. These organisms can be excreted through the udder directly into the milk or may originate from the skin and mucous membranes of the animal or milker and contaminate the milk and milk utensils.

Healthy mammary glands are essential for the secretion of milk that is wholesome to drink and sufficient in quantity to be profitable to dairy men. Mastitis, or inflammation of the mammary gland, occurs in all mammalian species but is particularly important in dairy cattle. Mastitis is generally considered the most costly disease of dairy cows (Fetrow and Mann, 1991). It reduces the quantity and quality of milk and manufactured milk products. Lactose, fat, solids-not-fat and casein are reduced during and following mastitis. Whey proteins, chloride, sodium, pH, free fatty acids and milk somatic count (SCC) are increased. Total protein remains relatively stable, but albumin and immunoglobulins increase. It is estimated that mastitis in cattle in the USA causes a loss of approximately $2 billion per year (Philpot, 1984). In south Africa, the loss in dairy cows during 1987-1988 was R 526 million. The public health implications of the consumption of milk from mastitic cows and other dairy products derived from such milk are well documented (Galbraith *et al.*, 1984; Barrett, 1986).

Mastitis as a disease has received little attention in Ethiopia so far, especially the subclinical form. Efforts have only been concentrated on the treatment of clinical cases. Due to the heavy financial implications involved and the inevitable existence of latent infection, it is obvious that mastitis is an important factor limiting dairy production. The disease is worth studying due to the financial loss involved as a result of reduced milk yield, discarded milk following antibiotic therapy, early culling of cows, veterinary costs, drug costs, increased labor, death of peracute cases and replacement costs. Additional economic incentives to control mastitis include consumer acceptance and product shelf-life factors. Although all of these factors result in considerable economic loss, decreased milk production is the single most important economic consideration.

The objectives of this study were:

- to determine the prevalence and incidence rate of bovine mastitis in selected farms of the Addis Ababa Dairy Farms Association .
- to isolate and identify the types of aetiological agents of clinical and subclinical mastitis
- to estimate the resistance pattern of bacterial isolates of mastitis to commonly used antimicrobials
- to analyze risk factors for mastitis related to the husbandry management of milking cows.

2.. LITERATURE REVIEW

2.1. General

Mastitis continues to be one of the major disease problems facing dairy farmers (Funk et al., 1982). In spite of control efforts (including intensified treatments with antibiotics and extensive research), mastitis is still the most important disease problem in the dairy industry (Dodd, 1985, Fetrow and Mann, 1991; Lipman, 1995; Omore, 1996;). For this reason, more and exact knowledge from extended epidemiological analysis of mastitis and other udder diseases is needed for creating better control programs.

Although mastitis is caused by both non-infectious (traumatic or toxic) and infectious agents, microorganisms are the most important aetiological agents. Mastitis may be classified as clinical or subclinical depending on the degree of inflammation (Philpot, 1967). Clinical mastitis is defined by the diagnosis of abnormal changes (acute, local and systemic) in the body, udder and milk, with concurrent decrease of at least 25% in daily milk production (Shpigel *et al.*, 1994). Clinical mastitis may further be classified according to the severity of the inflammatory response as mild, acute, peracute or chronic. Infection of each mammary gland occurs via the teat canal, the infection originating from two main sources, the infected udder and the environment. In dairy cattle, important infections are those which persist readily in the udder. Bacteria which are normal inhabitants of the environment cause mastitis much less frequently but, when they do, the disease is much more resistant to hygienic measures. Subclinical mastitis on the other hand, cannot be detected by visual observation, though it can be identified by tests that detect infecting microorganisms or the products of inflammation such as somatic cells. Subclinical mastitis is 15 to 40 times more prevalent than the clinical form, it is of long duration, reduces milk production and adversely affects milk quality (Philpot, 1967).

2.2. Etiology

The occurrence of mastitis involves the complex interaction among three major factors: the agent, host, and environment (Radostits *et al.*, 1996). Host factors include stage of lactation, age, anatomy of the udder, parity, intramammary defence mechanisms and periparturient diseases (Shook, 1989; Radostits *et al.*, 1996). At the pathogen level, factors such as the nature of the organism, its virulence and numbers, toxins and antimicrobial resistance are important, whilst management, climate, feeding, housing, milking technique and malfunction of milking machines are environmental risk factors (Anon, 1987). Milking machines may damage the teat, allowing pathogens easier access to the teat canal, and transfer pathogens from one cow to another via contaminated clusters (Du Preez, 1991). Factors which adversely affect the normally efficient barriers to infection of the udder, namely the skin of the teats, teat canal and mammary cisterns, predispose the udder to mastitis. Ticks cause skin damage which predisposes to abscessation and mastitis.

2. 2. 1. Pathogens causing mastitis

The microbial causes of mastitis include a wide variety of bacteria (aerobic, facultatively anaerobic, micro-aerophilic and anaerobic), mycoplasmas, yeasts, fungi, moulds, algae, viruses and rickettsias (Allen, 1985; Du Preez, 1981). Lumpy skin disease, foot-and-mouth disease and ephemeral fever viruses are the most important viral causes of mastitis in cattle (Afshar and Bannister, 1979). Some organisms, such as *Coxiella burnetii* and *Brucella abortus*, may be shed in the milk for weeks without causing apparent lesions in the mammary gland (Baumgartner, 1966).

For simplicity, mastitis causing organisms are broken down into: *contagious* organisms that colonize the mammary gland and can be spread by milking machines and milkers, and *environmental* pathogens that do not normally infect the mammary gland but can do so when the cows' environment, the teats and udder (or injuries thereof), or the milking machine is contaminated with these organisms and they gain access to the teat cistern (William, 1995). Contagious organisms include: *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Staphylococcus aureus*, and *Mycoplasma* sp. Contagious organisms are spread by milking procedures, contaminated machinery, and the hands of milkers. Most contagious organisms cause new infections within the first 2 months of lactation. Environmental organisms include: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia* sp., *Proteus* sp., *Pseudomonas* sp. and other Gram-negatives, coagulase-negative *Staphylococci*, environmental *Streptococci*, yeast or fungi, *Prototheca*, *Actinomyces pyogenes* and *Corynebacterium bovis*.

Since the introduction of antibiotics in the 1940s, there has been a gradual change in the predominant bacteria causing mastitis, from *Streptococci* to *Staphylococci* (Dodd, 1985; Shpigel *et al.*, 1994). This applies particularly to countries where various udder disinfectants and regimens of intramammary antibiotic therapy for mastitis in lactating and non-lactating dairy cattle are used intensively. Microorganisms other than *Streptococci* and *Staphylococci* generally play a comparatively minor role, but mycoplasmas, coliform bacteria, or a combination of *Actinomyces pyogenes*, *Peptococcus indolicus* and *Streptococcus dysgalactiae* may cause sporadic epidemics of clinical mastitis.

2. 2. 2. Major pathogens

Sources indicate that 80% to 99% of all mastitis infections are caused by four types of organisms: 1) *Staphylococci*, 2) *Streptococcus agalactiae*, 3) other Streptococcal species, 4) coliforms (Dodd, 1971). These major pathogens are mainly spread from cow to cow through milkers' hands, udder cloths, residual milk in teat cups, etc. Major pathogens causing environmental mastitis, for example, coliforms and the other *Streptococci*, the most prevalent species being *Escherichia coli*, *Streptococcus uberis* and *Streptococcus dysgalactia*, gain access to the teats due to failure of proper hygienic procedures. The other environmental infections are of an opportunistic nature, namely those caused by *Pseudomonas* spp., yeast agents, P*rototheca* spp., and *Nocardia* (Watts, 1988). The major mode of transmission is from the environment to the cow by inadequate management of the environment.

2. 2. 3. Minor pathogens

These are environmental pathogens and include, coagulase negative staphylococci, *Actinomyces bovis*, *Bacillus cereus*, and *Serratia marcescens*. Minor pathogens are rarely associated with clinical changes and often induce only a moderate somatic cell response (Brooks *et al*., 1983).

2. 2. 4. Features of important agents

2. 2. 4. 1. Contagious causes

Staphylococcus aureus

Enterotoxin-producing staphylococcal species, *Staphylococcus aureus* in particular, are the leading cause of food-borne illness throughout the world (Harmon and Langlois, 1989). Milk and milk products can become contaminated unless good hygiene (including mastitis) control occurs on farms, the milk is adequately pasteurized, and precautions are taken to prevent contamination and subsequent growth of *Staphylococci* during the manufacturing processes and in the finished product (Heesche, 1994). The pathogenicity of *Staphylococcus aureus* has been recognized for many years and it may cause mastitis or skin diseases in milk- producing animals or lead to food borne intoxications in consumers of milk and milk products (Quinn *et al.*, 1994). Once established within a herd, *Staphylococcus aureus* is difficult, if not impossible, to eliminate (Watts, 1988). Chronic infections, resistance to antibiotics, and difficulty in diagnosis typify the organism. *Staphylococcus aureus*, still remains a common mammary pathogen. In most cases mastitis begins as a consequence of the penetration of pathogenic bacteria through the teat duct into the interior of the mammary gland (Quinn, 1994). The pathogens may be found in infected quarters, the udder surface, the hands of milking personnel, contaminated towels and the milking machine. The infected mammary gland and lesions of teats are the main sources of infection with *Staphylococcus aureus*. *Staphylococci* of the vagina, the tonsils or the skin are of minor importance. Most contamination occurs during milking (Matos *et al.*, 1991). Most of the remaining *Staphylococcus* species are coagulase-negative and are considered to be minor pathogens (Harmon and Langlois, 1989). If the bacteria multiply within the gland, the by-products of this growth can cause irritation to the delicate tissues and ensues an inflammatory response. The most common form of staphylococcal mastitis is a chronic subclinical or relatively mild disease that is characterized by an occasional acute flare-up. *Staphylococci* can also produce a severe form of infection known as peracute mastitis that can terminate in gangrene and sloughing of the gland. They have the capacity to penetrate into tissue, producing deep-seated foci. The pathogen induces mastitis by the production of injurious toxins and by the establishment of deep seated pockets of infection which become walled off by scar tissue (Cifrian *et al.*, 1996). This scar tissue is partially responsible for the poor antibiotic cure rates. *Staphylococcus aureus*. Intra Mammary Infection (IMI) are more likely to persist into the new lactation, compared with infections by other mastitis pathogens. Despite the availability of a variety of antibiotics and numerous reports on the sensitivity of *Staphylococcus aureus* to antibiotics, treatment success during lactation is still very low (Matos *et al.*, 1991; Packard *et al.*, 1992). Therefore, persistence of *Staphylococcus aureus* infections during lactation could be of great economic importance.

Streptococcus agalactiae

Streptococcus agalactiae is highly adapted to the bovine mammary gland. It is a highly contagious cause of mastitis within a herd (William, 1995). Mastitis due to this organism is largely subclinical with occasional flare-ups. The main reservoir of bovine strains is the infected udder of cows. Generally, milk becomes contaminated after multiplication of the organism in the udder and the pathogen will be spread to other cows by milking (Quinn *et al*., 1994). The major reservoir of *Streptococcus agalactiae* is milk of infected cows, but it also can occur on the surfaces of materials and objects including bedding, milking machines, and milkers' hands recently soiled by contaminated milk (Watts, 1988). *Streptococcus agalactiae* causes mainly contagious subclinical mastitis which is usually spread by milking and leads to considerable losses of milk quality and yield. Young calves fed with mastitic milk containing *Streptococcus agalactiae* and housed in common pens have a high incidence of *Streptococcus agalactiae* mastitis at freshening (William, 1995). This probably results from calf suckling. Spread to uninfected quarters occurs most frequently at milking and in the absence of good hygiene and effective control measures (Dodd, 1971). *Streptococcus agalactiae* is the only organism in the Lancefield B serological grouping of streptococcus species and is an important pathogen of both animal and man. The advent of antibiotics, and the realization that over 90% of infections could be eliminated with a single course of therapy, resulted in the introduction of the so called 'blitz therapy' as a method of elimination of *Streptococcus agalactiae* from herds (Dodd, 1983). Herd mastitis due to *Streptococcus agalactiae* even with low or moderate prevalence causes profound elevations in somatic cell counts that quickly affect the level of somatic cell counts in the bulk tank. Herd problems with *Streptococcus agalactiae* should be suspected when bulk tank somatic cell counts skyrocket (William, 1995).

Streptococcus dysgalactiae

Although contagious, *Streptococcus dysgalactiae* tends to have a lower prevalence than that observed in *Streptococcus agalactiae* infections, it may become overtly clinical earlier than typical for *Streptococcus agalactiae* (Watts, 1988). Injured teat ends and improper milking hygiene promote the spread of the organisms within the herd. The signs are non-specific. Subclinical cases may be suspected when clots and flakes are observed in fore strippings or when a positive CMT is detected (Quin *et al*., 1994). Clinical cases have mild fever and a swollen, warm, doughy quarter with abnormal secretion (Watts, 1988). Definitive diagnosis requires culture.

2. 2. 4. 2. Environmental causes of mastitis

Streptococcus uberis

Streptococcus uberis is ubiquitous throughout the farm environment because of fecal contamination by cows harboring the organism in their rumen. The organism may also be found on the cow's lips, vagina, external genitalia, and skin of the teats and udder (Watts, 1988). Therefore, *Streptococcus uberis* contained in contaminated bedding, filthy environments, and colonizing teat skin or teat injuries present a potential pathogen (Quinn *et al.*, 1994). Poor cleansing and preparation of the udder before milking predispose to mastitis caused by *Streptococcus uberis* as with other environmental pathogens. Most infections occur early in lactation or late in the dry period, although failure to use dry-cow treatment may allow earlier infection during the dry period. It has been reported to be the most common infection acquired during the dry period (Bramley, 1975). Injuries to the teat or chapping of the teat skin encourage colonization of the skin by *Streptococcuus uberis* and increase the risk of infection. *Streptococcus bovis* and other environmental streptococci, including enterococci, are also found in the rumen, faeces, and barn environment of cattle. However, *Streptococcus uberis* was the pathogen most frequently isolated from clinical cases which occurred in the dry period (Wilesmith, et al., 1986; Deutz and Obritzhauser, 1996). The relative importance of environmental streptococci, especially *Streptococcus uberis* has been on the rise and their incidence exceeds that of *Streptococcus agalactiae* which has largely been eliminated from herds with low somatic cell counts (Schukken *et al.*, 1989). They found that the incidence ranged from 12.3 to 26.2%, as compared to 0.2% for *Streptococcus agalactiae* in such herds.

Coagulase negative Staphylococci

Coagulase-negative *Staphylococci* (CNS) are normal flora of the skin-including the skin of the teat and external orifice of the streak canal. Factors that contribute to teat skin irritation or injury increase the numbers of coagulase-negative *Staphylococci* at these locations (William, 1995). Coagulase-negative *Staphylococci* are usually the most prevalent species of bacteria isolated from mammary secretions of lactating dairy cows (Harmon and Langlois, 1986). Infections may occur during lactation or late in the dry period. Coagulase negative

Staphylococci are generally associated with subclinical cases of mastitis that result in decreased production and elevated somatic cell counts in infected glands (Bramly, 1975). It has been suggested that quarters harboring minor pathogens, in particular CNS, may be more resistant to subsequent infection by more pathogenic bacteria than uninfected quarters (Lam *et al.*, 1997; Edwards and Jones, 1966). Jones *et al.* (1982) found that 23.3% of quarter IMI were caused by CNS. Subclinical CNS intramammary infections are associated with elevated SCC, with reduced milk yield (Timms and Schultz, 1987). Although CNS intramammary infections elevate SCC and may decrease yield, some studies (Bramly, *1975;* Linde *et al., 1980*) have shown that CNS and *Corynebacterium bovis* provide protection against major mastitis pathogens. This protective effect has been attributed to: 1) elevated SCC in quarters infected with CNS and *Corynebacterium bovis* (Linde, et al., 1980; Rainard and Poutrel, 1988). 2) production of an antibiotic-like substance that is inhibitory to the more pathogenic bacteria (Edwards and Jones, 1966). 3) a factor that is inhibitory to growth and haemolytic patterns of *Staphylococcus aureus*. The results of more recent studies have suggested a reduction in the rate of intramammary infection for *Staphylococcus aureus* by experimental challenge in quarters infected with CNS compared with bacteriologically negative quarters (Pankey *et al.*, 1985; Poutrel and Lerondelle, 1980). Other than latent decreased production, positive CMT results and elevated SCC, no clinical signs exist that specifically identify CNS.

Coliforms

The Gram-negative facultative anaerobes are classified into three families: the enterobacteriaceae, vibrionaceae and pasteurellaceae. Enterobacteriaceae are often isolated from bovine intramammary infections (Watts, 1988). *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella* spp, *Citrobacter* spp, *Serratia* spp and *Proteus* species are the most frequently isolated Enterobacteriaceae from bovine intramammary infections (Eberhart *et al.*, 1979; Smith *et al.*, 1985; Watts, 1988).

Coliform mastitis is a classic example of an environmental cause of mastitis. Management factors that contribute to a build up of coliforms increase the risks of coliform mastitis. Deep mud and excessive moisture in barnyards greatly increase the likelihood of coliform organisms contaminating the udder (William, 1995). Summer heat and humidity contribute to multiplication and persistence of coliforms in the environment such that the incidence of coliform mastitis usually is increased during the summer months. However, because of the wide-spread use of free-stall housing for cattle, the damp barn environment present in free stalls predisposes to coliform mastitis, regardless of seasonality. Coliform mastitis therefore is an "occupational hazard" in free-stall housed cattle (Eberhart, et al., 1979). Excessive build up of manure, failure to clean or maintain stalls, and poor ventilation further contribute to high levels of coliform organisms in free stalls (William, 1995). Bedding has been a subject of controversy in the pathogenesis of coliform mastitis, but sawdust bedding, especially green or wet sawdust, has been known to harbor high levels of coliforms (Rendos *et al.*, 1975). Recently-fresh cows are at greater risk for coliform mastitis (Erb, 1984). Cows in herds with low somatic cells counts had the highest incidence of clinical mastitis within the first 30 days of lactation (Erskine *et al.*, 1988). Udder edema, incomplete milkout, and leaking milk between milkings are important contributing factors to coliform mastitis in these fresh cows. Leaking of milk allows the environmental causes of mastitis to gain access to the teat cistern and gland. Concurrent matabolic diseases such as hypocalcemia that cause the cow to remain recumbent also may increase the exposure to environmental coliforms (Smith *et al.*, 1985a). Other concurrent diseases in the post-partum period such as fatty liver disease or retained placenta may depress neutrophil function, thereby altering the defense mechanism of the mammary gland.

Dry cows are at greatest risk of infection just after drying off and just before calving. The rate of bovine Gram-negative bacterial IMI is three to four fold greater during the dry period than during lactation (Smith *et al.*, 1985b). This rate of infection is not constant across the dry period, but rather, it is elevated during the first 2 weeks of the dry period and the 2 weeks prior to parturition. Studies on naturally occurring IMI indicate that the majority of *Escherichia coli* IMI associated with the dry period are first detected immediately prior to or at parturition (Smith *et al.*, 1985). In contrast, the origin of *Klebsiella* spp. IMI was equally divided between the first and second halves of the dry period.

Coliform organisms contaminating the teats and udder simply need an opportunity to gain access to or bypass the streak canal. Milking procedures and teat end injuries are probably the most important contributing factors to coliform mastitis (Watts, 1988). Poor preparation or washing of the udder to remove environmental contamination is an obvious problem. Similarly, failure to dry teats and udder or using excessive water when preparing the udder contribute to environmental causes of mastitis. High bulk tank coliform counts indicate poor udder preparation, much filth in the environment, or both. Teat-end injuries due to abrasive surfaces, skin chafing, excessive milking vacuum, over milking, injuries, infections, and irritants also all predispose to colonization by environmental organisms (William, 1995).

Actinomyces pyogenes

Actinimyces pyogenes causes "dry cow " or "summer " mastitis. The infection is extremely purulent and abscessation of affected glands is common. Most, but not all, infections occur during the dry period and the incidence of infection is increased by filthy, wet, or muddy environments for dry cows (Brooks,et al., 1983). Because *Actinomyces pyogenes* is a common skin organism of cattle, it is routinely isolated from abscesses, wounds, and a variety of tissues in cattle (Watts, 1988). The organism may be spread by flies and fly bites of the teat end during summer months but disease occurs year-round in some operations. Most infections begin after the udder has been dry for 2 weeks or more, and muddy or wet and when dirty environments usually are present (Pankey, 1985). Epidemics also are possible with up to 25% of the dry cows being affected (William, 1995). Swelling of the infected quarter usually is acute and results in a very firm or hard, inflamed, painful gland or glands. Diagnosis is greatly assisted by the clinical signs and the patient being a dry cow, it is confirmed by culture of secretion from the quarter.

2. 3. Diagnosis of mastitis

Complete mastitis surveillance requires a combination of cowside and laboratory tests.

2. 3. 1. Cow side Tests

Strip cup or plate

Several simple tests performed on the farm (cow side), or more complicated laboratory tests are used to diagnose mastitis in individual cows or on a herd basis (Anon, 1987). Clinical mastitis may be detected by examining the udder for warm, swollen quarters, which are indicative of acute mastitis, or for misshapen, hard, atrophied and fibrotic quarters, indicating permanent damage caused by chronic mastitis. The strip cup test is a practical and effective method of identifying cows with clinical mastitis. Abnormal milk is usually discolored, watery, or contains flakes, shreds or clots, but in chronic mastitis the milk is not always macroscopically abnormal. The strip cup test in these cases is not sufficiently sensitive to detect subclinical mastitis.

California Mastitis Test (CMT)

The California Mastitis Test is another cow side test that can be used for the detection of mastitis. It is an indirect test that grossly measures the amount of DNA, primarily a function of the number of nucleated white blood cells in the milk (Quinn *et al.*, 1994). It is more sensitive than the strip cup test and enables subclinical mastitis to be detected. The basis of the CMT is the reaction which occurs when the reagent comes into contact with the increased quantity of cell material derived from increased numbers of somatic cells in the milk (Bramly, 1975). Somatic cell numbers in milk tend to increase during milking, and remain high for several hours thereafter, even in uninfected quarters (Anon, 1987). For reliable results, the CMT therefore should be conducted just before milking, after stimulating the cow, and having discarded the fore milk. Based on the amount of gelling that occurs as equal amounts of milk and reagent interact, the test (reaction) is subjectively read (scored) as O (negative), t (trace), 1 (slight), (moderate) 3 (heavy). These scores equate well with somatic cell levels (Philpot and Nickerson, 1991). The CMT is most helpful in detecting subclinical mastitis and serves little purpose in acute clinical mastitis. A CMT tends to have a high score in recently fresh cows and in cows at the end of lactation just prior to drying off. A CMT is also elevated in secretions from cows whose milk production has dropped precipitously due to illness (William, 1995). Because results of the test are interpreted subjectively, discrepancies may arise between evaluators, and estimates of somatic cell count that correlate with CMT scores vary greatly (Barnes Pellensen *et al.*, 1985). Loss of production correlates directly with CMT scores. This factor may be useful when convincing owners to use mastitis detection aids. Production losses from quarters with CMT trace values may be 5% or more and losses from quarters having CMT +3 values may be 25 to 50% (Philpot, 1984).

PH Indicator Papers

These test strips detect the more alkaline pH in quarters with mastitis. Normal milk has a pH of approximately 6.5 to 6.7, whereas mastitic milk often approaches plasma pH of 7.4 (William, 1995).

Palpation of the udder

Palpation of the udder is very helpful in detecting areas of fibrosis associated with chronic subclinical *Staphylococcus aureus* infection. Palpation also is very useful to rule out mastitis when concern is raised by a positive CMT score, simply from acute drying off due to systemic illness. In this latter case, the udder palpates normally and is not infected. The acute dry off due to illness simply causes lack of fluid dilution of somatic cells causing the CMT to be positive. Palpation also is valuable following resolution of acute clinical mastitis to detect glandular changes that might be associated with infarction, abscessation, or chronic infection (Radostits, et al.,1996).

Somatic cell counts

The somatic cell count has become the most widely used index of the level of infection within individual cows and herds (Bartlett, et al., 1992). Monitoring individual quarters or composite samples from all four quarters allows specific information, helpful in decisions regarding treatment or culling. Infection is the major factor that elevates the somatic cell count (Erskine, et al., 1988). Somatic cells consist primarily of leukocytes that are present in the udder in response to infection and to repair damaged tissue. Somatic cells also include epithelial cells which make up the internal lining of the mammary gland tissue and are normally replaced during the events of lactation (Harmon, and Langlois, 1986). When the udder or teat is severely injured there are large increases in SCC. Some elevation in counts in these cases is in response to the increased prevalence of mastitis with injury (De Graaf and Dwinger, 1996). Neutrophils increase dramatically to compose the majority of cells and may increase to 95% of the somatic cell count with infection of the gland (Kirk, 1984). Mononuclear cells and sloughed alveolar epithelial cells also contribute to the somatic cell count, but neutrophils compromise the majority of cells. Lower somatic cell counts are associated with higher herd milk production (Miller, and Bartlett, 1991). Somatic cell counts tend to be higher for individual cows during the first 2 weeks and last 2 weeks of lactation and lower during peak lactation. A precipitous decrease in milk production associated with illness also may raise the somatic cell count due to concentration of cells (Kehrli, et al., 1982).

The direct microscopic somatic cell count (DMSCC) is the procedure of evenly spreading a measured volume of milk over a calibrated area of a microscope slide, staining the film and counting somatic cells within a specified area of the film (Packard *et al.*, 1992). The count is then converted to cells per milliliter (ml) by a factor which is determined by magnification and area counted. Electronic means of counting somatic cells in milk are becoming popular, speed and repeatability are the two main benefits. Although the equipment is expensive to purchase, large numbers of samples can be run in a short period of time, at low cost per sample.

2. 3. 2. Culturing

Bacteriological cultures of milk samples from individual quarters or of composite samples from all four quarters of individual cows are required to determine the aetiological agents involved (Anon, 1987). In herds with a large number of subclinical cases of mastitis, a reliable diagnosis can be made by culturing samples of milk from cows selected on the basis of increased CMT scores or somatic cell counts. Most of the bacterial pathogens causing mastitis grow on ox or sheep blood agar. A MacConkey agar plate is streaked in parallel to detect *Enterococcus faecalis* and any Gram-negative bacteria that are able to grow on the medium. Edwards medium is highly selective for *Streptococci* and also acts as an indicator medium for haemolysis and for the hydrolysis of aesculin. A Sabouraud dextrose agar plate can be inoculated if a fungal pathogen is suspected (Quinn *et al.*, 1994; Carter, 1996).

2. 4. Epidemiology

In recent years, veterinary epidemiologists have undertaken several explanatory studies to investigate factors influencing health and production in livestock populations. Given the structure of livestock populations, herds are usually the sampling units. The unit of interest may be the individual animal, the herd, or both (McDermott and Shoukri, 1991).

2. 4.1. Microbial factors

The microbial factors which affect mastitis occurrence are: the ability to survive in the immediate environment of the animal, the ability to colonize the teat duct, the ability to adhere to mammary epithelium and not be flushed out with the milk flow, the degree of invasiveness (for example streptococci cause little pathological change to secretory cells while

Staphylococci initiate degenerative changes), the ability to resist phagocytosis and antibacterial substances in the udder, including resistance to antibiotics (Quinn *et al.*, 1994).

2. 4. 2. Host factors

It has been demonstrated that genotypes favorable for milk yield are more susceptible to mastitis (Shook, 1989). The long term selection pressure for milk production may have had a negative effect on the polymorphisms of genes linked to the major histocompatibility complex (BoLa) in dairy breeds (Lewin, 1989; Lipman, 1995). Genetic variation in conformation of the udder, teats, sphincter tone and anatomy of the teat canal is determined in part by heredity and may be considered one component of genetic resistance (Shook, 1989). Pendulous udders also are susceptible to mastitis (Young and Legates, 1960). Once inside the teat cistern, pathogens encounter a group of non-specific bacteriostatic and bacteriocidal factors. When these fail, phagocytic cells aided by immunoglobulins are called into action. Variation among cows has been observed in most of these mechanisms, a portion of which is attributable to heredity (Shook, 1989). Stage of lactation is a further determinant of both subclinical and clinical mastitis. Mastitis due to environmental organisms is most common in the first few days after calving and regresses as lactation progresses (Radostits et al., 1996). This is due to a reduction in the immune function soon after calving (Kehrli *et al.*, 1989). On the other hand, subclinical mastitis increases as the stage of lactation progresses. It also increases at higher lactation numbers. Parity has been shown to be a further risk factor for both subclinical and clinical mastitis (Schukken *et al.*, 1989). It is postulated that younger animals have a decreased susceptibility through a more effective host defense mechanism (Dulin *et al.*, 1988). Older cows, especially after four lactations, are more prone to mastitis. Periparturient diseases such as dystocia, parturient paresis, retained placenta and ketosis also have been identified as risk factors for subsequent development of mastitis (Radostits *et al.*, 1996). The other host factors which affect mastitis occurrence are immunological factors such as the level of IgA, IgG1, lactoferrin and phagocytes in the mammary gland; presence of lesions on the teats that may predispose to inadequate milking or may harbor mastitis-producing bacteria (Quinn *et al.*, 1994).

2. 4. 3. Environmental factors

Certain environmental risk factors become apparent as the level of intensification changes. It has been observed that well managed herds are at a higher risk of environmental mastitis than contagious mastitis where intramammary treatment for all non-lactating cows is practised (Erskine *et al*., 1988). An increasing number of hours that cows spend outside protects against environmental mastitis (Smith *et al*., 1985). Tied dairy cows showed an increased frequency of mastitis than exercised cows. Coliform mastitis is much more frequent in housed cattle than those grazing. Health in general was positively influenced by exercise (Gustafson, 1993). Herd size is another factor associated with the incidence of clinical mastitis. As herd size increases, manure disposal and sanitation problems may increase exposure to coliforms and environmental streptococci (Bartlett *et al*., 1992). The existence of organic materials, moisture and temperature favor the growth of environmental organisms (Zehner and Farnsworth, 1986). Presence of large numbers of potential pathogens in the immediate environment of the animal may induce mastitis. Milking management and milking technique have been shown to be essentially important risk factors, with machine milking being more risky than hand milking and calf suckling (Hamann *et al*., 1991). Milking machine malfunction or inadequate design, milking-shed environment including poor hygiene predispose cows to mastitis.

2. 5. Mastitis control

The objective of any dairy is to produce as much milk (cost-effectively) as possible. Mastitis not only reduces the productive capacity of cows, it is also expensive to treat. Therefore its prevention should be a priority. Prevention of mastitis depends primarily on good hygiene (before, during and after milking) practices and effective animal management which includes treatment of clinical cases as they occur, use of udder disinfectants, pre-milking strip cup, post milking teat dipping and dry cow therapy (Radostits *et al*., 1996). Pre-milking hygiene includes cleansing the teats with clean, uncontaminated water with or without sanitizers. Water is both a help and a hindrance because it is necessary for effective cleaning when the teats and udder are dirty but it also can carry bacteria down the teat from a wet udder and thus allow contamination of milk during the milking procedure (Bushnell, 1984). Common washing solutions, sponges, or rags are very dangerous and may easily spread contagious organisms. Following preparation, cow should be dried with an individual paper towel that then is discarded. Forestripping should be completed before or during the pre-milking cleansing. This removes bacteria from the teat end and streak canal and allows surveillance for subclinical or clinical mastitis through the use of a black-faced strip cup or black floor tile.

Post-milking teat dips are well recognized and proven to be important adjuncts to decrease new intramammary infections from contagious organisms and, to a lesser degree, environmental pathogens (Philpot and Nickerson, 1991). Both dips and sprays are available. Sprays protect against contamination of dip cups but require careful application to provide proper contact. Any teat dip should be replaced frequently and dip cups disinfected to prevent opportunists such as *Serratia* species from contaminating the applicator (William, 1995).

2. 6. Zoonotic significance of milk and its products

Milk allows the growth and multiplication of a number of potentially harmful microorganisms (Heeschen, 1994). It is a vehicle for the transmission of many zoonotic diseases. The major infections transmitted through milk are: tuberculosis, brucellosis, typhoid, paratyphoid, dysentery, staphylococcal intoxication, scarlet fever and diphtheria (Galbraith *et al.*, 1984). Rare cases of milkborne diseases are *Escherichia coli* enteritis, *Clostridium perfringens* food poisoning and Q- fever. Milkborne diseases with increasing incidence are salmonellosis and campylobacteriosis. Raw milk is the biggest source of milkborne diseases.

2. 7.Economic losses

Economic losses due to mastitis are recognized worldwide as a major problem on dairy farms. These economic losses include decreased production, drug costs, veterinary fees, loss due to discarded milk and labor and replacement costs due to increased culling (Bunch *et al.*, 1984). The problem is that at the sub-clinical level, symptoms of the disease may not be visually evident, and dairymen could be unaware of the problem (Blosser, 1979). The main economic loss from subclinical mastitis is lost milk production- generally a loss of up to 75% (Fetrow, 1980). Mastitic milk further has physico-chemical changes that adversely affect the quality of the milk. These changes may also affect the suitability of the milk for industrial processing

(Janzen, 1970). Additional economic incentives to control mastitis include public health, consumer acceptance, and product shelf-life factors. Although all of these factors result in considerable economic loss, decreased milk production is the single most important economic consideration (De Graves and Fetrow, 1993; Omore, 1996).

2. 8. Mastitis in Ethiopia

Few epidemiological studies were carried out for mastitis in Ethiopia. Bishi (1998), indicated the overall prevalence of subclinical and clinical mastitis to be 30.2% and 5.5% respectively, in a study conducted in urban and peri-urban dairy production systems in and around Addis Ababa. A more restricted study by Biru (1989) indicated a combined prevalence of 67.4% of both clinical and subclinical mastitis in a herd of 43 cows. The main isolates were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Actinomyces bovis* and *Actinomyces pyogenes*. In another study conducted on smallholder farms in the Wondogenet area of Ethiopia, 39% of the cows had mastitis with a quarter infection rate of 16% (Abdella, 1996). Nesru *et al.* (1997), reported a clinical and subclinical mastitis prevalence of 5.3% and 19% respectively, in a study conducted at the Adamitulu and Holetta livestock research centers, with isolated genera of *Staphylococci*, *Streptococci*, *Actinomyces*, *Pasteuralla* and *Nocardia*. The Faculty of Veterinary Medicine of the Addis Ababa University has conducted several unpublished studies on the mastitis complex.

3. MATERIALS AND METHODS

3.1. The study area

The study was conducted in the urban and peri-urban areas of Region 14 (Addis Ababa). Addis Ababa is the capital city of Ethiopia, a center for the Federal Government Administration and also hosts numerous International Organizations. The human population is approximately 3 million. It is a high land area with an altitude of 2500 meters above sea level, having an average annual rainfall of 1800 mm and an average temperature of 21 °C. The city experiences a bimodal rainfall.

3.2. Study population

The target animals sampled were milking cows within the Addis Ababa Dairy Producers Association. Some of the farms selected were large, with more than ten cows and the others small with less than five cows (Tables 4.1 and 4.2). Most of the milking animals were exotic breed crosses.

3.3 Questionnaire survey

A questionnaire was circulated to all farmers and completed in the study period. For each herd, it gives details of the following:

(i). Herd description and farm structure

(ii). Management and control measures

(iii). Social factors and farm establishment

(iv). Farm inspection and environmental management

Table 3.1 Number of dairy farms in the Addis Ababa under the Dairy farms Association (1999)

Zone	District	I	II	III	Total
1	3	1	3	-	4
	4	2	3	-	5
	5	-	-	-	-
	6	-	-	-	-
Subtotal		3	6	-	9
2	20	2	1	-	3
	21	-	1	-	1
	22	-	-	-	-
	23	3	-	-	3
	24	1	1	-	2
Subtotal		6	3		9
3	17	6	1	1	8
	18	6	1	1	8
	19	31	6	1	38
	28	11	3	-	14
Subtotal		54	11	3	68
4	1	1	3	1	5
	9	2	-	-	2
	11	9	1	-	10
	12	10	1	1	12
	13	3	2	1	6
	15	6	4	3	13
	16	4	-	4	8
Subtotal		35	11	10	56
5	2	4	-	-	4
	7	-	2	2	4
	8	2	1	1	4
	10	-	-	-	-
	14	2	2	1	5
	25	3	2	-	4
Subtotal		10	7	4	21
6	26	1	-	-	1
	27	-	-	-	-
Subtotal		1			1
Grand total		**109**	**38**	**17**	**164**

I = Small farms (1 - 5 milking cows)
II = Intermediate (6 – 10 milking cows)
III = Large (more than 10 cows)

Table 3.2 Cattle population in the Addis Ababa Region under Dairy Farms Association (1999)

Zone	District	Cow	Heifer	Female calf	Total
1	3	22	10	6	38
	4	26	13	8	47
	5	-	-	-	-
	6	-	-	-	-
Subtotal		48	23	14	85
2	20	14	2	4	20
	21	6	2	3	11
	22	-	-	-	-
	23	11	4	4	19
	24	10	4	3	17
Subtotal		41	12	14	67
3	17	41	15	9	65
	18	64	9	14	87
	19	161	44	49	254
	28	47	16	18	81
Subtotal		313	84	90	487
4	1	37	19	7	63
	9	5	1	2	8
	11	36	10	13	59
	12	53	2	9	64
	13	25	10	5	40
	15	88	16	10	114
	16	69	23	13	105
Subtotal		313	81	59	453
5	2	15	2	4	21
	7	75	1	-	76
	8	32	9	7	48
	10	-	-	-	-
	14	57	11	6	74
	25	22	8	8	38
Subtotal		201	31	25	257
6	26	3	1	1	5
	27	-	-	-	-
Subtotal		3	1	1	5
Grand total		**919**	**232**	**203**	**1354**

3.4. Sampling Strategies

Based on the sampling frame of the dairy farms of the Dairy Producers Association in region 14 (zones 3 and 4), 76% of the farms in those two zones were proportionally sampled. It was felt that the two zones form a representative sample with regards to the total number of animals in Addis Ababa within the Association. All farms and all cows in the two zones were included in the study. This resulted in a sample of 626 cows on 114 farms; 479 of the cows were milking.

3.5. Study design

3.5.1. Cross-sectional study

In the study herds, prevalences of clinical and subclinical mastitis were determined at the beginning and end of the study period, using clinical observations, the results of the California Mastitis Test (CMT), Somatic Cell Counts (SCC), and results of microbiological examination of samples from all quarters. Prevalences of clinical and subclinical mastitis at the cow level were calculated as:

$$\textbf{Prevalence} = \frac{\textbf{Number of cows with the disease}}{\textbf{Total number of lactating cows}}$$

3.5.2. Longitudinal study

A follow-up (incidence) study was conducted in all study herds for a period of 3 months (March to May) with regular inspection intervals of four weeks using clinical observation, culturing and SCC. CMT positive samples in the study period were cultured for isolation and identification of etiological agents. CMT negative samples were cultured in two subsequent samplings if they were positive at the first sampling. Clinical cases were identified by making wise use of observations and by strip cup, CMT and culturing results. The procedures followed for subclinical mastitis were: milk sampling, CMT, SCC, culturing, isolation and identification. The subsequent cumulative incidence rates (CI) for clinical and subclinical mastitis were calculated as:

$$CI = \frac{\textbf{Number of cows developing mastitis in 3 months time period}}{\textbf{Number of cows at risk at the beginning of the 3 months period}} \times 100$$

The incidence density rates (ID) for clinical and subclinical mastitis, based on animal time, were calculated using the formula:

$$ID = \frac{\textbf{Number of cows developing mastitis in 3 months period}}{\textbf{The average length of time each cow was at risk of developing mastitis}} \times 100$$

3.6. Screening tests

3.6.1. California Mastitis Test

The California Mastitis Test was used as a screening test for somatic cells and for selection of samples for culture for the cows under study. The test was conducted just before milking, after stimulating the cow and having discarded the foremilk. The reaction was then scored as 0 (negative), T (trace), 1(slight) 2 (moderate), 3 (heavy), depending on gel formation as shown in Table 4.3 (Philpot and Nickerson, 1991). The estimates of the somatic cell levels as they relate to CMT scores are also given.

Table 3.3 Interpretation of CMT findings.

Score	Gelling	Interpretation	SCC mean score	Range '000 cells/ml
0	None	Negative	100000	< 200
Trace (T)	Slight	Suspicious	300000	150-500
1 (+)	Slight to moderate	Positive	900000	400-1500
2 (++)	Moderate	Positive	2700000	800-5000
3 (+++)	Heavy	Positive	8100000	>5000

3.6.2. Somatic cell count

To estimate the somatic cell count of milk samples, the direct microscopic somatic cell count (DMSCC) was employed. A measured volume of milk was evenly spread over a calibrated area of a microscope slide, the film was stained and somatic cells counted within a specified area of the film (Kirk, 1984). The counts then were converted to cells per milliliter by multiplication with a factor which was determined by magnification and area counted. General agreement rests on the values of less than 300,000 cells/ml for uninfected cows and greater than 500,000 for cows infected with significant pathogens such as *Staphylococcus aureus* or *Streptococcus agalactia* (Jones *et al.*, 1982). Cows with SCC between these values may have recovered from an infection, sustained an injury or being infected with a less important organism such as *Corynebacterium bovis* (De Graaf and Dwinger, 1996).

3.7. Investigation procedures

Clinical cases of mastitis were recorded at the time of milk sampling. Clinical mastitis was diagnosed when there were visible signs of inflammation in the udder or when changes were observed in the secretions. In doubtful cases, CMT was carried out. Milk samples from affected quarters subsequently were collected into sterile bottles. All cows were screened using the CMT. From CMT positive cows quarter milk samples were collected into sterile vials after the teats had been thoroughly washed with water, wiped dry with towels and well swabbed with 70% ethanol. Samples were transported to the laboratory in an ice box for further examination. Due to the non-availability of an automatic cell counter, SCC was determined by the standard methods for the examination of dairy products using clean glass slides (Packard *et al*., 1992).

Farm inspection activities (questionnaire, direct observation) were systematically recorded for identifying risk factors for udder health performance. Culturing techniques included primary plating (inoculation) onto 7.5% sheep blood agar, McConkey and sabourauds dextrose agar. The speed of growth, morphology of colonies and haemolytic characteristics were recorded. All colonies were Gram-stained and subjected to biochemical tests for further identification as described by Barnes-Pellesen *et al*., (1985), Carter (1996) and Quinn *et al*. (1994) (Fig. 4.1, 4.2, 4.3).

Fig 3.1 Microbiological diagnosis for routine culture

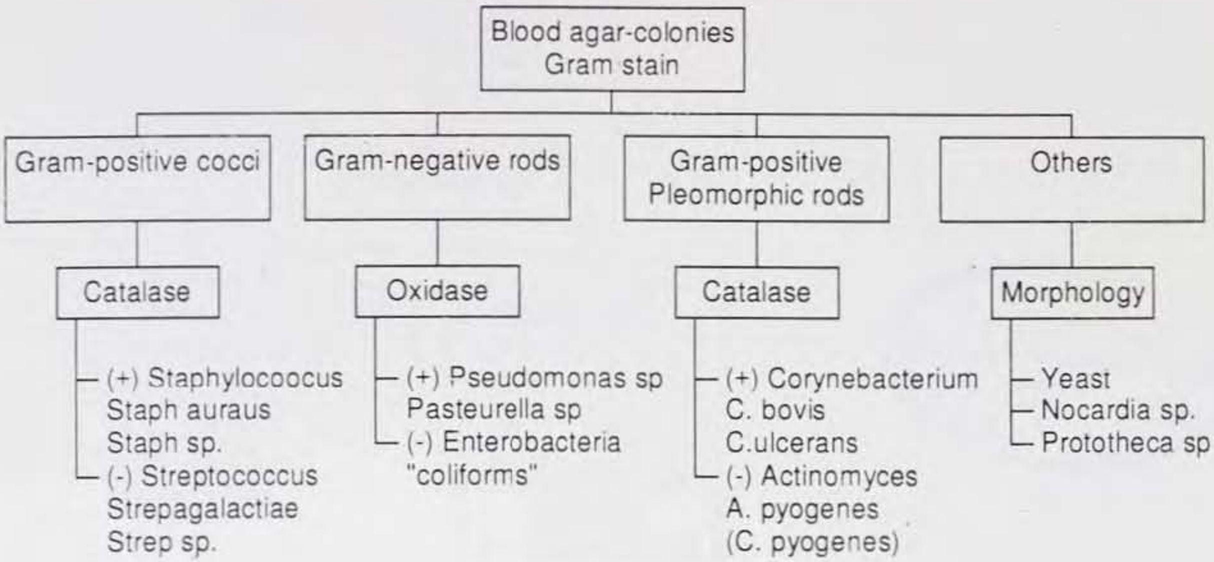


Fig 3.2 Microbiological diagnosis for Gram-positive organisms

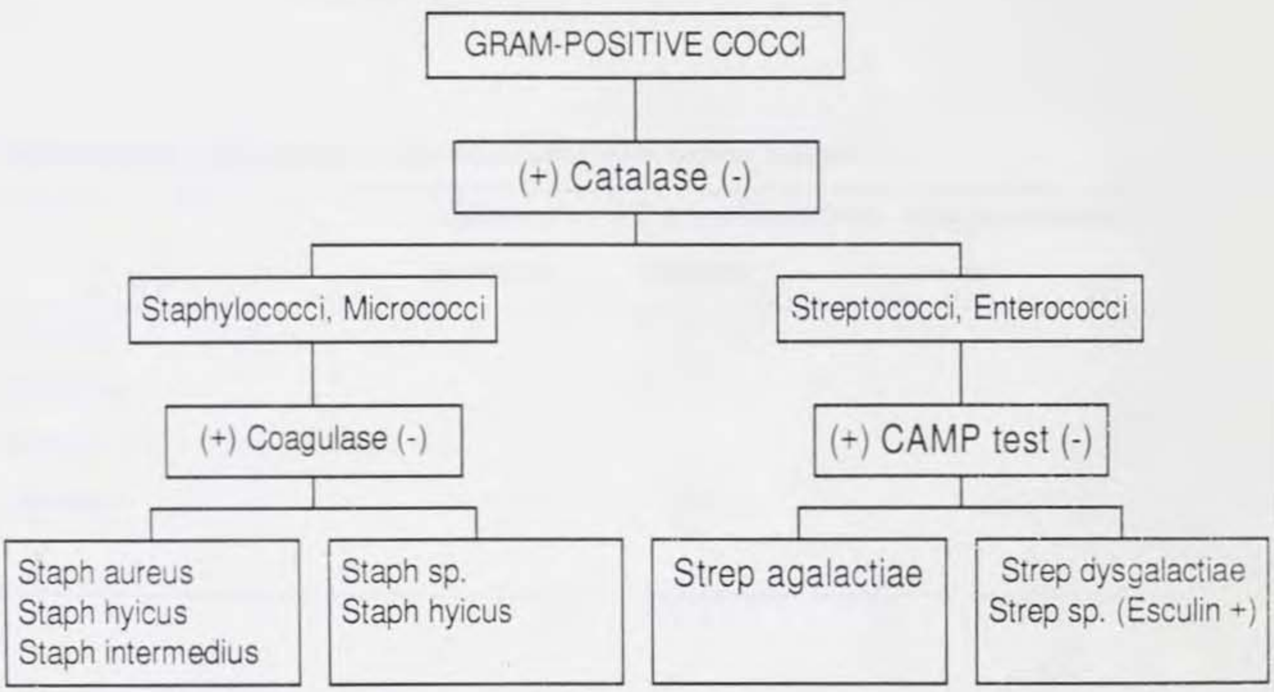
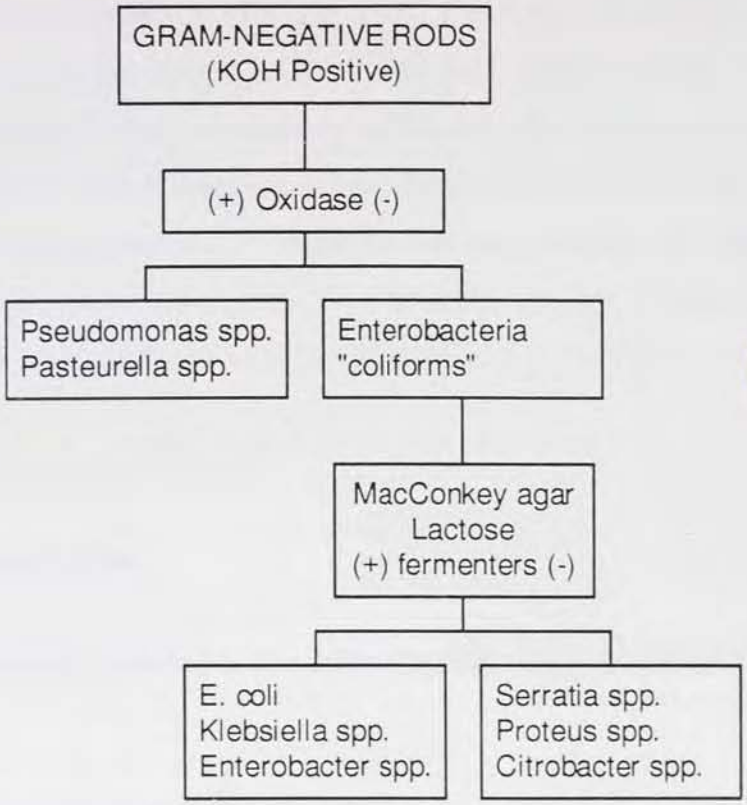


Fig 3.3 Microbiological diagnosis for Gram-negative organisms



Gram-positive pleomorphic rods associated with bovine mastitis

	Actinomyces pyogenes	Corynebacterium ulcerans	Corynebacterium bovis
Catalase	-	+	+
Hemolysis	+	+	-
Growth in 9% Sodium Chloride	-	-	+

3.8. Antibiotic sensitivity test

In vitro antibiotic sensitivity tests (Kirby-Bauer disc diffusion method) were carried out on 246 isolates using the following 11 antimicrobial drugs: ampicillin, oxytetracycline, chloramphenicol, penicillin streptomycin, tetracycline, erythromycin, polymyxin B, sulphamethoxazole, gentamycin and neomycin. Mueller-Hinton agar was used as plating medium. Antibiotic impregnated paper discs (Oxoid, United Kingdom) were applied, and plates were incubated at 37°C for 18 h. The basis of the test was for making treatment decisions in mastitis cases (to identify the most effective drugs). The diameters of zones of growth-inhibition were measured in millimeters and reported as susceptible, intermediate, or resistant. Sixty four of the isolates were *Staphylococcus aureus*, 28 *Actinomyces pyogenes*, 27 *Streptococcus agalactiae*, 17 *Streptococcus dysgalactiae*, 16 *Proteus* spp, 16 *Enterobacter* spp, 16 *Streptococcus uberis*, 12 *Klebsiella* spp, 10 Pseudomonas spp, 5 *Enterobacter faecalis*, 3 *yeasts*, 2 *Pasteurella* spp, 20 CNS and 10 *Corynebacterium bovis*.

3.9. Data collection

Information was recorded on the following major aspects of farm production:

3.9.1. Farm structures and resources

Data on modalities of relevant farm structure variables included: location, dominant cow breed, other agricultural activities, percentage of land allocated for animal feeds and other products, type of housing, duration of housing, manure handling, number of workers and record keeping.

3.9.2. Animal resources

Information was gathered on the number and different classes of animals. Some of the information collected included: breed, age, parity, stage of lactation, herd size, etc.

3.9.3. Feed resources and utilization

Information on feed resources and feeding systems were generated from the farms studied.

3.9.4. Mastitis

Cows were clinically examined for udder abnormalities at regular intervals. Milk sampling and analysis was conducted as described under 3.7 above. In addition, information on pre-milking cow handling, udder and teat conformation, presence of blind teats, milking equipment, milking techniques and level of production were collected.

3.10. Analysis

Methods of analysis included:

Prevalence of subclinical and clinical mastitis

Incidence density rates of subclinical and clinical mastitis

Mean frequencies of pathogens isolated in subclinical mastitis cases

Uni-and multivariate analysis of risk factors using the SPSS statistical program

Regression analysis to evaluate the relationships between variables

4. RESULTS

4.1. Questionnaire survey

Results of the questionnaire survey, based mainly on management, social and farm structure are presented.

4.1.1. Farms distribution

The study was carried out in 2 zones and 11 districts (woredas) of the Addis Ababa Region. one hundred and fourteen dairy farms were investigated. Sixty eight of them were peri-urban and forty six urban dairy farms (Table 6.1). The largest number of small farms were found in the peri-urban areas of the 2 zones (twice that of the urban areas). The intermediate and large farms are larger in number in the urban areas than in the peri-urban out skirts (Fig. 6.1). All farms had individual owners.

Table 4.1 Distribution of farms in study zones and districts (Addis Ababa, Ethiopia)

Zone	District	No. of farms	Urban Size of farm			Peri-urban Size of farm		
			Small	Intermediate	Large	Small	Intermediate	Large
3	17	5	5					
	18	8	5	1	2			
	19	32	5	2		23		2
	28	13				13		
4	1	5	4		1			
	9	2	2					
	11	10	1			8	1	
	12	17				16	1	
	13	5		3	2			
	15	13	9	3	1			
	16	4				2	1	1
	Total	**114**	**31**	**9**	**6**	**62**	**3**	**3**

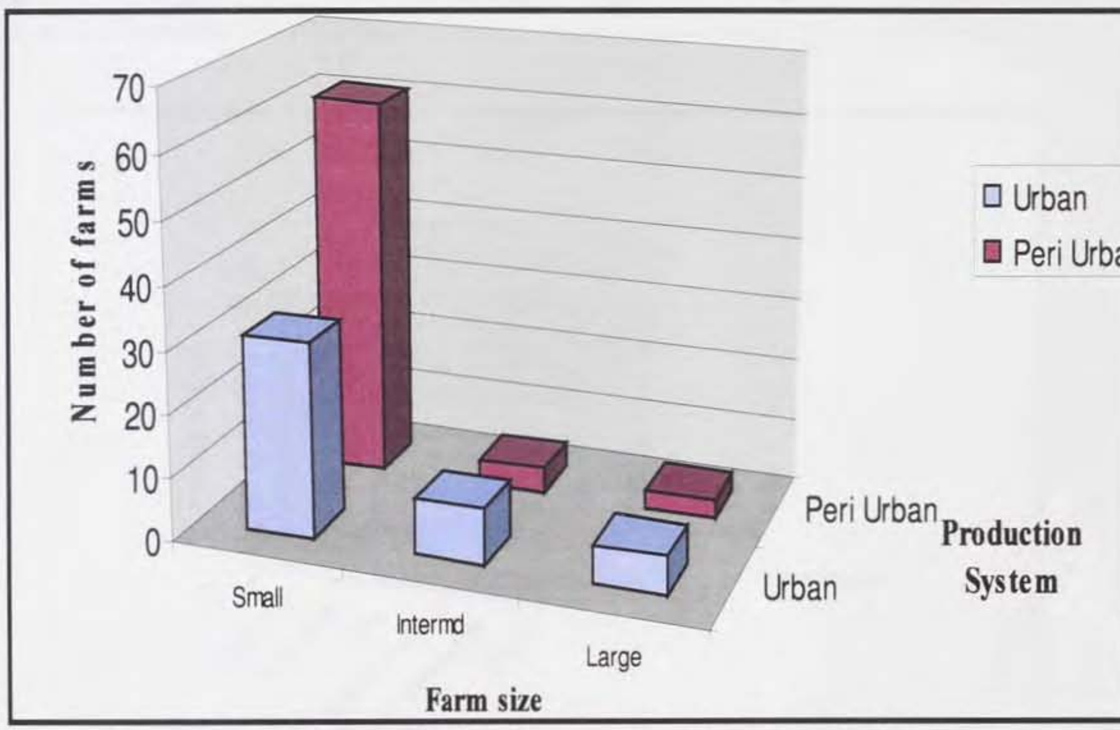


Fig. 4.1: Farm distribution according to area and size.

4.1.2. Farm establishment

The time of farm establishment (year) ranged from 1949 up to 1999, a span of 50 years (Table 6.2 and Figure 6.2). The majority of farms were established between the years 1971 and 1990 (77%). The increased dairy development activities (with foreign aid) within that period by the Ministry of Agriculture (MOA) and other Non-Government Organizations (NGOs) contributed to this increase in private dairy farm development.

Table 4.2 Year of farm establishment (Addis Ababa, Ethiopia)

Year	Urban			Peri-urban			Total
	Small	**Intermediate**	**Large**	**Small**	**Intermediate**	**Large**	
1940-1950			1				1
1951-1960			2		1		3
1961-1970	2	1	3				6
1971-1980	14	7		10	1		32
1981-1990	14	1		38	1	2	56
1991-1999	2			12	1	1	16
Total	**32**	**9**	**6**	**60**	**4**	**3**	**114**

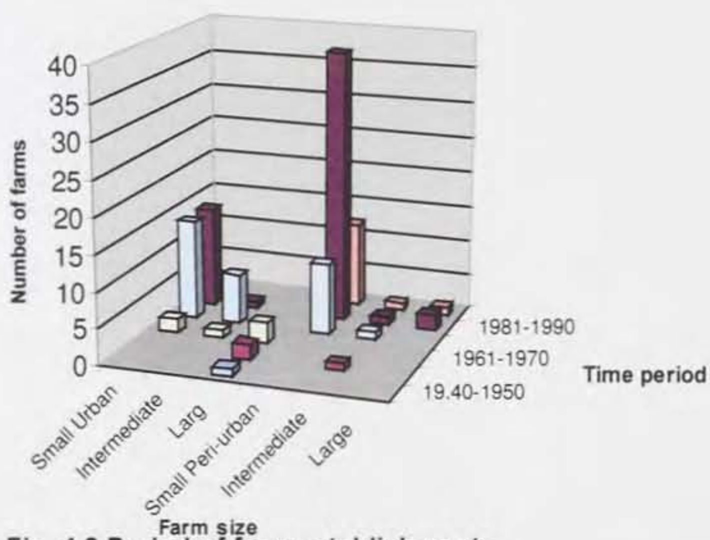


Fig. 4.2 Period of farm establishments

4.1.3. Occupation and education level of farm owners

The social status of owners ranged from retired vice ministers, former king councelors, retired higher military personnel to daily laborers (Table 4.3, Fig. 4.3)). All of the farmers were individual owners, no farm was owned by a partnership, in the form of rent or lease.

Table 4.3 Occupation and educational levels of farm owners

Ser.no	Occupation	No	Education level	No
1	Business men/women	42	No formal education	16
2	House wives	23	Some grade school	53
3	Dairy farmer	15	Some high school	17
4	Retired military personnel	10	High school graduate	16
5	Civil servant	10	Some college training	6
6	Retired civil servant	6	College diploma	4
7	Private enterprise employee	6	Some university	1
8	Daily laborer	2	University degree	1
Total		**114**		**114**

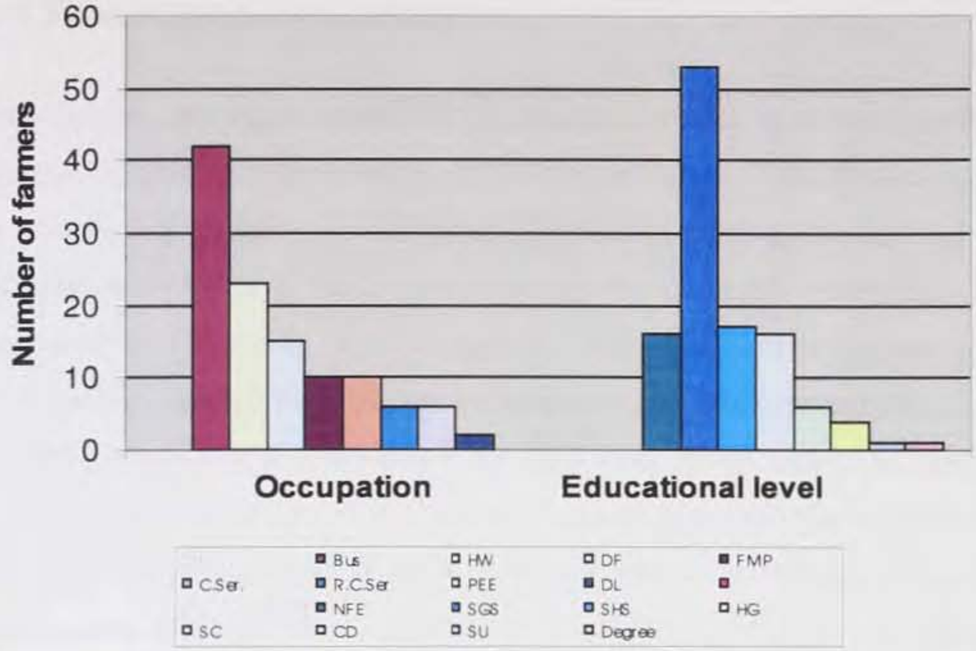


Fig. 4.3: Occupation and educational level of farmers

Bus--	Business men/women	NFE--	No formal education
H.W--	House wives	SGS--	Some grade school
D.F.--	Dairy farmers	SHS--	Some high school
FMP--	Former military personnel	HG--	High school graduate
C.Ser.--	Civil servant	SC--	Some college
R.C.Ser-	Retired civil servant	CD--	College diploma
PEE --	Private enterprise employee	SU- -	Some university
DL---	Daily laborer	Degree--	University graduate

The number of milking cows in the farms ranged from 1 up to 29 cows. With the exception of 3 farms (morning, mid day and evening milking) all cows in all farms were milked twice a day (morning and evening). The most common sources of the limited amount of information on dairying available on farms were veterinarians, the dairy association, regional dairy specialists, extension agents and sometimes other dairy producers. The three most frequent health concerns recorded for all farms were mastitis, reproductive inefficiency and calf mortality.

None of the routine mastitis control measures such as post-milking teat dipping, (using teat dip antiseptics), dry cow therapy, use of separate towel for each cow, washing of milkers hands between milkings, were used in any of the dairy farms.

4. 1. 4. Farm structure and social aspects

Farm structures and social aspects are summarized in Table 6. 4. Sixty (53%) of the dairy farms owners were females of which 23 (38%) were house wives. The average age of farm owners at the time of study was 58.3 years. The average farm size was 128 m^2 (sizes ranging from $12m^2$ up to $2000m^2$). The average farm size for the small, intermediate and large farm classes were 41, 175, and 1120 m^2, respectively. The majority of the farmers (except two) had their dairy farms located close to their living quarters (in one precinct). Four farmers (2 peri-urban and 2 urban) owned extra land in the rural areas. It was mainly used for pasture fodder and crop production. Only 2 farms had other animals on their farms which were chicken, sheep and donkeys. The availability of dairy workers was satisfactory in most of the farms. However, lack of knowledge and skills of the employed workers was complained by the majority (68%) of the farmers. Forty six per cent of the principal farm operators (owners) had an education level of some grade school. Only 6 farm owners were holders of college diploma or university levels of education. Their farms, however, were equally affected by mastitis as those of others having no formal education.

Table 4.4 Summary results on farm structures and social aspects

	Urban			Peri-urban		
Details	**Small**	**Intermediate**	**Large**	**Small**	**Intermediate**	**Large**
Sex of owner:						
M	19	4	2	24	3	3
F	13	5	4	36	1	0
Average age (years)	57.7	61	69	56.3	56	59
Occupation:						
Businessman/woman	13	5	5	17	1	1
Professional	6	0	0	9	1	0
Retired	8	0	0	4	2	0
House wife	3	0	0	19	0	0
Full time farmer	2	4	1	9		2
Daily laborer	0	0	0	2	0	0
Average farm size (sq. m)	50.5	72	907	32	278	1333
Do you own land elsewhere?						
Yes	0	1	1	0	2	0
No	32	8	5	60	2	3
Do you've other animals on the farm?						
Yes	0	0	1	0	0	1
No	32	9	5	60	4	2
Are you engaged in crop farming?						
Yes	0	0	1	0	2	0
No	60	9	5	60	2	3
Availability of dairy workers:						
Satisfactory	23	6	1	44	3	3
Moderate	8	2	2	16	1	0
Unsatisfactory	1	1	3	2	0	0
Knowledge & skills of dairy workers:						
Satisfactory	2	1	1	6	0	0
Moderate	4	3	1	18	2	0
Unsatisfactory	26	5	4	38	2	3
Common source of dairy information:						
Veterinarian	26	9	4	52	4	2
Dairy association	20	8	6	46	4	1
Extension agents	10	2	2	21	1	0
Other dairy producers	8	2	0	14	0	0
Average years of experience:	17	22	38	13	20	22

4.1.5. Health problems, management and control measures

Questionnaire results of the prevalent health concerns and treatments are presented in Table 6. 5. The most frequent health concerns of all the dairy farms investigated were silent estrus, mastitis and calf mortality, in order of importance. These were pressing problems in both the urban and peri-urban areas. Except for 3 farms which were milked trice daily, all the other farms milked their cows (hand milking) twice a day, that is morning and evening hours. Due to the high rate of silent estrus and hence of long intercalving periods, about 30% of the cows under study were milked for more than a year. Four farmers were registered as culling cows due to multiple teats blindness and chronic mastitis. The most common problems for culling or selling dairy cows were reproductive failure, debilitating diseases and acute shortage or high cost of feed stuffs. The small holders used family labor for milking. The intermediate and large farm holders used mixed labor as well as paid labor. All farmers washed udders prior to milking. Twenty (17.5%) farmers gave exercise to their animals. The rest kept their animals in total confinement of stalls. The hands of milkers were washed before milking in almost all farms. Only 20% of them, however, used one common towel for several cows to dry the washed udders and teats; in none of the farms separate towels were used for each cow. On some cows, reckless washing seemed to spread the filth from the udder to the teat ends.

Farmers do consult animal health personnel whenever their cows get mastitic. Thirty seven percent of the farmers, however, did not know as to which drugs to use for using mastitis. Sixteen (14%) farmers did not encounter mastitis in their herds before. The common drugs sold in domestic markets as intramammary infusions were used haphazardly. They were Penstrep, Gentamast®, Tetracycline, Multiject® and Mastiject®. A single tube of each infusion tube costed USD 1.48, USD 1.54, USD 1.35, USD 1.6 and USD 1.54, respectively. A full dose for a mastitic cow required 4 tubes. All small and intermediate stock owners complained about the high prices of drugs. For that reason, 84% of farmers responded that they do not treat mastitic cases as they occur. The long established (experienced) farmers usually administered the drugs themselves.

In 98 (86%) farms, cows with mastitis were milked at any time (rather than milking after the healthy ones) due to lack of knowledge of the mechanism of contamination of teats.

Table 4.5 Results of questionnaire survey on management and control measures

Questions	Urban			Peri-urban		
	Small	Intermediate	Large	Small	Intermediate	Larg
Most frequent health concerns:						
Mastitis	25	8	6	38	4	3
Silent estrus	32	9	6	54	4	3
Calf mortality	20	7	6	36	4	3
Most common reasons for culling animals:						
Disease	13	4	2	53	3	3
Reproduction	26	3	6	52	4	3
Mastitis	2	1	0	1	0	0
Number of times cows are milked in a day:						
Once	0	0	0	0	0	0
Twice	32	8	6	60	4	3
Three times	1	1	0	1	0	0
Do you give exercise to the animals?						
Yes	3	5	4	3	2	3
No	29	4	2	57	2	0
Is udder cleaned (washed) before milking?						
Yes	32	9	6	60	4	3
No	0	0	0	0	0	0
Are hands washed before milkings?						
Yes	32	9	6	60	4	3
No	0	0	0	0	0	0
Are separate towels used for each cow?						
Yes	0	0	0	0	0	0
No	32	9	6	60	4	3
Do you use a teat dip antiseptic?						
Yes	0	0	0	0	0	0
No	32	9	6	60	4	3
Do you have knowledge about dipping?						
Yes	2	0	0	6	0	0
No	30	9	6	54	4	3
Do you treat mastitic cases as they occur?						
Yes	2	3	3	6	2	2
No	30	6	3	54	2	1

Table 4.5 continued

Questions	Urban			Peri-urban		
	Small	Intermediate	Large	Small	Intermediate	Large
When do you milk cows with mastitis?						
First	0	0	0	0	0	0
Last	2	1	4	6	2	1
Any time	30	8	2	54	2	2
Do you practice dry cow therapy?						
Yes	0	0	0	0	0	0
No	32	9	6	60	4	3
Do you have knowledge of dry cow therapy?						
Yes	0	1	0	0	0	0
No	32	8	6	60	4	3
Drugs used for mastitis treatment:						
Tetracycline	9	3	4	6	0	2
Gentamast	6	4	5	14	2	2
Penstrip	3	6	4	2	6	1
Multiject	0	0	0	0	0	0
Mastiject	3	3	0	11	0	0
Do not know	10	6	1	22	2	1
Never treated before	0	0	0	3	0	0

4.1.6. Farm inspection and environmental management survey

Table 4.6 below summarizes the findings of the farm inspection and environmental management survey.

4.1.6.1. Building structures

Thirty three per cent of the dairy farms building materials were modern (made of cement, iron sheets, timber perlins and planks). The rest of the houses were a mixture of traditional (wood and mud) and modern. All housing types were free stall.

4.1.6.2. Floor types

The uneven floor surfaces were clear hazards to the animals and were not suitable for cleaning. As a result, foot-rot was common in such barns. Twenty six per cent of the farms used arranged stones for floors. All of them were small farms. All large farm barns, in contrast, had concrete floors. Only 4 farms had earth floors with complete wet and muddy ground throughout the study period.

4. 1.6. 3. Hazards /space

Twenty one per cent of cow stalls did contain hazards to the animals. Hazards were such things as defective stalls with protruding wood perlins, uneven cracked or cratered floors, defective doors made of wood or iron, biting flies, roofs which are not rainproof, inadequate space, etc. Seventy seven per cent of the farms had adequate space for their animals.

4.1.6.4. General farm hygiene / ventilation / drainage

Seventy nine (69.3 %) dairy farms had poor hygienic standards, while fifteen farms (13%) were in a good hygienic condition. The analysis of hygienic standards related to mastitis occurrence (significant at $p < 0.001$) is presented in Table 4.9. Ventilation also was highly related to mastitis prevalence ($p < 0.001$) (Table 4.10). Sixty percent of the farms investigated had poor ventilation with some houses being completely sealed, resulting in humid hot conditions inside. Fifty seven percent of farms had poor drainage systems resulting in accumulation of waste materials. Only seventeen (15%) farms had good drainage systems. Most of these farms were located next to a moving stream or to river sides so that farm wastes could drain into the water.

Fifty nine percent of the cows showed serious soiling of udders. This was clearly related to the poor hygiene and drainage conditions of the farms. Twenty four percent of the dairy cows showed apparent soiling of udders. Sixty six percent of cows showed moderate fly infestations. Nineteen percent of cows, however, suffered seriously from too many flies. Poor drainage systems and infrequent cleaning seemed to increase the number of flies. Some farms were observed of spraying insecticides when flies numbers were alarmingly high.

Table 4.6 Results of questionnaire survey on farm inspection and environmental management

Table 4.6 Results of questionnaire survey on farm inspection and environmental management

Questions	Urban			Peri-urban		
	Small	Intermediate	Large	Small	Intermediate	Large
Building material used is:						
Traditional	0	0	0	0	0	0
Modern	10	3	5	16	1	3
Mixture	22	6	1	44	3	0
Housing type is:						
Earth	1	1	0	2	0	0
Concrete	23	8	6	32	4	3
Arranged stone	8	0	0	22	0	0
Does sun shine enter barn?						
Yes	2	0	2	4	2	2
No	28	9	4	56	2	1
Is the roof structure rain proof?						
Yes	27	9	6	52	4	3
No	5	0	0	8	0	0
Are cow stalls free of hazards?						
Yes	26	6	4	49	3	2
No	6	3	2	11	1	1
Are cow stalls of adequate size?						
Yes	23	6	6	47	3	3
No	9	3	0	13	1	0
The drainage system						
Good	2	3	0	11	0	1
Satisfactory	13	2	1	12	2	2
Poor	17	4	5	37	2	0
Ventilation						
Good	3	0	0	12	0	1
Satisfactory	12	2	3	12	0	1
Poor	17	7	3	36	4	1

Table 4.6 Continued

Questions	Urban			Peri-urban		
	Small	Intermediate	Large	Small	Intermediate	Large
General farm hygiene & orderliness						
Good	3	0	0	12	0	0
Satisfactory	6	3	0	10	1	0
Poor	23	6	6	38	3	3
Soiling of udders is:						
In apparent	3	3	2	18	1	0
Moderate	2	4	0	11	3	0
Serious	27	2	4	31	0	3
Flies on the cows:						
Negligible	2	0	0	14	0	0
Moderate	27	6	6	32	2	2
Too much	3	3	0	14	2	1

4.2. Prevalence study

The total number of milking cows was 479, total quarters numbered 1916. Some of the animals tested had one or more lost quarters due to previous attacks of mastitis and therefore were not able to secrete any milk (102 quarters were blind). Sixty two blind teats were found in small farms in 43 animals, 14 in intermediate farms in 10 animals and 26 in large farms in 23 animals. Out of these, 56 were single blind teats of 56 cows, 28 were double blind teats of 14 animals and 18 triple blind teats of 6 animals. The total number of teats examined was 1814.

Mastitis prevalence was determined by the CMT, SCC and microbiological culture at the animal and quarter levels (Table 4.7, Figure 4.4). Four hundred thirteen (85.6%) and 392 (81.2%) cows were positive for CMT and SCC, respectively. Out of the CMT positive animals 178 (37.2%) did harbor a causal agent for mastitis. One thousand one hundred and forty four (63%) quarters were positive for CMT, 983 (54.4%) for SCC and 280 (15.4%) in microbiological culture (Table 4.7). There was no significant difference in the number of quarters affected with respect to their anatomical positions. The number of quarters affected in the first prevalence study were: right fore, 10 and 77 right hind, 8 and 62 left fore, 6 and 54 left hind, 9 and 54 for clinical and subclinical mastitis, respectively.

Table 4.7 **Prevalence of mastitis (both clinical & sub-clinical) based on CMT, SCC and culture**

Farm category	No. of farms	No. of cows	CMT +	SCC +	Agent +	CMT+ Prevalence (%)	SCC + prevalence (%)	Agent prevalen e (%)
Small	93	201	169	160	92	84.0	79.6	45.8
Medium	12	96	82	77	36	85.4	80.2	37.5
Large	9	182	159	152	50	87.4	83.5	27.5
Total	**114**	**479**	**410**	**389**	**178**	**85.6**	**81.2**	**37.2**

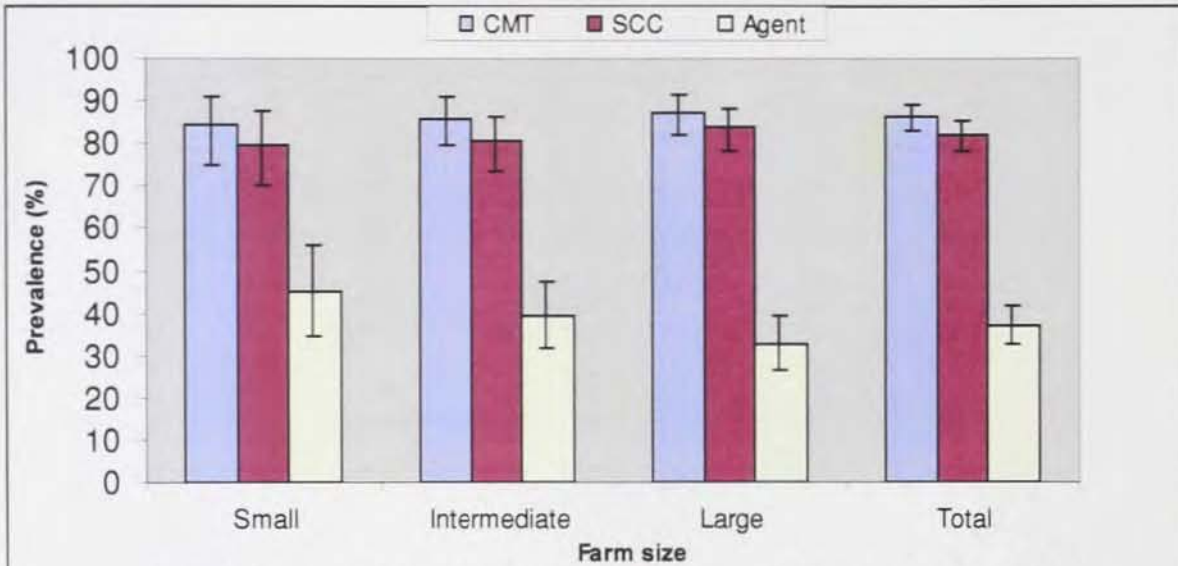


Fig. 4.4 :Prevalence of mastitis (clinical and subclinical) based on CMT, SCC and bacteriological findings

4.2.1. Prevalence of clinical mastitis

Twenty four cows (5%) were found to be clinically infected. Four of the total blind teats (102) were from these clinically infected cows. Seventy five (81.5%), 65 (71%), and 33(1.8%) quarters were positive for CMT, SCC and mastitis causing agents respectively. The majority

(18) of the clinically affected cows were from the small and large dairy farms (Table 4.8). The difference in clinical mastitis prevalence between farms was not statistically significant at the cow level, but at the quarter level. The highest prevalence of quarters infection was from the intermediate farms.

Table 4.8 Clinical and subclinical mastitis at animal and quarter levels

Farm size	No.of farms	No. of cows	Clinical mastitis (animals)	Subclinical mastitis (animals)	Clinical mastitis (teats)				Subclinical mastitis (teats)			
					RF	RR	LF	LR	RF	RR	LF	LR
Small	93	201	8	84	2	1	2	2	47	37	27	29
Intermediate	12	96	6	30	5	3	1	3	9	11	10	8
Large	9	182	10	40	3	4	3	4	21	14	17	17
Total	**114**	**479**	**24**	**154**	**10**	**8**	**6**	**9**	**77**	**62**	**54**	**54**

4.2.2. Prevalence of subclinical mastitis

One hundred and fifty four cows (32.2%) were subclinically infected. Ninety eight of the total blind teats were from these cows. The number of open quarters examined was 1722. One thousand three hundred and seventy eight (76%), 1240 (68.4% of) and 247 (13.6%) open quarters were positive by CMT, SCC and bacteriological investigation respectively.

The distribution of clinical and subclinical mastitis in the different farm categories is shown in Table 4.8. The highest prevalence of subclinical mastitis was recorded in the small farms (41.8%) followed by intermediate(31.3%) and large farms (22%). This difference in subclinical mastitis between farm size categories was statistically significant at the cow and quarter levels. The highest quarter infection was recorded in the small farms (Table 4.8).

4.2.3. Risk factors analysis

Risk factors were identified and analyzed for their association and effect with mastitis using the Epi Info Program Version 6.02. Potential risk factors identified included general hygiene and cleanliness of barns, cows, workers and cubicles, ventilation of barns, drainage of the barns, parity, age, stage of lactation and herd size. Comparison of rates of mastitis related to the exposure of animals to these risk factors (measures of magnitude of association) was carried out by 2 x 2 tables containing the risk factor-disease categories and using prevalence ratios (PR), (Tables 4.9, 4.10, 4.11,.4.13, and 4.14). Statistical analysis was carried out by use of Chi square analysis and logistic regression, using the SPSS program.

Table 4.9 Prevalence of mastitis related to hygiene

Hygiene	Positive	Negative	Total	Point prevalence (%)	95% CI
Good	3	36	39	7.7	1.6 – 20.9
Moderate	32	70	102	31.4 **	22.5 – 41.3
Poor	143	195	338	42.3	37.0 – 47.8
Total	**178**	**301**	**479**	**37.2**	**32.8 – 41.7**

$\chi^2 = 19.80$, d.f = 2 ** significant at $p < 0.001$ Prevalence ratio = 5.2; the prevalence in exposed animals was 5.2 times higher than in unexposed animals.

Table 4.10. Prevalence of mastitis related to ventilation

Ventilation	Positive	Negative	Total	Point prevalence (%)	95% CI
Good	5	56	61	8.2	2.7 – 18
Moderate	36	70	106	34 **	25.0 – 43.8
Poor	137	175	312	44	38.3 – 49.6
Total	**178**	**301**	**479**	**37.2**	**32.8 - 41.7**

$\chi^2 = 28.47$, d.f. = 2 ** significant at $p < 0.001$ Prevalence ratio = 5.0

Table 4.11 Prevalence of mastitis with respect to drainage

Drainage	Positive	Negative	Total	Point prevalence (%)	95% CI
Good	16	59	75	21.3	12.7 – 32.3
Moderate	58	134	192	30.2 **	23.8 – 37.2
Poor	104	108	212	49	42.0 – 56.0
Total	**178**	**301**	**479**	**37.2**	**32.8 – 41.7**

$\chi^2 = 24.87$, d.f.= 2 ** significant at $p < 0.001$. In the multivariate analysis, drainage was found to be not statistically associated with mastitis ($p = 0.8251$). Prevalence ratio = 1.9.

Table 4.12 Prevalence of mastitis related to parity

Parity	Positive	Negative	Total	Point prevalence (%)	95% CI
1	35	150	185	18.9	13.55-25.32
2	43	106	149	28.9 **	21.7-36.8
3	100	45	145	68.96	60.8-76.4
Total	**178**	**301**	**479**	**37.2**	**32.8-41.7**

Parity 1 represents those cows which calved up to 2, parity 2 cows which calved 3 – 4.times and parity 3 cows which calved 5 times or more. $\chi^2 = 93.52$, d.f = 2 ** Significant at $p < 0.001$. Age is obviously expressed by parity. Test results of age are: $\chi^2 = 81.30$ d.f. = 9,** significant at $p < 0.001$. The majority (67%) of the mastitic animals were within the age range of 6 to 10 years. Prevalence ratio = 2.57

Table 4. 13 Prevalence of mastitis related to stage of lactation

Stage of lactation	Positive	Negative	Total	Point prevalence (%)	95% CI
1	55	52	107	51.4	41.54-61.18
2	23	45	68	33.8	22.79-46.32
3	22	51	73	30.0 **	19.94-42.00
4	78	153	231	33.8	27.69-40.26
Total	**178**	**301**	**479**	**37.2**	**32.82-41.66**

Stage of lactation 1 represents cows within 1 month of lactation, 2 represents those within 2 – 3 months, 3 within 4 – 6 months and 4 those which are within more than 6 months of lactation. $\chi^2 = 12.30$, d f = 3 ** Significant at $p < 0.01$. Prevalence ratio = **1.23**

Table 4.14 Prevalence of mastitis related to herd size

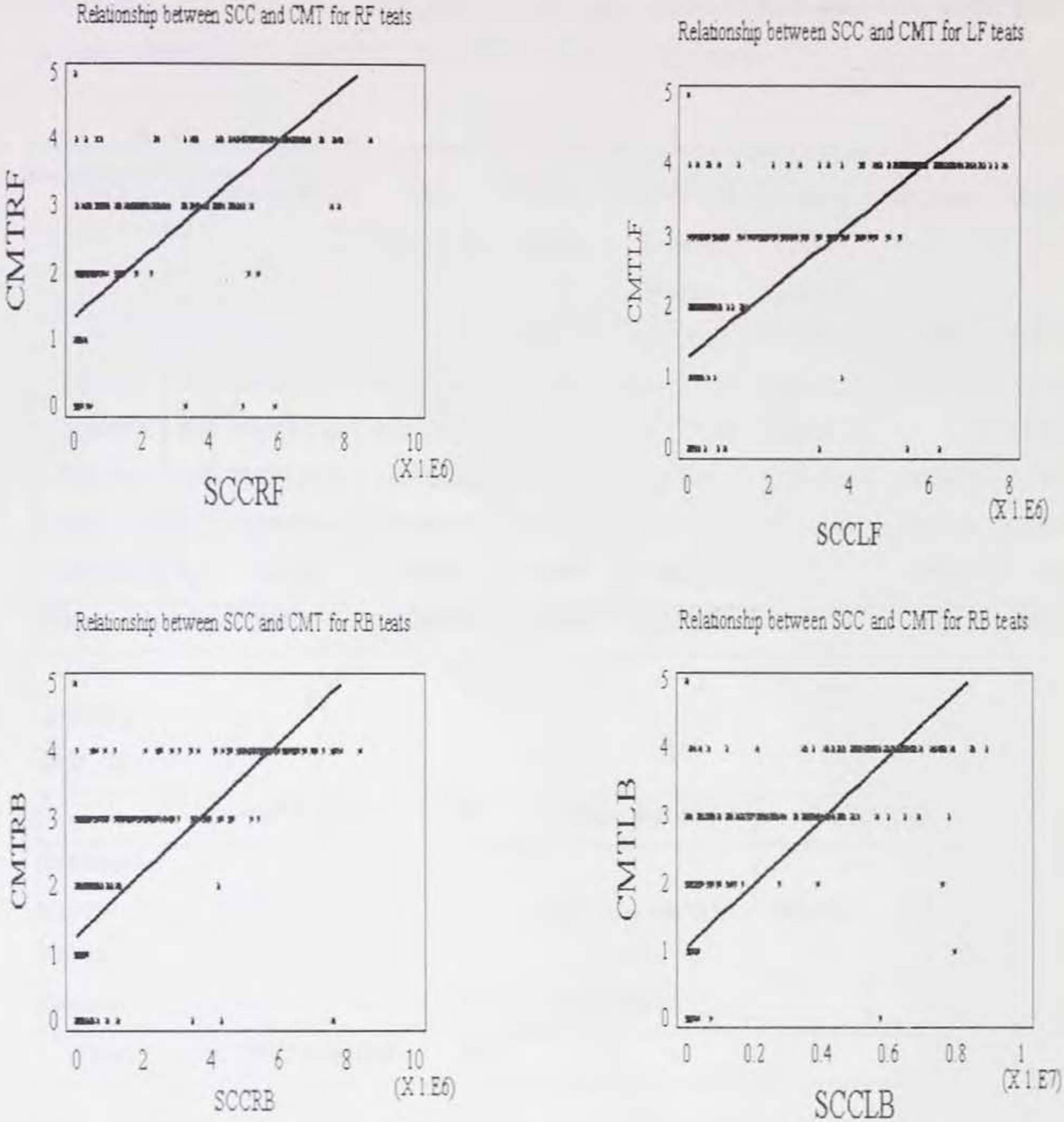
Farm size	Positive	Negative	Total	Point prevalnce (%)	95% CC
Small	92	109	201	45.77	38.7-52.9.
Intermediate	36	60	96	37.5 **	27.8.-48.0
Large	50	132	182	27.5	21.1-34.6
Total	**178**	**301**	**479**	**37.2**	**32.8-41.7**

χ^2 =,13.7 d.f.= 2 ** Significant at $p < 0.001$. Prevalence ratio = 1.35

Multivariate analysis using logistic regression by (SPSS program) was conducted on the major risk factors (hygiene, ventilation, stage of lactation, herd size and parity). The method used was Forward Stepwise Regression (LR). Odds Ratio (OR) for hygiene was 2.1, ventilation 11.95, parity 13.2, stage of lactation 2.44, herd size 1.15. In this adjusted analysis, drainage and location (at urban, peri-urban or zone levels) were found to have no statistical significance on mastitis occurrence ($p > 0.05$).

4.2.4. Association of CMT and SCC

For evaluation of association between SCC and CMT, linear regression analysis was carried out. Fig. 4.5. shows the results of fitting a linear model to describe the relationships between the two screening tests for the 4 teats (RF, RR, LF and LR). There is a statistically significant ($p < 0.01$) relationship between CMT and SCC at the 99% confidence level. The R–squared statistic indicates that the model fitted did explain 40.6%, 44.3%, 39.0% and 52.7% for RF, RR, LF and LR quarters, respectively, of the variability in the corresponding CMTs. The correlation coefficients were 0.64, 0.67, 0.63 and 0.73 for RF, RR, LF and LR quarters, respectively, indicating a moderately strong relationship between the two variables.



CMTRF = 1.36991 + 4.35413E-7*SCCRF   CMTRB = 1.24971 + 4.76917E-7*SCCRB

CMTLF = 1.31524 + 4.61672E-7*SCCLF   CMTLB = 1.06636 + 4.71198E-7*SCCLB

Fig. 4.5 Relationships between CMT and SCC results for the 4 quarters

Results for the RF teat (taken randomly as an example of the four teats), based on CMT versus SCC results are presented in Table 4.15. A direct association between the two screening tests is evident. The mean somatic cell counts increased with an increase in CMT grades. Mean plots of the two screening tests for RF are presented in Figure 6.6. A clear association between

CMT and SCC is observed. An increase in the CMT grades is accompanied by an increase in the somatic cell counts. The value 5 on the CMT axis represents blind teats from which milk was not available; this value is different from the zero CMT values.

Table 4.15 Mean somatic cell counts of the RF teat in relation to CMT grades

CMT	N	Mean SCC	Std. Deviation	Std. Error	95% CI for mean Lower Bound	Upper Bound	Minimum	Maximun
.00	139	159438.85	698955.05	59284.5	42215.24	276662.3	11300.0	5900000.
1.00 (t)	18	128666.66	54786.215	12913.2	101422.36	155911.2	12000.0	287000.0
2.00 (+)	62	740766.12	961567.91	122119	496573.42	984958.7		5400000.
3.00 (++)	119	2286176.4	1597559.8	146448	1996169.0	2576183	19000.0	7800000.
4.00(+++)	117	5529726.4	1493851.9	138106	5256188.2	5803264	21000.0	8721000.
5.00(blind	24	0000	0000	0000	0000	0000	0000	0000
Total	**479**	**2065633.6**	**2453546.4**	**112105**	**1845353**	**2285913**	**.00**	**8721000**

ANOVA

SCC RF

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2193612718317784	5	438722543663556.	303.432	.000
Within Groups	683894846711068	473	1445866483532.91		
Total	**287750756502885**	**478**			

The mean somatic cell counts increased with the increase in CMT grades. The CMT grades of ++, +++, and the somatic cell counts higher than 500,000 were found to be good predictors of positive bacterial culture of milk samples. The differences between the sample means for cell counts of CMT grades were significant.

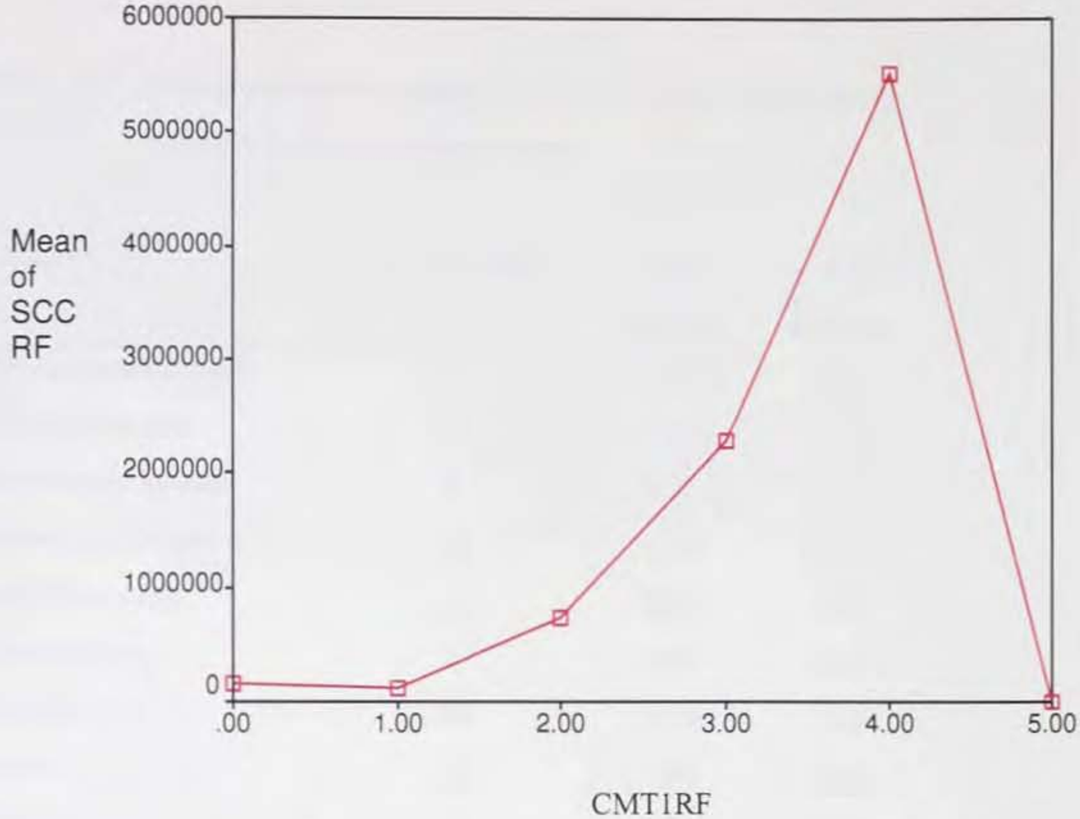


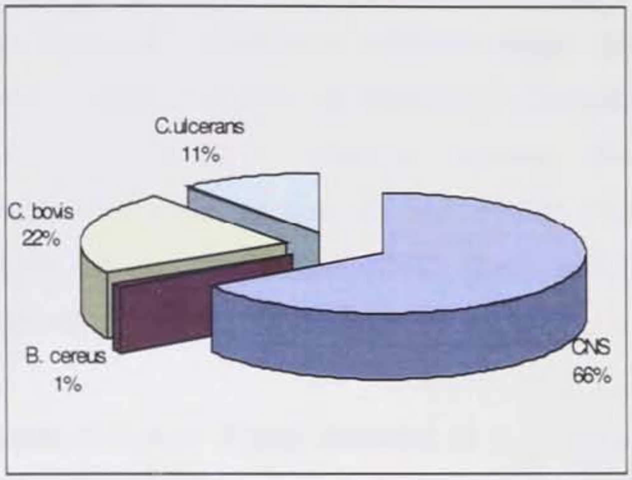
Fig. 4.6. Plot of mean for SCC and CMT values of the RF teat

4.2.5. Etiological agents isolated in mastitic animals

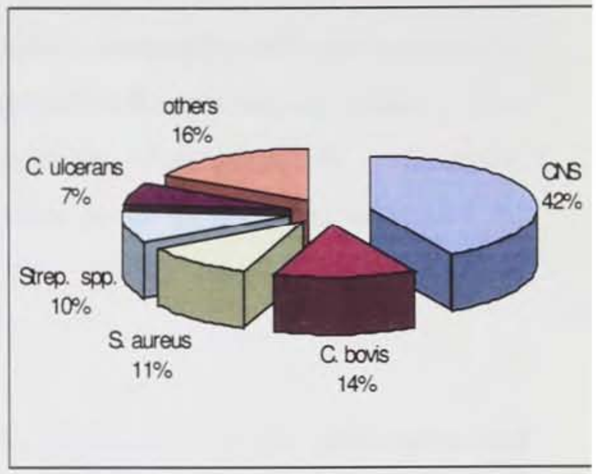
A total of 683 agent isolations was done in the initial prevalence study, 249 identification for major pathogens and 434 for minor pathogens (Table 4.16.). In this study, emphasis was given to the major pathogens. *Staphylococcus aureus* and *Streptococcus* species were the pathogens most frequently encountered as major causative agents. They comprised 58% of the total major pathogenic microorganisms isolated. The most commonly isolated agents were CNS (41.9% of total isolates). When organisms of environmental origin were discovered in the laboratory investigation, milk was resampled from the respective cows aseptically to resolve doubts that the agents did originate intact from the udder and not from milkers' hands. The proportions of isolates are presented in Fig. 4.7 (a, b, c). Differences in the distribution of aetiological agents were not statistically significant at the location, farm and farm size levels.

Table 4. 16 Pathogenic microorganisms isolated from quarter milk Samples in the prevalence study

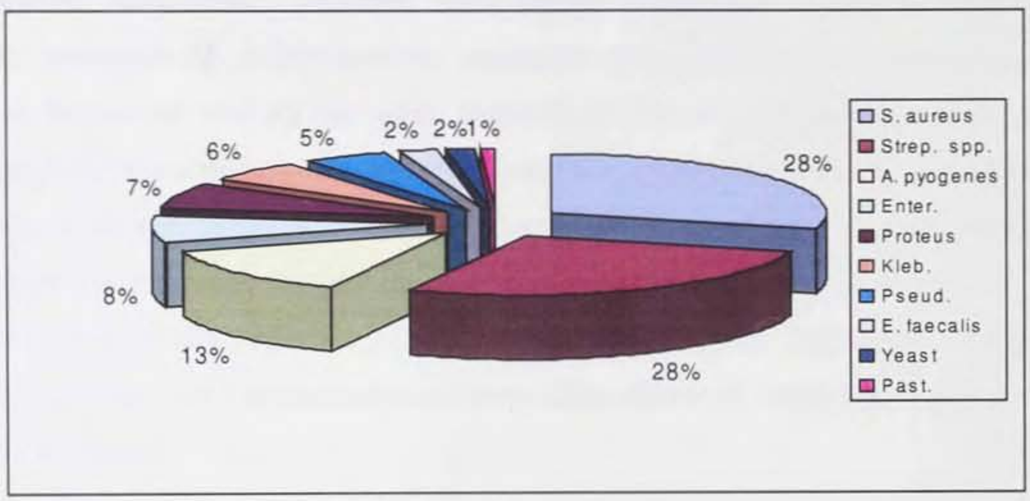
Pathogens	No. of isolates	% of total samples	% of total isolates
Staphylococcus aureus	73	4.02	10.69
Streptococcus spp.	69	3.80	10.10
Enterococcus faecalis	6	0.33	0.88
Actinomyces pyogenes	32	1.76	4.68
Pseudomonas spp.	12	0.66	1.76
Pasteurella spp.	2	0.10	0.29
Klebsiella spp.	14	0.77	2.05
Proteus spp.	18	1.00	2.63
Enterobacter spp.	19	1.00	2.78
Yeast	4	0.22	0.59
CNS	286	15.77	41.87
Bacilus. Cereus	4	0.22	0.59
Corynebacterum bovis	96	5.30	14.06
Corynebacterium ulcerans	48	2.6	7.03
Total	**683**	**31.9**	**100.00**



a. Proportions of minor pathogens



b. Proportions of total isolates



c. Proportions of major pathogens

Fig. 4.7 (a, b, c): Proportions of microorganisms isolated in the prevalence study

4.2. 6. In vitro antibiotic sensitivity tests

A total of 246 isolates of major pathogenic and minor organisms isolated were tested for their *in vitro* antibiotic susceptibility to 11 antimicrobial drugs (Table 4.17). The drugs used were found as commonly used intramammary infusions bought in local markets. Generally, erythromycin, polymyxin B, sulphamethoxazole, gentamycin and oxytetracycline and neomycin were found to be effective drugs. Ampicillin, tetracyclin and streptomycin, in contrast, were found to be the weakest of all the drugs used in the antibiogram. Streptomycin, however, was still effective against *Streptococci*. Polymyxin B, neomycin, sulphamethoxazole, gentamycin and erythromycin were found to be drugs of choice for *Staphylococcus aureus* and CNS. These agents were mostly resistant to tetracycline, streptomycin, ampicillin, oxytetracycline, and penicillin.

Streptococci were highly sensitive to polymyxin B, sulphamethoxazole, gentamycin and neomycin. They were also relatively sensitive to penicillin, streptomycin, oxytetracycline and chloramphenicol. *Actinomyces pyogenes* were highly susceptible to sulphamethoxazole, gentamycin, polymyxin B, oxytetracycline, neomycin and erythromycin. Tetracycline and streptomycin did not act well on this agent. *Enterobacter faecalis* was sensitive to all drugs except ampicillin, chloramphenicol, streptomycin and tetracycline. All Gram-negative isolates were susceptible to erythromycin, polymyxin B, sulphamethoxazole, gentamycin, neomycin and oxyteracycline. However, the best drugs of choice for these bacteria were polymyxin B and oxytertacycline (100% effective). Drugs of high sensitivity for *Actinomyces pyogenes* were also effective against *Corynebacterium bovis* (78% effective). Yeast was resistant to all of the antibiotics used.

Table 4. 17 Antibiotic sensitivity of pathogenic organisms from clinical and subclinical cases of mastitis

Pathogens	Total isolates	No. tested	AMP S/R	OXY S/R	CHL S/R	PEN S/R	STR S/R	TET S/R	ERY S/R	PLY S/R	SUL S/R	GEN S/R	NEO S/R
Staph. Aureus	73	64	22/42	48/16	141/23	28/36	18/46	12/52	58/6	63/1	60/4	59/5	61/3
Strep. agalactia	31	27	9/18	23/4	22/5	24/3	22/5	20/7	25/2	27/0	27/0	26/1	26/1
Strep. dysgalactia	20	17	12/5	14/3	15/2	15/2	13/4	11/6	15/2	17/0	17/0	16/1	17/0
Actin. Pyogenes	32	28	4/24	27/1	19/9	19/9	12/16	12/16	26/2	27/1	26/0	27/1	26/2
Strep. uberis	18	16	5/11	15/1	15/1	9/7	12/4	8/8	15/1	16/0	14/2	15/1	16/0
Ent. Faecalis	6	5	3/2	5/0	3/2	4/1	2/3	2/3	4/1	5/0	5/0	5/0	5/0
Pasteuralla spp.	2	2	1/1	2/0	1/1	2/0	1/1	1/1	2/0	2/0	2/0	2/0	2/0
Pseudomonas spp.	12	10	8/2	10/0	10/0	10/0	4/6	5/5	10/0	10/0	8/2	10/0	10/0
Klebsiella spp.	14	12	6/6	12/0	8/4	4/8	5/7	4/8	10/2	11/0	12/0	8/4	10/2
Proteus spp.	18	16	3/13	16/0	13/3	7/9	7/9	9/7	15/1	16/0	16/0	10/0	16/0
Enterobacter spp.	19	16	2/14	16/0	14/2	6/10	6/10	14/2	12/4	16/0	16/0	15/1	16/0
CNS	286	20	11/9	8/12	10/10	11/9	8/12	9/11	19/1	20/0	19/1	20/0	20/0
Coryne. Bovis	96	10	4/6	10/0	7/3	6/4	5/5	4/6	10/0	10/0	10/0	10/0	10/0
Yeast	4	3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Total	**631**	**246**	**90/156**	**206/40**	**178/68**	**136/110**	**115/131**	**111/135**	**221/25**	**240/6**	**232/14**	**229/17**	**235/11**

AMP- Ampicillin (2 µ g)
CHL – Chloramphenicol (30µg)
OXY- Oxytetracycline (30µg)
PEN - Penicillin G (10 IU)
STR - Streptomycin (10µg)
TET - Tetracycline (10µg)
ERY - Erythromycin (5µg)
PLY - Polymyxin B (300 IU)
SUL - Sulphamethoxazole (25µg)
GEN - Gentamycin (5µg)
NEO - Neomycin (10µg)
S/R - Sensitive/Resistant

4.3. Longitudinal study

All farms included in the initial prevalence study were also involved in the incidence study.

4.3.1. Incidence of clinical mastitis

The incidence rates for clinical mastitis investigated for 3 months at inspection intervals of one month are presented in Table 4.18. The farms follow the same categories as in the prevalence study (small, intermediate and large). For the 3 months period, the overall incidence rate was 4%. The clinical incidence per cow month was 1.33%. The cumulative incidence was 3.97%. Mean farm incidence rates between the different farm sizes were significantly different ($p < 0.001$). The incidence rate of clinical mastitis was highest in the intermediate farm size category followed by the large and small farm categories. The number of new cases decreased from the first to the last interval in all farm size categories. As a result, the incidence of clinical mastitis showed a down ward trend from the first to the last interval.

Table 4.18 Incidence of clinical mastitis in the different farm categories

Farm size	Cows at risk at the beginning	Cows at risk at end of period	No. of new cases over 3 m	Incidence density rate in the 3 m (%)	Cumulative incidence rate (%)	Incidence per cow-montl (%)
Small	193	188	7	3.67	3.6	1.20
Intermediate	90	84	4	4.6	4.4	1.53
Large	170	166	7	4.2	4.0	1.4
Total	**453**	**438**	**18**	**4**	**3.97**	**1.33**

4.3.2. Incidence of subclinical mastitis in farm categories

At the beginning of the study, 304(63.3 %) cows were at risk of developing subclinical mastitis out of a total milking cow population of 480 animals. The number of cows at risk at the end of the 3 month period was 155. The corresponding incidence rate for the 3 month period therefore was calculated as 33.6 % and the cumulative incidence as 25.3%. The incidence rates per-cow month in the small, intermediate and large farms were 14.2%, 10% and 8.53%, respectively (Table 4.19). These mean farm incidence rates between the different farm sizes were significantly different ($p < 0.001$). The incidence in the small farms (42.5%) was higher than in the intermediate (30.2%) and the large (25.6%) farms, taken separately. As with clinical mastitis, the number of new cases decreased from the first to the last study interval in all farm size categories.

Table 4.19 Incidence of subclinical mastitis in the different farm categories

Farm size	Cows at risk at the beginning	Cows at risk at end of period	No. of new cases over 3 m	Incidence density rate in the 3 m (%)	Cumulative incidence rate (%)	Incidence per cow month (%)
Small	112	81	41	42.5	36.6	14.2
Intermediate	60	26	13	30.2	21.7	10.0
Large	132	48	23	25.6	17.4	8.53
Total	**304**	**155**	77	**33.6**	**25.3**	**11.2**

4.4. Final prevalence study

At the end of the longitudinal study, a final cross-sectional study was again carried out with the mere intention of assessing the intensity and direction of mastitis on the 114 farms. The number of lactating cows sampled was 464. Ten animals (2.2%) were found with clinical mastitis. The number of teats affected by clinical mastitis was 13, total blind teats registered were 70. The number of normal teats sampled was 1786. The prevalence of clinical mastitis at quarter level was, therefore, 0.7%. A hundred and twelve cows (24%) with 184 teats (10.3%) were subclinically infected.

There was a clear drop of mastitis prevalence (clinical and subclinical) in the farms investigated when compared with the initial prevalence study 4 months before. As in the initial study, the highest prevalence was found in the small farms (33%) and the lowest in the large farms (19%). In the intermediate farms, the prevalence was 27%. The profiles of the etiological agents identified were similar with those recovered in the initial study, with the exception that *yeasts* were not detected at all.

The advice forwarded to the farmers on proper hygienic procedures probably were more or less turned into practicality by the large and some intermediate farm owners.

5. DISCUSSION

The conventional outlook is that as herd size increases mastitis prevalence and incidence also increases; herd size in particular is said to be associated with the incidence of clinical mastitis. With increasing herd size, manure disposal and sanitation problems may also increase exposure to coliforms and environmental streptococci (Bartlett et al., 1992). In this study, significant differences in mastitis prevalences between different farm sizes (especially for subclinical mastitis) were observed. However, the highest mastitis prevalence was established for the small farms and the lowest for the large farms. Knowing about and using more often antibiotics and also due to the great attention which is paid to the large farms, may have contributed to this effect. Side-line jobs of farmers were not attached with the large farms, owners rather were always found busy and giving full attention to their stock. Their earnings were entirely based on their farms and hence the general management of the large farms was relatively better than in the intermediate and small farms. Owners seemed to understand the consequences of leniency in managing their herds.

The largest number of small farms were located in peri-urban areas, while the greater parts of the intermediate and large farms were located in the urban centers. This seems to be contrary to the conventional view that larger farms mostly are located at city outskirts. However, this diversion is a result of the inconsistent and discouraging land ownership policy of the socialist administration of Ethiopia in the seventies and eighties, where large areas of land were rarely given to individuals. The owners of the large farms in the urban centers are those who attained their holdings before the military regime came into being.

Diverse occupations and educational levels of farm owners were recorded during the study. This signifies that various social groups have found interest in dairy enterprises, inspite of their limited know-how on routine activities in intensive production and with high yielding cattle.

Fifty three percent of the dairy farm owners, including those of the large farms, were females. This is not an unusual phenomenon for Ethiopia, for females are highly attached to dairy activities in most areas, including the rural hinterlands. From the total owners only 13% of them were full time dairy farmers. These were the ones who were highly devoted to dairying, exerting full time labor, paying attention to professional advice and endeavoring to draw maximum benefit out of their efforts. Conversely, for the majority of farmers, their dairy farms were side-line jobs, only to add to their income from other major occupations. The educational standard did not seem to have a major effect on dairying in the farms studied. Even academically well trained farm owners did not take advantage to fully use their skills to improve their farm outputs. Their farms were equally affected by mastitis as those belonging to owners without any education. This fact points to the need for a consistent extension services to address farmers on dairy activities. The time of establishment of some dairy farms in Addis Ababa goes back to the 1950s (half a century), yet the dairy enterprise is still poorly developed. While the ideal highland climate is most favorable for the development of small holder dairying, unfavorable national and international policies have probably done much to discourage such dairy enterprises (Delgado, 1991, Walshe *et al.*, 1991, Winrock International, 1992).

The sizes of small, intermediate and large farms were 41, 175 and 1120m^2, respectively. Only the portion of land allotted to the animals (barns, exercising area, the portion of land for manure disposal and feed storage) in this investigation was taken as farm size; owners' dwellings beside the barns were not taken as portion of barns. The majority of the farm owners (except two) were living in the same compound beside their dairy animals. The operation of farms area therefore is very small, especially for small farms. Problems of effluent disposal, ventilation and drainage consequently were clearly observed, especially in the small and intermediate farms; these constraints pose a potential menace, both for man and animals. Neighbors grievances and outcries due to irritating smell of farms and effluent dispersion were registered around most of these small and intermediate farms, particularly in the urban areas. Some neighbors even have undergone legal proceedings against farm owners because of the discomforts they have.

Four large farm owners owned extra land in rural areas, all of them had vast expanses of land (not less than 0.06 km^2) and two of the owners did possess additional dairy farms on these extra lands. Three of them were also engaged in crop farming. They produce excess hay and crop residues to the extent of selling it to other farmers.

Most farms were satisfied with the availability of dairy workers, but complained about their lack of knowledge and skills. Small dairy holders almost exclusively used family labor for handling and milking their animals. This undoubtedly moderated the staggering economic balance of these farms. Intermediate and large farms used paid labor as well. Lack of dairying information was evident in almost all farms. The meager know-how, if at all existing, was from veterinarians, the dairy association, regional dairy specialists, extension agents and sometimes other dairy producers.

The three most frequent health concerns recorded in all farms were reproductive inefficiency, mastitis and calf mortality. The other problem of farms was the acute shortage of feed stuffs which aggravated recently due to absence of rains in the short rainy season. The market price of roughage and concentrates did increase more than 3 fold and some farmers were even threatened to close their farms.

Due to reproductive failures about 30% of cows were milked longer than a year. Only 4 farmers were identified that did cull cows due to multiple teats blindness and chronic mastitis. This condition in the majority of farms does not only result in economic loss to farmers but also poses a serious risk of infection for clean cows (Smith, et al., 1985a, Radostits *et al.*, 1996). Twenty farmers exercised their animals, the rest kept their animals in total confinement of stalls. Hooves outgrowth and dirty grounds were common observations in such farms. Deep mud and excessive moisture in such barn yards greatly increase the likelihood of coliform organisms contaminating the udder (William, 1995). An increasing number of hours spent outside protects against environmental mastitis (Smith *et al.*, 1985a). Tied dairy cows showed increasing frequencies of mastitis than exercised cows. Coliform mastitis is much more frequent in housed cattle than in those grazing and health in general is positively influenced by exercise (Gustafson, 1993). Fresh verdant lush grass was only given to cows in some small farms during the wet seasons and only such cows were able to get an adequate supply of vitamin E (Smith *et al.*, 1985b).

Knowledge about post-milking teat dipping, use of disinfectants for cleaning udders, hands and utensils and dry cow therapy were generally missing from all farms. This requires urgent solution.

Few farm owners did milk mastitic cows last, eighty six percent of the farmers rather milked their mastitic cows at any time during the milking hours. This practice obviously facilitates cow-to-cow transmission of contagious as well as environmental pathogens (Matos *et al.*, 1991). The major pathogens are mainly spread from cow to cow through milkers' hands, udder cloth and residual milk in teat cups, etc. (Dodd, 1971). Udder and milkers hands were washed before milking in almost all farms. In no farm, however, separate towels were used for drying udders of milking cows, old rags rather were most commonly used for most cows before milking. Hand washing with only water without disinfectants may reduce pathogenic microorganisms but does not guarantee their elimination. Water is both a help and a hindrance because it is necessary for effective cleaning when the teats and udder are dirty but it also can carry bacteria down the teat from a wet udder and thus allow contamination of milk during the milking procedure (Bushnell, 1984). On some cows reckless washing seems to aggravate teats contamination, pathogens spread from cow to cow during milking (Quinn *et al.*, 1994, Matos *et al.*, 1991).

Dairy farmers, especially those of small and partly of intermediate farms complained about the current high price of intramammary drugs available in local markets. However when the financial situation of farmers is taken into consideration, they are actually able to treat their mastitic cows. In order to treat a single cow by gentamycin, with two mammary glands affected, it would cost a farmer USD 11.84 (Birr 96.2). This expense can be covered by milk sale from a single cow of eight days. An average milk yield of a crossbred cow in urban and peri-urban small holding is usually 6-10 liters a day (Azage and Alemu, 1998) and the current average milk price in Addis Ababa is USD 0.25 (2 Birr). However, if a cow with two mammary glands affected is not treated, half of its production in one lactation period gets lost. Therefore, it is imperative that farmers should be made aware of these facts.

Sixty seven percent of the farms were constructed with traditional and some modern (mixture) materials. This trend is encouraging, given at the current low status of living conditions of farmers in the city. The number of farms with concrete floors also was 67%. This condition is encouraging as well, for it allows adequate cleaning. Those farms with floors of arranged stones and mud, in contrast, were hazardous, stressing the cows and hindering proper cleaning. Eighty eight percent of farms barns did not have access to sun light and failed to make use of it. Sun light has a beneficial effect against environmental mastitis causing organisms (Smith *et al.*, 1985b). Most farms had rain proof roofs (89%). In the remaining farms (11%), the wet seasons have horrible consequences, the grounds turn floody, and the refuse is spreading to every corner of the barns. The existence of organic materials (urine, dung, milk, feed left overs), moisture and temperature favor the growth of environmental organisms (Zehner and Fansworth, 1986). It is worth mentioning that most of the hazards registered in the study were due to negligence of the farm owners.

Risk factors for mastitis of dairy cattle (of hosts and of the environment) were carefully scrutinized. The analysis indicates that herd size, parity, stage of lactation, hygiene, ventilation and age were significantly related to mastitis occurrence. Similar observations have been reported by e. g Bushnell, (1984), Schukken et al., (1989), Hamann et al., (1991). Parity in particular has been shown to be risk factor for both subclinical and clinical mastitis (Schukken *et al.*, 1989). It is postulated that younger animals have a decreased susceptibility through a more effective host defense mechanism. Older cows, especially after four calvings, are more prone to mastitis (Dulin *et al.*, 1988). Mastitis due to environmental organisms is most common in the first few days after calving and regresses as lactation progresses (Radostits *et al.*, 1996). This is due to a reduction in the immune function soon after calving (Kehrli *et al.*, 1982). On the other hand subclinical mastitis increases as the stage of lactation progresses.

Of the farms studied, only few had good hygienic conditions. Hygienic procedures in general are very important in controlling mastitis (Bushnell. 1984). Presence of large numbers of potential pathogens in the immediate environment of the animal might have induced mastitis. Milking management and milking technique have been shown to be important risk factors, with machine milking being more risky than hand milking and calf suckling (Hamann *et al.*, 1991).

Most of the dairy farms had poor ventilation and drainage, thus did predispose animals to mastitis. Damp barn environment present in free stalls predisposes to coliform mastitis, regardless of seasonality. Coliform mastitis is an "occupational hazard" in free-stall housed cattle. Excessive build up of manure, failure to clean or maintain stalls, and poor ventilation contribute to high levels of coliform organisms in free stalls (William, 1995).

Both CMT and SCC were used as indirect tests for the detection of especially subclinical mastitis. The clinical and subclinical mastitis prevalence rates recorded in this study are remarkably high. The results are, however, lower than those reported in some previous studies (Biru, 1989; Abdella, 1996), the high prevalence rate, in particular, agrees with the results of Bishi (1998), where 5.5% and 30.2% prevalences for clinical and subclinical mastitis, respectively, were reported for Addis Ababa and Debrezeit.

The majority of microorganisms isolated did belong to the group of minor pathogens, especially CNS (42%). This is in agreement with previous reports (Harmon and Langlois, 1986, Birru, 1989, Bishi, 1998). Coagulase positive staphylococci were mostly found in subclinical cases in combination with other major pathogens. It has been suggested that quarters harboring CNS may be more resistant to subsequent infections by more pathogenic bacteria than uninfected quarters (Lam *et al.*, 1997; Edwards and Jones, 1966).

The major pathogens were *Staphylococcus aureus* and *Streptococcus* species (59%). *Staphylococcus aureus* was recovered both in clinical and subclinical cases of mastitis while *Streptococcus agalactia* was mainly recovered in subclinical cases of mastitis. There are several reports to support this result (William, 1995; Abdella 1996; Birru, 1989). Agents of environmental origin contributed 30% of the major pathogens, a clear indication for the needs of proper hygienic procedures. Deep mud and excessive moisture in barn yards greatly increase the likelihood of coliform organisms, contaminating the udder (William, 1995). Excessive buildup of manure, failure to clean or maintain stalls and poor ventilation also contribute to high levels of coliform organisms in free stalls. Thirteen percent of the major pathogens were *Actinomyces pyogenes*, half of them were recovered in clinical cases. Most of the infections due to these organisms occur in dry cows, hence relate to "dry cow mastitis" (Brooks, 1983; Pankey et al., 1985); Watts, 1988. Dry cows, however, were not examined in this study. It is well possible that the prevalence of mastitis due to these organisms will be higher in dry cows. This is an area for further investigation.

Farmers used drugs for treatment of mastitis without selection. The disk sensitivity test did provide guidance to the choice of effective antibiotic therapy. Antibiograms of the isolated microorganisms against antibiotics commonly available as intramammary preparations in domestic markets showed that erythromycin, polymyxin B, sulphamethoxazole, gentamycin, neomycin and oxytetracycline were effective drugs. *Staphylococcus aureus*, however, one of the major pathogens isolated showed the widest spectrum of resistance, next to yeasts. Similar observations have been reported by Matos *et al.* (1991) and Packard *et al.* (1992). Farmers have to be advised to select effective drugs for the treatment of mastitis.

The incidence rate of the clinical mastitis registered in this study is lower than that reported by Bishi, (1998). It was similar, however, with results of a recent study of Omore (1996) in Kenya where they reported a clinical mastitis incidence of 13.3% per annum. The clinical incidence rate in this study was significantly different among the different farm sizes. The attempt to record cases within the intervals of consecutive visits failed due to insufficient information derived from the farm owners. The incidence of subclinical mastitis was significantly different in the 3 farm size categories. The highest was in the small farms, followed by the intermediate and the large farms. As in the first cross-sectional study, this stands against the hypothesis that greater risks of mastitis prevail in larger than in small farms. The reverse situation found here might be due to the same explanation given in the prevalence study. The values of subclinical mastitis incidence rates in this study were also lower than those reported by Bishi, (1998) and by far lower than those reported by Erksine et al. (1988) and Wilesmith *et al.* (1986). The number of new cases decreased from the first to the last interval in all farm size categories for both clinical and subclinical incidences. This finding is more or less contrary to the report of Bishi (1998). The decrease in the number of new cases through time was most probably due to the improved management taken by farmers when they were repeatedly visited by animal health personnel. They asked for advice and guidance. Giving a positive response for their request was unavoidable.

The regression analysis conducted on the SCC and CMT results revealed the relationship between the two screening tests. Apart from research purposes, in the absence of electronic cell counter, DMSCC can hardly be used for monitoring herd udder health using individual quarter or bulk milk samples. With all the drawbacks that DMSCC has, it is hard to set a threshold for mastitis infection of a herd in a single study. Repeated counts, if possible, with electronic cell counters are necessary. In this study, quarter samples with more than 500000 cells seemed to show infection. This is supported by the findings of Kehrli (1982) and De Graaf and Dwinger (1996).

5.1 RECOMMENDATIONS

Mastitis was found to be one of the major constraints to dairy production in the intensive dairy farms studied. Management factors are highly responsible for the success of such enterprises. The importance of these factors increased with the leniency of owners due to their involvement in other jobs (dairy as a side line income generating activity). In most of the farms studied, the hygienic conditions consequently were generally poor. The economic losses in this situation could be substantial, but methodologies to examine the true impact of these problems on urban and peri-urban milk production have not been adequately developed. Such investigations are indispensable for the formulation and implementation of effective control measures. The common observations in many of the farms studied were poor sanitary conditions and practices, problems of nutritional management, confinement of animals in over-crowded and poorly ventilated and constructed housing. The standard of milking hygiene equally was very low and preventive measures like the use of udder disinfectants, strip cup, post-milking teat dipping, dry cow therapy and treatment of clinical cases as they occur were a rare sight on these farms. These factors had a significant effect on the occurrence of mastitis in the herds. Surprisingly, in the farms investigated, little efforts and work are done to detect subclinical mastitis of the dairy herds. This should be done at a regular interval and clinical cases should be treated as they occur. Although many farmers complain about the current high price of antibiotics, effective drugs for the treatment of mastitis are available in local markets. It is obvious that the market orientation of production only addresses the supply of milk to markets. The necessary complementary input side, involving production into the dairy operation, is not yet considered by most milk producers. In this regards the systems are not yet "modern". Given this gap, there is high need that the dairy farmers get support from the government, dairy development agencies, extension services or other Non-Government Organizations (NGOs) by all kinds of training and on-farm support.

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7. ANNEX

7.1. Annex 1. Questionnaire

A. Herd description and farm structure

Farm owner-------------------------------------sex--------age-----------years
Occupation-----------------------------------
Farm address-----------------------------------Tel.--------------------
Owner's address------------------------------- Tel.--------------------
Year farm established---------------------------
Location : urban------------------
peri-urban-------------
Farm size----------ha.
Species owned-----------------,-----------------,----------------,---------------
No of each species (respectively) -----------,-----------,----------,----------
Breed of cattle owned -----------,-----------,-----------,-----------,------------
No of each breed -----------,-----------,-----------,-----------,-------------
Cows----------Heifers-----------Bulls-----------Oxen-----------Calves---------.
Do you own land elsewhere eg. rural area? yes no.
What do you do on this land? --

B. General production information

1. Please indicate the number of cows of each breed that you are milking today.
-----------------Local zebu
-----------------cross bred
-----------------pure exotic
2. Please indicate the number of dry cows in your herd today. ------------------
3. Please indicate the number of milking cows in your herd that are currently in the:
----------------first lactation.
----------------second lactation.
----------------third lactation
----------------fourth lactation.
----------------fifth lactation.
----------------sixth lactation.
----------------six and above.
4. Do you plan to remain in the dairy industry over the next 5 years? Yes no unsure
5. If you plan to leave the dairy industry, does a current associate or member of your immediate family plan on continuing? Yes no unsure
6. Are bred heifers and dry cows grouped together? Yes no

C. Social factors

7. Please circle the number indicating your level of satisfaction with:
   a. Availability of dairy workers:

      very dissatisfied   very satisfied
      1   2   3   4   5
      [ ] does not apply.

   b. Knowledge and skills of available dairy workers:

      very dissatisfied   very satisfied
      1   2   3   4   5

8. Please check up to five of the following as your most common sources of dairy information.
   - District (woreda) agriculturalist.
   - Veterinarian.
   - Farm inspector.
   - Colleges and Universities.
   - Research Institutes.
   - Regional dairy specialist.
   - Dairy association.
   - Private consultant.
   - Extension agents.
   - Other dairy producers.

   Other, please specify--.

   Comments---

   ---.

9. How many years have you been the principal operator?
   - < 5 yr.
   - 6-10 yr.
   - 11-15 yr.
   - 16-20 yr.
   - > 20 yr.

10. Please indicate the highest level of education achieved by principal operator:
    - no formal education.
    - some grade school.
    - some high school.
    - high school graduate.
    - some college training.
    - college diploma.
    - some University.
    - University degree.

    Other, please specify--.

11. Age of principal operator:
    - < 30
    - 31-40
    - 41-50
    - 51-60
    - >60

12. Please check the following categories that apply to your dairy farm:
    individual owner
    owned by a partnership
    rented
    leased
    Other, please specify---.

13. How many families, including employees' families, depend on the dairy enterprise as their major source of earnings?
    one
    two
    three
    four
    five or more
14. Please indicate below what percentage of your gross income the dairy enterprise contributes to your farm operation (not including off-farm income).
    1-20%.
    21-40%.
    41-60%.
    61-80%.
    81-100%.

D. <u>Health problems</u>
15. Please indicate your three most common reasons for culling animals over the last year:
    production
    feet and legs
    conformation
    reproduction
    mastitis
    size
    temperament
    disease
    Other, please specify---.
16. Please indicate your three most frequent health concerns:
    ketosis
    retained placenta
    milk fever
    mastitis
    cystic ovaries
    foot problems
    metritis
    silent estrus
    calving difficulties
    Other, please specify---.

E. Management

17. How many times do you milk your cows in a day?
    - once
    - twice
    - three or more times
18. Is the udder cleaned before milking? Yes No

19. Do you use a teat dip anticeptic? Yes No
20. When do you dip the teats?
    - before milking
    - after milking
    - both
21. When do you milk cows with mastitis?
    - first
    - last
    - at any stage
22. Do you treat mastitic cases (clinical) as they occur? Yes No
23. Do you practice dry cow therapy? Yes No
24. Milking management

Are udders and teats washed before milking?	Yes	No
Are udders and teats dried before milking?	Yes	No
Is a separate towel used for each cow?	Yes	No
Are hands washed between milkings?	Yes	No
Is a disinfectant used for washing hands?	Yes	No
Is a teat dip used for each cow?	Yes	No
Does the teat dip applicator appear clean?	Yes	No

F. Farm inspection report.

25. Housing.
    Building material used is:
    - traditional
    - modern
    - mixture
26. Housing type.
    - pen
    - barn
    - stanchion
    - free stall
27. Floor type.
    - earth
    - concrete
    - stone

    Other, please specify---.

28. Does the building structure allow sunshine into building?
Yes No

Is the roof structure rain proof?	Yes	No
Are cow stalls free of hazards?	Yes	No
Are parturition pens clean and well bedded?	Yes	No
Are cow stalls of adequate size? (220cm x 110cm)?	Yes	No

29. The drainage system:
    - excellent
    - satisfactory
    - poor
30. Ventilation in building:
    - excellent
    - satisfactory
    - poor
31. General farm hygiene and orderliness:
    - excellent
    - satisfactory
    - poor
32. Cow cleanliness:
    - soiling of the udders,
        - not apparent
        - moderate
        - serious
    - flies on the cows,
        - negligible
        - moderate
        - too much

7.2. Annex.2. Map of Addis Ababa showing the woredas and Study area

CURRICULUM VITAE

Personal Data

Name	Nesru Hussein
Date of birth	10 / 07/ 63
Place of birth	Butajira, Ethiopia
Nationality	Ethiopian
Marital status	Married
Profession	Veterinarian
Permanent address	P. O. Box 20682, Code 1000, Addis Ababa, Ethiopia.

2. Educational background

1968-1974	Butajira elementary and junior high school
1975-1978	Yirgalem high school
1981-1986	Addis Ababa University; Faculty of Veterinary Medicine

3. Professional experience

1986-1993	Research Officer grade 2, Institute of Agricultural Research,
1994-1995	Research Officer grade 3, Oromia Agricultural Bureau
1996-1997	Animal health division head, Adamitulu Research Center

4. Membership

Member of the Ethiopian Veterinary Association and Ethiopian Society of Animal Production

5 Publications

1. Nesru, H., Teshome, Y., Getachew, T. (1997): Prevalence of mastitis in different local and exotic breeds of milking cows. Eth. Jou. Agric. Scie. **16**: 53-60.

2. Nesru, H., Abubeker; H., Zeleke, A. (1997): Efficacy of panacur®, rintal® and deuxamine® on GIT parasites of goats. Proceedings, Ethiopian Veterinary Association, Addis Ababa, Ethiopia, 11-18.

3. Nesru, H. (1997): Studies of calf mortality. Proceedings, Ethiopian Society of Animal Production, Addis Ababa, Ethiopia, 252-261.

4. Nesru, H. (1992): Prevalence of bovine brucellosis around Adamitulu. Research Newsletter of IAR, Ethiopia. **7**(1), 4.

5. Nesru, H. (1993): Studies of calf mortality at Adamitulu. Research Newsletter of IAR, Ethiopia. **10**(2), 5-6.

Cross Sectional & Longitudinal study of Bovine Mastitis in Urban & Peri-Urban dairy systems in the Addis Ababa Region, Ethiopia.

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