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

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Alec Simukai Bishi

January, 1998

FREIE UNIVERSITÄT BERLIN AND ADDIS ABABA UNIVERSITY

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A thesis submitted in partial fulfilment for the degree of
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at the Freie Universität Berlin and Addis Ababa University

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To my dear wife Angeline and my parents for everything

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ABSTRACT

This study was conducted to establish the prevalence and incidence of clinical and subclinical mastitis in peri-urban and urban dairy production systems in and around Addis Ababa and Debre Zeit, Ethiopia. A questionnaire survey was also conducted to gain an insight into the farming systems in operation, and to study some management and social factors that may have an influence on the occurrence of mastitis. The main aim of this study was to demonstrate the effect of intensification (farm size in particular) on the occurrence of mastitis.

The prevalence study was based on 434 currently milking cows from 40 different farms. For the purposes of this study the farms were categorized into two groups, as smallholder and large farms. There were nine large farms with 366 lactating cows and 31 smallholder farms with 68 lactating cows.

The California Mastitis Test (CMT) was used to screen quarters for infection. Milk samples were only collected from quarters that reacted positively to CMT. These samples were subjected to standard microbiological procedures whereby microorganisms were identified to the species level or the genus level.

Two hundred and sixty-seven quarters (15.9%) were positive on the CMT. Of these 27 were clinically affected (1.6%). The proportion of subclinically affected quarters that were positive on microbiological culture to known agents for mastitis was 85.4%. The overall prevalence of subclinical mastitis at the cow level as determined by the CMT and microbiology was 34.3% and 30.2% respectively. The mean prevalence rates taken at the farm level were significantly different between the two farm groups. The prevalence was higher in the large farms (30.6%) than the smallholder farms (27.9%) as determined by microbiology. The overall prevalence of clinical mastitis was 5.5% at the cow level. There was a significant difference in mean prevalences at the individual cow level between the two farm groups. It was higher in the large farms (5.7%) as compared to 4.4% in the small farms.

Two hundred and eleven isolates were obtained from subclinical cases. Fifty-four per cent were due to coagulase negative staphylococci, 27% *Streptococcus agalactiae*, 9.5% *Staphylococcus aureus* and 5.2% coryneforms. Streptococci species other than *Str. agalactiae* accounted collectively for 3.8% of the infections. The mean frequency rates of CNS, *Str. agalactiae*, *S. aureus* and coryneforms were significantly different between the large and smallholder farms.

A total of 25 isolates were obtained from clinical cases. *Str. agalactiae* was the most frequent cause of clinical mastitis and contributed 32.3% of the isolates. CNS accounted for 22.6% of these isolates, 12.9% *Staphylococcus aureus*, 9.6% environmental streptococci (9.6%) and 3.2% gram positive bacilli. Six of the quarters (19.4%) yielded no growth.

Sixty-seven isolates obtained from both subclinical and clinical mastitis were subjected to the in vitro antimicrobial susceptibility tests against 14 antibiotics. The bacterial species tested were 17 isolates of *S. aureus*, 19 environmental streptococci, 10 coryneforms and 19 CNS species.

Staphylococcus species were 100% susceptible to gentamycin, erythromycin, neomycin, and oxacillin and highly susceptible to lincomycin (94%). Only 12% were susceptible to

ampicillin and 18% to penicillin. Erythromycin was the most effective against environmental streptococci, with 89% of the isolates being susceptible. Only 68% of the isolates were susceptible to ampicillin. Erythromycin was the most effective drug against coryneforms with 80% being sensitive followed by polymixin B at 70%. CNS were highly susceptible to neomycin (90%), gentamycin, erythromycin and oxacillin (86%), respectively.

Economic losses due to mastitis were estimated to cause a 6.9% drop in milk volume or, on average a daily loss of 0.55 liters per day during the entire lactation period of every cow.

The incidences of clinical and subclinical mastitis were determined by the CMT in three, three week intervals. One hundred and seventy-four cows out of 309 cows from 23 farms were considered to be at risk at the beginning of the study period. The study period coincided with the rainy season which runs from June to August. The overall incidence of subclinical mastitis was 39.5% over the nine week period which translated to 19.0% per cow month at risk. The mean incidence rates between the large and smallholder farms were significantly different. The incidence was higher in the large farms (18.7%) than in the small farms (12.1%) per cow month at risk. The incidence of subclinical mastitis over the study period showed an upward trend in the large farms whilst it was on the decline in the smallholder sector.

The overall incidence of clinical mastitis was found to be 9.4% for the 9 week period or 4.2% per cow month at risk. The mean incidence rates calculated at the farm level between large and small farms were significantly different. It was higher in the small farms (6.8%), compared to 3.7% per cow month at risk in the large farms. The incidence of clinical mastitis was on the increase in both farm groups during the study period.

The most important findings of the questionnaire survey were: there was a general lack of knowledge of basic mastitis control measures such as dry cow therapy, teat dipping, culling and monitoring; the use of disinfectants was almost absent; nearly all the farmers washed the udder prior to milking. Eighty percent of the farmers consulted a veterinarian although 70% of them treated the cows themselves. The most commonly used antibiotics were penicillin/streptomycin combinations, gentamycin and tetracycline. The use and knowledge of antibiotics were lower in the smallholder sector as compared to the large farms.

1 INTRODUCTION AND OBJECTIVES

Ethiopia is a land-locked country in the horn of Africa with a total area of approximately 1,2 million km^2. The heart of Ethiopia is a high table-land known as the Ethiopian plateau. The climate varies according to elevation above sea level from the tropical zone below 1 830 m receiving about 510 mm rainfall, to the temperate zone with a rainfall of over 1 500 mm and over 2 500 m above sea level.
The country has an estimated human population of about 51.1 million (1993 estimates). Agriculture accounted for 48% of the GDP in 1992, and engaged 73% of the economically active population. The country has the highest livestock population in sub-Saharan Africa with an estimated 27 million cattle, 24 million sheep, 18 million goats, 7 million equines, 1 million camels and 52 million chickens (FAO, 1993). Over 60% of the cattle and sheep are found on the highlands.

The development of the smallholder dairy production in Sub-Saharan Africa regarding Eastern and Southern Africa, began in earnest within the last 30 to 40 years. In some countries in the region, the development of this sector was initiated by governments. This was after the realization that the future, sustained and long-term ability for adequate national dairy production lay in the hands of the smallholder sector. They had vast potential in terms of their numerical advantages, livestock and land ownership. Examples of such active government participation are, for example, the Smallholder Development Project of Zambia formed in the early 1970's (Kaluba, 1992), the Kenya National Dairy Development Project formed in 1980 (Miriuki, 1992), and the Dairy Development Program (DDP) formed 1983 in Zimbabwe (Mupunga and Dube, 1992).

In Ethiopia, the development of dairy production has evolved through similar lines with the assistance of such institutions as, the Institute of Agricultural Research (IAR), Arsi Regional Development Program (ARDP), teaching institutions, Ministry of Agriculture and the Ministry of State Farms Development (Kebede, 1985).

However, the impact of these programs on the dairy industry has been minimal considering the livestock numbers available and the ideal agroclimatic conditions of the Ethiopian Highlands. The FAO (1993), estimates that some 645 000 to 700 000 tons of milk are produced per annum from the national herd. Tedla (1997 personal communication), identified four major systems, the traditional smallholder system, dairy cooperatives, state dairy farms, private small and medium scale dairy farms in urban and peri-urban locations. The traditional sector accounts for 97% of total production. This sector uses indigenous breeds whose productivity is as low as 1.5 to 2 liters per day over a 150 to 180 day lactation and cows calve once every two years. Recognition of this low potential production has necessitated the importation of high yielding exotic breeds. The Ministry of Agriculture estimates that meanwhile 80 000 of the cattle population are improved crossbred animals that produce 6 to 10 liters of milk per day over a lactation period of 270 to 300 days.

The main reasons for the poor achievements are that the research and development programs have been conducted without a clearly defined strategy or program and lack of cohesiveness amongst the various institutional organizations (Kebede, 1985). This is in contrast to the Kenyan experience where well organized institutional policy has resulted in relatively good success in the Kenyan Highlands. The Kenya dairy industry is dominated by the smallholder farmers who account for over 75% of the industry's output (Miriuki, 1992). However, dairy

production using high yielding crossbred animals encounters numerous problems in obtaining production inputs, services, and information on advanced production technology and as such finds it difficult to operate at the appropriate level of intensity to fully utilize production potential (Batz, 1995).

The failure of artificial insemination delivery and the risk of East Coast Fever in most areas and of Trypanosomiasis in others, are major biological constraints preventing many smallholders adopting dairy farming as an enterprise in Kenya (Thorpe *et al.*, 1992). The major constraints in dairy production in Ethiopia, cited by Kebede (1985), were, among other factors, low genetic potential of indigenous breeds, poor husbandry practices, poor feed resources, diseases and poor marketing infrastructure.

Major disease epidemics such as Rinderpest, Trypanosomiasis, Contagious Bovine Pleuropnemonia and Foot and Mouth Disease have been cited as major livestock diseases in Ethiopia (FAO, 1993). While it is acknowledged that epidemics are a major constraint in the viability of dairy enterprises, with increasing control and eradication of these major disease epidemics, and increasing intensification of production methods, there will be a shift in disease patterns from major epidemics to the more difficult to control resident opportunistic diseases such as pneumonia, mastitis, lameness, calf scours etc., which can prove to be prohibitively costly if left uncontrolled (Schwabe, 1984). Milk production under extensive production systems such as the use of essentially indigenous breeds on pasture has, for example, been associated with low prevalence of mastitis (Perry *et al.*, 1987).

Despite these numerous problems, three main forces are driving change in the agriculture system and in the livestock sub-sector. These are population growth, changes in demand for livestock products as well as changes in the availability of inputs and technology. Marketed dairy production is already increasing in most African countries in response to these circumstances as well as development efforts to promote smallholder dairy production. The increase in the human population is inevitably leading to increased land scarcity and a tendency towards intensification of production methods. Although extensive productions are more capital and labour efficient and more appropriate to the smallholder resources, the increasing human population is leading to increasing pressure on land. This is expected to lead to much greater intensification of land use and therefore a move to cut-and-carry technologies (Thorpe *et al.*, 1992).

The occurrence of mastitis involves the complex interactions of pathogens, the host and the environment. These three factors are influenced to greater or lesser extent as the level of production intensification increases. The introduction of environmentally poorly adapted exotic breeds, high yielding cows, increasing herd sizes, poor sanitary conditions and practices, the use of mechanized milking systems, problems of nutritional management, confinement of animals in overcrowded poorly ventilated and poorly constructed housing may be the consequence of attempts of intensification. These factors, among many others, are bound to have an effect on the occurrence of diseases in these herds where mastitis is not an exception.

The aims and objectives of this study were to focus on the problems of mastitis in the smallholder, zero-grazed, landless type dairy production systems right through to the more intensive commercial and larger dairy systems. In brief, listed below are the areas the study emphasized its investigations on:

- An appraisal of the current production systems and production conditions,

- Establishment of the prevalence and incidences of bovine clinical and subclinical mastitis as well as the causative agents of the disease complex,
- Determination of antibiotic profiles of isolates from mastitis cases.

2 LITERATURE REVIEW

2.1 General

Mastitis is an inflammatory reaction of the mammary gland in response to traumatic injury or to the presence of infecting microorganisms that have gained entrance into the udder. In the vast majority of cases, the disease is caused by microorganisms. Mastitis may be classified as clinical or subclinical depending on the degree of inflammation (Philpot and Nickerson, 1991).

Clinical mastitis may further be classified according to the severity of the inflammatory response as mild clinical, acute clinical, peracute or chronic.

On the other hand subclinical mastitis is subtle and 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. Because of its subtleness it may be overlooked but it is very important for the reasons that, it 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 and Nickerson, 1991).

Non specific mastitis also referred to as aseptic mastitis also occurs when microorganisms cannot be isolated from a milk sample.

2.2 Etiology and Epidemiology

The occurrence of mastitis involves the complex interaction among three major factors; the agent, host, and environment (Radostits *et al.*, 1996). At the host level, factors such as anatomy, intramammary defense mechanisms, lactation stage, parity and periparturient diseases are important (Shook, 1989; Radostits *et al.*, 1996). At the pathogen level factors such as virulence, toxins, antimicrobial resistance are important, whilst management, climate, feeding, housing, and milking technique are environmental risk factors for mastitis.

2.2.1 Mastitis causing pathogens

From the etiological point of view a total of 137 microbial species, subspecies and serovars have been isolated from the bovine udder (Watts, 1988). However, epidemiology has allowed their classification into the following:

2.2.1.1 Major pathogens

Major pathogens can further be classified as causes of either contagious or environmental mastitis.

The major pathogens causing contagious mastitis are *Streptococcus agalactiae* and *Staphylococcus aureus*. These are mainly spread from cow to cow through milkers' hands, udder wash cloths, residual milk in teat cups etc. Major pathogens causing environmental mastitis are for example coliforms and the other streptococci, with the most prevalent species being *E. coli*, *Streptococcus uberis* and *Streptococcus dysgalactia*. The other environmental infections are of an opportunistic nature, namely those caused by *Pseudomonas spp.*, yeast agents, prototheca spp., and Nocardia (Watts, 1988). Environmental mastitis occurs from ubiquitous organisms commonly found in the environment. The major method of transmission is from the environment to the cow by inadequate management of the environment. Major pathogens cause both clinical and subclinical mastitis.

2.2.1.2 Minor pathogens

Minor pathogens fall in the group of environmental pathogens and include, coagulase negative staphylococcal species, *Actinomyces bovis*, *Bacillus cereus*, and *Serratia marcens*. Minor pathogens are rarely associated with clinical changes and often induce only a moderate somatic cell response (Brooks *et al.*, 1983). The minor pathogens have been credited for maintaining the high Somatic Cell Count (SCC), and with increasing the resistance of the udder to invasion by a major pathogen (Rainard and Poutrel, 1988).

2.2.1.3 Some important features of causative agents

Staphylococcus aureus

Staphylococcus aureus 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 (Philpot and Nickerson, 1991; Cifrian *et al.*, 1996). This scar tissue is partially responsible for the poor antibiotic cure rates. These micro-abscesses are chronic active and release the bacteria to other areas of the gland in a continuous cycle resulting in larger abscesses which become clinically palpable.

On rare occasions *S. aureus* may produce a toxin that causes blood vessel constriction, producing peracute gangrenous mastitis which leads to the loss of the quarter and sometimes death of the cow (Philpot and Nickerson, 1991).

The microbiological classification of *S. aureus* as a gram-positive, β-hemolytic, catalase positive and coagulase positive coccus is insufficient to distinguish it from other staphylococci that have these characteristics, such as *S. hyicus* and *S. intermedius* (Sischo *et al.*, 1993). These two bacteria also cause mastitis. This classification results in the over-estimation of the incidence or prevalence of *S. aureus* by about one percent (Sears *et al.*, 1990). For the purposes of prevention and control, however, all coagulase positive staphylococci are routinely identified as *S. aureus* (Sears *et al.*, 1993). *S. aureus* together with *Streptococcus agalactiae* have been incriminated as the causes of most of intramammary infections in most dairy herds and are major contributors to herd somatic cell counts (Erskine, 1987; Sischo *et al.*, 1993). Smith, (1983) citing several other authors reports that these two were causes of 85 to 90% of bovine mastitis prior the development of teat dipping and dry cow therapy.

Streptococcus agalactiae

Streptococcus agalactiae is the only organism in the Lancefied B serological grouping of streptococcus species and is an important pathogen of both animal and man . It is considered

as an obligate parasite in cows (Schalm *et al.*, 1971;), although it is also found in 25% of normal human beings who may harbour it in the genital and gastrointestinal tract (Watts, 1988). It may cause septicaemia and meningitis in humans (Parker, 1979 cited by Shigidi and Mamoun, 1981). In dairy cattle it usually causes chronic subclinical mastitis, and heavy increases in somatic cell counts and significantly reduces milk production by as much as 18% (King, 1969; cited by Yamagata, 1987, Sischo *et al.*; 1993).

Dodd (1983), reports that, before the advent of antibiotics, it was already possible to reduce infection to only a few cows or eradicate *Str. agalactiae* within a two-year period provided segregation and culling was implemented in self-contained herds. 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 *Str. agalactiae* from herds (Dodd, 1983). Therapy could achieve cure rates as high as 98% and substantial benefits of therapy were realizable up to 121 days in a 305 day lactation, (Yamagata, 1987).

Dinsmore (1991), while he contented that *Str. agalactiae* was usually shed in high numbers from infected quarters, pointed to the fact that there were times when so few organisms were shed that a single sample may be culture negative. It was therefore essential, in an eradication programme, that repeated herd samplings were done to ensure that all *Str. agalactiae* infected cattle were identified and treated or culled. *Str. agalactiae* is presumptively identified based on colony morphology and hemolytic characteristics on blood agar , the camp reaction and the non-hydrolysis of esculin (Sears *et al.*, 1993).

Coagulase negative Staphylococci

Coagulase negative staphylococcus pathogens (CNS) mainly *Staphylococcus hyicus* and *S. epidermidis* have received very little attention until recently (Myllys *et al.*, 1994). Most CNS mastitis cases are subclinical or teat duct colonisations that slightly increase milk somatic cell counts, but may provoke clinical disease that may be difficult to cure (Myllys *et al.*, 1994). Myllys *et al.* (1994), also report that CNS together with *S. aureus* were the largest group of bacteria found in a Finnish mastitis survey.

Rainard *et al.* (1990), in a seven year incidence study found that CNS were the most prevalent cause of quarter infections and that most (75%) persisted until drying off. They also established that their contribution to somatic cell counts was significant (18.1%) as compared to major pathogens (46.6%). However, dry cow therapy could eliminate 86.5% of these infections. Another survey by Millar *et al.* (1993), showed that economic losses attributable to CNS could be higher than those from coagulase positive staphylococci. Since subclinical mastitis is economically important, there is a need for further research on CNS (Myllys *et al.*, 1994).

Coliforms

The present classification categorises the gram negative, facultative anaerobes into three families, the *enterobacteriaceae, vibrionaceae* and *pasteurellaceae*. Of these the *enterobacteriaceae* are often isolated from bovine intramammary infections (Watts, 1989). *Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella spp., Citrobacter spp. Serratia spp* and *Proteus species* are the most frequently isolated *Enterobacteriaceae* from the bovine intramammary infections (Eberhart *et al.*, 1979; Smith *et al.*, 1985; Watts, 1988).

Coliforms are the single most common etiologic agents isolated from cows with clinically severe mastitis (Hogan *et al.*, 1994), and although studies have suggested that only 10 to 29% of clinical coliform cases were severe in nature, severe coliform cases could result in as many as 6% of the cows in herds with low somatic cell counts (Erskine *et al.*, 1991). Earlier findings by Smith (1983), showed that only 4.2% of coliform infections resulted in acute mastitis. The majority of the clinical cases could best be described as mild (Smith, 1983; Wilesmith, *et al.*, 1986). Coliform infections are also of short duration with only 24% exceeding 30 days (Smith, 1983).

Mastitis due to coliforms is associated with severe systemic and local signs, and these signs are known to be due to the production of lipopolysaccharide endotoxins, and the spontaneous elimination of bacteria from the quarter is high (Pyörälä *et al.*, 1994).

Coliform mastitis, though generally an acute or chronic disease of high producing older cows in early lactation, occurs occasionally in cows and heifers before or at freshening. It has a strong relationship with milk fever and as many as 50% of cows with milk fever may develop coliform mastitis (Bushnell, 1980).

Of all the coliforms, *E. coli* has recently assumed the greatest importance (Jones, 1980; Wilesmith *et al.*, 1986). A number of authors have inferred an association between long-term use of control measures such as dry cow therapy, blitz therapy for the eradication of *Str. agalactiae* and teat dipping and the high incidence of mastitis caused by *E. coli* (Wilesmith *et al.*, 1986; Schukken *et al.*, 1989b; Jones 1990; Guterbock *et al.*, 1993).

E.coli was the predominant cause of mastitis in early and late lactation and although isolated throughout the year, it was the most predominant organism during summer in British herds (Jones and Ward, 1989). In *E. coli* mastitis the use of antimicrobials is widely believed to be no more effective than supportive therapy alone (Stampfli *et al.*, 1994; Pyörälä and Pyörälä, 1994).

Environmental streptococci

Many streptococci have been implicated as causes of mastitis, however the most important species are *Str. uberis* and *Str. agalactiae* (Radostits *et al.*, 1996). One of the major causes of lowered milk production is low grade chronic mastitis caused by streptococcal species other than *Str. agalactiae* (Hinckley *et al.*, 1985). *Str. uberis* was the pathogen most frequently isolated from clinical cases which occurred in the dry period (Wilesmith, 1986; Deutz and Obritzhauser, 1996). *Str. dysgalactiae* was also shown to predominate as the cause of most clinical mastitis in some months of the year in some British herds (Wilesmith *et al.*, 1986). The relative importance of environmental streptococci, especially *Str. uberis* and *Str. dysgalactiae* has been on the rise and their incidence exceeds that of *Str. agalactiae* which has largely been eliminated from herds with low somatic cell counts (Schukken *et al.*, 1989a). They found that their combined incidence ranged from 12.3 to 26.2% as compared to 0.2% for *Str. agalactiae* in such herds.

These bacteria usually were associated with acute catarrhal as well as parenchymatous mastitis (Deutz and Obritzhauser, 1996).

Although streptococci other than *Str. agalactiae* are considered as being of environmental origin, Krutz and Bramley (1982), were able to show that *Str. uberis* was consistently shed from the gastro-intestinal tract of some cows and that this may be a major source of the

organism among dairy cattle. This was also true of the enterococci such as *Str. faecalis* and *Str. bovis*.

2.2.2 Diagnosis of mastitis

2.2.2.1 Screening tests

Although unable to be definitive in most cases, the most obvious and important diagnostic procedure is clinical examination in the detection of clinical mastitis. It is, however, limited by its inability to detect subclinical mastitis. The diagnosis of subclinical mastitis has been made possible at the cow-side through a number of screening tests. Examples of the screening tests are: the California Mastitis Test (CMT), the N-Acetyl-B-D-Glucosaminidase test (NAGase), direct microscopic and electronic somatic cell counting, electrical conductivity, specific antibody tests, adenosine triphosphate, bovine serum albumin, and antitrypsin concentration (Radostits *et al.*, 1996). These tests are indirect methods for detecting the presence of inflammation. They are unable to distinguish the infecting microorganism but rather determine the concentration of by-products of the inflammatory response (Philpot and Nickerson 1991; Radostits *et al.*, 1996). As they are only screening tests, they should not form the basis for which treatment can be instituted (Sears *et al.*, 1993 Radostits *et al.*, 1996).

2.2.2.2 Microbiological diagnosis

Microbiological procedures should be used as confirmatory tests, and whenever possible therapeutic decisions and control measures should be based on known etiology and antimicrobial profiles of the offending microbes.
Microbiological diagnosis begins with the collection of uncontaminated milk samples from the cow. Various authors emphasize the importance of the strict adherence to laid down procedures for milk sample collection, transportation, storage, isolation and culture methodology, and explain the possible consequences and outcomes of the results so obtained (Barnes-Pallessen *et al.*, 1985; Sears *et al.*, 1990; Sears *et al.*, 1991;. Dinsmore *et al.*, 1992; Sears *et al.*, 1993).

2.2.2.3 Isolation procedures

Successful isolation of some pathogens may prove to be difficult. It is documented for instance that *S. aureus* and to some extend *Str. agalactiae* may be shed in a cyclical manner (Dinsmore *et al.*, 1992; Sears *et al.*, 1990). This therefore necessitates repeated sampling to improve diagnostic sensitivity. For example, in the case of *S. aureus* diagnostic sensitivity will improve from 75 % for a single sample to 98 % after the collection of a third consecutive day sample (Sears *et al.*, 1990). With coliform mastitis, milk samples will be culture negative in approximately 20 % of clinical cases (Gonzalez *et al.*, 1990). A 0.01 ml sample would often be culture negative (Dinsmore *et al.*, 1992).

In order to improve the positive culture rate from milk samples with low concentrations of bacteria, larger volumes (0.05 or 0.1 ml rather than 0.01) are recommended (Smith *et al.*, 1982; Dinsmore *et al.*, 1992). Improved recovery of microorganisms can also be realised by culturing in nutrient broth or incubating the milk in a water bath for 6-8 hours before plating (Dinsmore *et al.*, 1992; Sears *et al.*, 1993).

The most widely accepted criterion for intramammary infection diagnosis is that an intramammary infection exists when the same organism is isolated from two consecutive samples taken at least one day apart. (Sears *et al.*, 1993).

2.2.2.4 Identification procedures

The identification procedures for organisms that are causative agents for mastitis follow logical and specific guidelines as outlined by the National Mastitis Council (Barnes-Pallessen *et al.*, 1985). Most mastitis pathogens can be identified tentatively using colony morphology and hemolytic patterns on blood agar media and examination of colonies by gram stain and by catalase and oxidase reactions (Sears *et al.*, 1993). The ultimate and definitive identification of these organisms requires the application of further biochemical and metabolic properties of the organisms.

2.2.3 Host factors

From the host standpoint, it has been demonstrated that genotypes favourable for milk yield are more susceptible to mastitis (Shook, 1989). It is speculated that 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). For instance, animals carrying the BoLa class II haploid b were significantly more susceptible to subclinical mastitis than those not carrying this haplotype (Arriens, 1994). This phenomenon has been verified by other workers (Schukken, 1990a; Roy *et al.*, 1993; Simmon, 1995).

Genetic variation in conformation of the udder and teats is determined in part by heredity and may be considered one component of genetic resistance (Shook, 1989). Pendulous udders are especially susceptible to mastitis (Young and Legates, 1960).

The teat end is important in mastitis defense for its role in preventing entry of pathogens. (Schukken *et al.*, 1989). Several physical and chemical properties of the streak canal constitute the first line of defence. 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 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. Subclinical mastitis is usually caused by contagious pathogens and therefore the increase in prevalence is attributed to an inability of mastitis control efforts to limit cow-to-cow transmission, rather than a physiological effect (Radostits *et al.*, 1996).

Parity has been shown to be a further risk factor for both subclinical and clinical mastitis (Schukken *et al.*, 1989a). It is postulated that younger animals have a decreased susceptibility through a more effective host defense mechanism (Dulin *et al.*, 1988).

Periparturient diseases such as dystocia, parturient paresis, retained placenta and ketosis have been identified as risk factors for subsequent development of mastitis (Radostits *et al.*, 1996).

2.2.4 Environmental factors

Certain environmental risk factors become apparent as the level of intensification changes. For example, 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.*, 1987; Gonzalez *et al.*, 1990).

It has also been observed that an increasing number of hours spent outside protects against environmental mastitis; and this may be attributed to a cleansing effect by sun/radiation, a decrease in exposure to microorganisms in the bedding or a general enhancement of the immune status through decreased stress (Smith *et al.*, 1985; Schukken *et al.*, 1988).

The higher incidence of environmental mastitis has been attributed to a higher bacterial count on bedding and an increase in heat stress caused by ventilation problems in barns (Smith *et al.*, 1985).

2.2.4.1 Daily exercise

Studies on the effect of daily exercise on the health of tied dairy cows showed increasing frequency of calving related problems, mastitis and leg problems than in exercised cows. Health in general was positively influenced by exercise (Gustafson, 1993). It has been shown by Bartlett *et al.* (1992), that mostly confined herds or herds almost always on pasture had the lowest logarithmic bulk somatic cell counts (LNBSCC). In contrast herds which switched back and forth between confinement and pasture had the highest LNBSCC which may indicate that repeated transition between confinement and pasture is an important risk factor for mastitis.

2.2.4.2 Milking herd size

Herd size is associated with the incidence of clinical mastitis. Epidemiological considerations make it difficult to control or eradicate contagious pathogens in a herd with a greater reservoir pool and a greater number of cow-to-cow contacts. Also, as herd size increases, manure disposal and sanitation problems may increase exposure to coliforms and environmental streptococci (Oz *et al.*, 1985; Schukken *et al.*, 1990b; Bartlett *et al.*, 1992).

2.2.4.3 Environmental sanitation

This refers to the removal of urine, feces, milk, and keeping the surroundings clean, dry and well ventilated. These factors were more important than the type of bedding used and it has been suggested that the existence of organic materials, moisture and temperature favoured the growth of environmental organisms (Zehner and Farnsworth, 1986). However, wood chips and saw dust bedding have been shown to be important determinants of coliform mastitis while straw bedding was the safest (Bartlett *et al.*, 1992).

2.2.4.4 *Milking management*

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). It was shown that machine milking can cause disturbances of circulation within bovine teat tissue, which impair its ability to withstand mastitis pathogens. Changes in teat end thickness can be used as a measure of circulatory impairment due to congestion and edema, and are always significantly greater with machine milking than with calf suckling or hand milking. Results of infection trials confirm that the risk of new mastitis infection is closely related to the magnitude of these changes (Hamman *et al.*, 1991). However, hand milking also has to meet certain basic hygienic standards. Titterton *et al.* (1979) demonstrated that a significant reduction in udder infections could be achieved by simple preventive measures provided that a teat lubricant containing 0.2% hexachlorophene in a vanishing cream base is used for hand milked herds.

2.2.4.5 *Nutrition*

Nutritional programmes that result in an improper anion-to-cation balance in the dry cow ration are foster periparturient hypocalcaemia which in turn increases the risk of new intramammary infection (Radostits *et al.*, 1996).

Dietary supplementation of Vitamin E and Selenium in contrast improves the mammary gland's resistance to infection (Erskine *et al.*, 1987). Vitamin E and Se containing enzyme glutathione peroxidase are antioxidants that protect neutrophils from the destructive action of toxic oxygen radicals. The neutrophils are necessary for intracellular killing of ingested pathogens. Dietary supplementation with Se results in more rapid neutrophil influx into the milk following intramammary microbial challenge (Hogan *et al.*, 1993).

2.2.5 Incidence and Prevalence of Mastitis

In the western hemisphere, rigorous control programmes (particularly teat dipping and total dry cow therapy) directed towards the control of mostly contagious pathogens have resulted in the predominance of environmental mastitis pathogens (Grommers *et al.*, 1985; Wilesmith *et al.*, 1986; Erskine *et al.*, 1987; Millar, 1993).
A cross-sectional study on 1032 large herds in the USA by Sischo *et al.* (1993), revealed the herd prevalence of contagious pathogens to be as low as 5% for *Str. agalactiae* but 45% for coagulase positive staphylococci. Field studies in herds with low somatic cell counts from Pennsylvania, Ohio and California also showed that the average incidence of clinical mastitis is 45-50 cases per 100 cows annually, with coliforms being isolated from 30-40% of the cases (Erskine *et al.*, 1991).

In general, the incidence of clinical mastitis is reported to be between 30% and 55% annually (Wilesmith *et al.*, 1986; Bartlett *et al.*, 1992; Millar *et al.*, 1993), in contrast to these findings the incidence of clinical mastitis has been very low in smallholder dairies in Eastern Africa. The incidence was reported to be as low as 13.3% per annum in the Kiambu district of Kenya (Omore *et al.*, 1996). The prevalence of subclinical mastitis, however, was very high (71%) when a threshold of 300 000 cells/ml was used. Studies done in the early seventies in Kenya generally showed very low incidences of clinical mastitis ranging only between 4 and 10% per annum (Hamir *et al.*, 1978). The most common isolates were *Staphylococcus aureus* and streptococcus species. Klapstrup and Halliwell (1977) found the herd prevalence for *Str. agalactiae* to be 4.39% in small herds in Malawi. The prevalence of subclinical mastitis in

this case was also low, ranging from 17 to 19%. The authors attributed this low prevalence mainly to hand milking and open housing systems.

2.3 Mastitis Control

As mentioned earlier, the occurrence of mastitis depends on the complex interaction of the three epidemiological components: host, agent and environment. Thus, for any control program to be successful it should be geared towards correcting mastitis problems associated with these three factors. Radostits *et al.* (1996) extensively reviewed the following as essential components of a comprehensive udder health program:

- Employ proper milking management methods
- Proper maintenance and use of milking equipment
- Dry cow management
- Appropriate therapy during lactation
- Cull chronically infected cows
- Maintenance of a clean environment
- Good record keeping
- Monitoring udder health status
- Periodic review of the udder health program
- Setting goals for udder health status

The specific steps for an udder health management program devised to fulfill the three basic epidemiological principles (Philpot and Nickerson, 1991; Radostits *et al.*, 1996) include:

1. **Elimination of existing infections.** This is achieved by such programs as proper milking hygiene and technique, teat dipping, dry cow therapy, treatment of clinical mastitis and culling. These procedures help to reduce either the rate of new infection or the duration of infection.
2. **Prevention of new infections**. This can be achieved through procedures such as good milking management, environmental and nutritional management, as well as by breeding methods.
3. **Monitoring of udder health.** This can be achieved through the application of cow side tests, for example the strip test to detect clinical mastitis, the California Mastitis Test for subclinical mastitis, automated electronic cell counters, NAGase test and others. These cow side tests should not be used to identify cows for treatment without culturing milk from individual cows, but rather should be used in monitoring progress and revealing deficiencies in mastitis control practices. (Philpot and Nickerson, 1991).

With an awareness created, and a system of monitoring udder health in place, a potentially successful mastitis control program should be:

1. known to be cost effective,
2. within the scope of the average dairy producer's understanding,
3. adaptable to the currently used dairy management systems.
4. and should lead to visible success by a rapid reduction in numbers of clinical cases, and a steady improvement in the parameters used for monitoring udder health status. (Philpot and Nickerson, 1991; Radostits *et al.*, 1996).

2.4 Zoonotic Significance of Milk and Milk Products

Milk and milk products can be serious sources of infection for consumers. Milk is a highly nutritious and highly perishable food and consequently allows for the growth and multiplication of potentially dangerous microorganisms. It is also a vehicle for the transmission of a number of zoonotic diseases. The list of pathogens transmissible through milk is long. In a review by Galbraith *et al.* (1984) in England and Wales, the following were listed as major infections transmitted through milk: tuberculosis, brucellosis, typhoid, paratyphoid, dysentery, staphyloccocal intoxication, scarlet fever and diphtheria. Rare cases of milkborne diseases were *Escherichia coli* enteritis, *Clostridium perfringens* food poisoning and Q-fever. Milkborne diseases whose incidence was on the increase were salmonellosis and campylobacteriosis.

Serious and often fatal outbreaks of *Str. zooepidemicus* have been reported and attributed to the consumption of raw milk (Hinckley, 1985; Edwards *et al.*, 1988). *Str. agalactiae* has also been shown to be transmissible from cow to man and may cause septicaemia and meningitis (Brglez *et al.*, 1987). Zoonotic listeriosis may also be contracted from milk or milk products (Hird, 1987). *Listeria monocytogenes* and other listeria species and strains may be isolated even from pasteurized milk and may be a source of human listeriosis (Garayzabal, 1986). Evidence has also been advanced indicating that the bovine oncovirus can be transmitted in milk and should be considered as the possible cause of multiple sclerosis and perhaps other neurological syndromes in man (Cunningham, 1986).

Raw milk is probably the biggest source of milk-borne diseases. For example of the 32 outbreaks of diseases associated with the consumption of milk and dairy products affecting 714 people in England and Wales, 27 were attributed to the consumption of raw milk (Barrett, 1986). The pasteurization or sterilization of milk is thus important in the curtailment of milkborne diseases and the effectiveness of this has been clearly shown (Sharp *et al.*, 1985).

The consumption of raw milk and not so well secured dairy products by the African smallholder farmer and his or her clients still is the rule rather than the exception. The occurrence of many of the zoonotic diseases listed above therefore is a well too known situation in this part of the world. Considerable work still remains to be done on consumer awareness and education in these societies.

2.5 Economic Losses due to Mastitis

Mastitis is generally considered to be the most costly disease facing the dairy industry (Blosser, 1979). Janzen (1970), in a comprehensive literature review, reported losses of between 5 to 25%. In the USA, Philpot and Nickerson (1991) estimated that national losses could be as high as 180 US$ per cow per year. The main sources for these losses were due to reduced milk production (64%), discarded milk (14%), early cow replacement costs (8%), reduced cow sale value (5%), drugs (5%), veterinary services (3%) and labour (1%).

Bartlett and Van Wijk (1991) reported from other literature sources losses as high as 88% from decreased production and from milk withheld from the market in cases of clinical mastitis. Crude milk production losses per cow with subclinical mastitis have been estimated at 1.56 kg/day and on average a 17.6% loss per affected quarter (De Graaf and Dwinger, 1996). Bartlett *et al.* (1990) also showed that the average herd lost an average of 1.17 kg of

milk per day associated with a somatic cell score greater than zero. Approximately 70 to 80% of all losses due to mastitis are due to subclinical mastitis (Philpot and Nickerson, 1991).

Blowey (1987) in a study covering a period of six years, demonstrated that a good mastitis control program is able to significantly reduce the losses due to mastitis and that the investment in veterinary services can be outstripped by returns by over twelve-fold. Grindal and Hillerton (1991) also suggested that the benefits of reduced infection rate from mastitis control are significantly underestimated since the animals are more susceptible than 40 years ago.

Not only are the losses attributable to a decrease in the volume of milk, but there are also physico-chemical changes that adversely affect the quality of the milk. These changes may also affect the suitabilty of the milk for industrial processing, (Janzen, 1970; Philpot and Nickerson, 1991).

2.6 Mastitis in Ethiopia

Comprehensive etiologic and epidemiological studies on the problems of mastitis in Ethiopia are generally lacking. Restricted studies by Geresu Biru (1989) indicated a combined prevalence of 67.4% of both clinical and subclinical mastitis in a herd of 43 cows and the main isolates being *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). Further screening tests (CMT) by ILRI (1995) showed an overall prevalence of 1.2% of clinical mastitis and 37.6% of subclinical mastitis in 725 dairy farms with a total of 2 735 dairy cows. These preliminary results indicated an increasing trend of the problem with increasing intensification (Tegegne, 1996 personal communication).

The Faculty of Veterinary Medicine of the Addis Ababa University has conducted several unpublished studies on the mastitis complex. A cross-sectional study done by Zerihun (1996) revealed a subclinical mastitis prevalence of 68.1% and production losses as high as 31%. In another study by Shirmako, (1996) significant differences in the prevalence of subclinical mastitis were found between an indigenous breed (the Arsi) and crossbred cows (Holstein-Arsi). The crosses had a prevalence as high as 53.6%, compared to 29.5% for the indigenous breed, confirming the vulnerability of the higher yielding cows. The main isolates from these and other studies were, in descending order, staphylococcal species with *Staphylococcus aureus* predominating, streptococcal species, mainly *Streptococcus agalactiae* and coliforms. Other less significant isolates were Actinomyces and Pseudomonas species.

3 MATERIALS AND METHODS

Background

This study was part of a project of the International Livestock Research Institute, (ILRI) Ethiopia, entitled: "The impact of intensification and reproductive wastage on the efficiency of African peri-urban milk production systems". The objectives of this broad study are to assess the importance of diseases of intensification, to develop methodologies and models to

investigate the biological and economic impact of these constraints, to study the epidemiology of, and risk factors associated with diseases of intensification, and to evaluate cost and benefits of alternative control measures.

3.1 Study design

3.1.1 Study area

The study was conducted in the urban and peri-urban areas of Addis Ababa and Debre Zeit. The human urban population is approximately 4,1 million in the two cities. Addis Ababa has a cool climate with an average rainfall of 1800 mm. The average temperature is 21°C. Debre Zeit is relatively warmer because of lower altitude. Both cities experience a bimodal rainfall pattern. Annex 1 shows the map of the study area.

3.1.2 Study population

Preliminary work already done by ILRI was used, providing the following information: there were at least 10 000 dairy farmers with about 40 000 dairy animals in the Addis Ababa region. A proportional and representative sample of farms was drawn from three identified subsystems (376 intra-urban; 36 peri-urban and 368 in the secondary town of Debre Zeit) with a total population of 2735 dairy animals. Preliminary analysis identified three farm clusters characterized by genotype, herd size, number of cows and types of housing. In clusters 1 to 3, the percentage of crossbred animals was 7, 12, and 91%, respectively; mean herd size and number of cows were 4, 10 and 28, and 1, 3 and 14 of farms respectively; and 29, 19 and 88% respectively, provided good floors and roofs. In general, farms in cluster 3 had the most intensive system. They had the highest percentage of crossbred animals, the largest number of dairy animals and relatively better housing than the other two groups. Overall, 34% of farm owners were female, with proportions increasing from 24 to 46% from the less to the most intensive system. About 36% of the owners were illiterate, and 34% had only elementary education.
The proportion of lactating cows were 74, 53 and 77% of the herd in clusters 1 to 3 respectively, and the respective proportion of pregnant animals was 23, 33 and 43%. Among cows that were not pregnant the mean interval from the previous calving ranged from 450 to 525 days.

From the 3 subsystems outlined above this study selected a total of 43 farms by way of two-stage stratified random sampling; the resulting study population comprised of:
1. Intra-urban system in the city of Addis Ababa, (17 farms),
2. Peri-urban system around the city of Addis Ababa (Sebeta and Kaliti), (6 farms)
3. Intra-urban system in the secondary town of Debre Zeit town (20 farms).
In these farms, there were approximately 1114 cross-bred dairy animals. Of these 436 were milking cows at the beginning of the study. In choosing the study herds an attempt was made to cut across all the 3 cluster groups outlined above.

3.1.3 Systems appraisal/questionnaire survey.

A questionnaire was served to all the farmers on the 40 farms. The main components of the questionnaire were based on

(i) Social factors (e.g. education, employment, innovativeness)
(ii) Management skills for dairy animals.
(iii) Application and knowledge of mastitis control measures
(iv) Application and knowledge of mastitis treatment measures.

A specimen of the questionnaire used in the study is shown in Annex 2.

3.1.4 Cross-sectional study.

In all the study herds the prevalence of clinical and subclinical mastitis was established with the use of clinical observation, the California Mastitis Test (CMT) and by microbiological etiologic diagnosis as well as antimicrobial sensitivity tests. Laboratory methods for these are outlined in section 3.6. First, all animals were screened using the CMT and only those positive in this test had individual quarter samples taken for microbiological investigation. During the cross-sectional study, base line information was gathered concerning factors such as general farm management, disease control measures (including mastitis), and the examination of environmental factors that may influence the occurrence of the disease).

The prevalence of clinical and subclinical mastitis at the cow level were calculated using the following formula:

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

An estimation of economic losses due to mastitis was also carried out from information gathered during the cross-sectional study. Milk production losses were estimated based on the findings of Philpot, (1967). He found that milk production was reduced by 2.8%, 11.4% and 45.5% in quarters of 178 jersey cows with CMT reactions of trace, 1, 2, and 3 respectively. His data also showed that CMT-negative quarters compensated (to a limited extend) for losses in production in CMT-positive quarters. In addition Philpot and Nickerson (1991) report that reduced milk production, discarded milk, early cow replacement, reduced cow sale value, drugs and veterinary services account for 64, 14, 8, 5, 5, 3, and 1% of the losses, respectively. The calculations were based on the findings of these workers and the following assumptions were made:

1. cows produced on average 8 liters per day when free from mastitis,
2. the lactation length was 7 months,
3. the infection status remained more or less constant throughout the period of lactation for all the cows,
4. clinical mastitis quarters produced milk that could not be consumed or sold,
5. the current cost of milk is 1.5 Ethiopian Birr (US$ 0.22),
6. the calculation was based on the cows that were currently lactating.

3.1.5 Longitudinal Prospective Study

A total of 31 farms with a total lactating cow population of 339 animals were included in this incidence study. The table below summarises the breakdown of the study population according to location.

Table 3.1 Summary of the breakdown of the study population of animals investigated in the incidence study

Location	Number of farms	Lactating cow population
Debre Zeit (large Farm)	1	21
Debre Zeit	9	22
Kaliti	3	102
Sebeta	3	104
Addis Ababa (smallholder farms)	13	27
Addis Ababa (large scale farms)	2	27
Total	31	339

Although all the farms listed above were included in the incidence study and were visited throughout, only 23 farms qualified for incidence calculations. These were 8 large holder and 15 smallholder farms. The other farms were excluded because all the animals were already infected right from the first visit and one large farm in Addis Ababa opted out of the study.

These farms (which were also in the cross-sectional study) were visited for a period of 9 weeks at regular intervals of three weeks. The cross-sectional study was was the starting point of the longitudinal study. At each subsequent visit, all currently lactating cows were screened using the CMT. Cow quarters that were positive on the CMT at each visit had milk samples collected in sterile vials for microbiological investigation. Cow quarters that were CMT positive on the previous visit, but CMT negative on the current visit were sampled again to ascertain freedom from microbiological infection. If they turned out to be negative on CMT screening on the subsequent visit thereafter, it was assumed that the quarter had become free of infection and therefore no further quarter milk samples were collected. This was done even if the sample from the CMT negative sample had turned out positive on culture.

Dry cows were considered not at risk for the purposes of this study, including all the animals that may have been culled from the herd for diverse reasons. New cows that moved in as they came into lactation or joined the herd also became part of the investigation and were considered at risk at a point that was assumed to be halfway between two consecutive visit.

This was done in order to monitor the dynamics of the disease occurrence over the period of 9 weeks. In addition to this the following activities were also carried out at each visit:

- Recording of any cases noted and treated by the farmer during the preceding fortnight.
- An inspection of the environment, and noting of any management changes.

The laboratory methods for this are outlined in the following sections.

The cumulative incidence rates (CI) for clinical and subclinical mastitis were calculated using the formula:

$$CI = \frac{\text{No. of cows developing mastitis in 9 weeks time period}}{\text{No. of cows at risk at the beginning of the nine week period}} \times 100$$

The incidence density rates (ID) for clinical and subclinical mastitis were calculated using the formula:

$$ID = \frac{\text{No. of cows developing mastitis in 9 weeks period}}{\text{The average length of time each cow was at risk of developing mastitis}} \times 100$$

The average length of time at risk was calculated by taking the mean value of the number of animals that were at risk of developing clinical or subclinical mastitis at the beginning of the period and of those at risk at the end of the 9 week period.

3.1.6 Field and laboratory investigative procedures

Field and laboratory procedures were used as outlined in the following sections.

3.1.6.1 Collection of milk samples

Milk samples were collected aseptically into sterile vials prior to milking and after the preparation of the udder by the farmer. The following technique was used:

1. A few streams of milk were stripped to the floor before collection.
2. The CMT then done as described in section 4.1.6.2.
3. The teat end was then swabbed with a pledget of cotton wool moistened in 70% ethyl alcohol until no more dirt appeared.
4. The cap was then removed from the sterile vial with the open end of the cap facing downwards.
5. With the vial tilted at 45 degrees while collecting, the sample (2 to 3 ml) was collected and the cap immediately replaced. It was discovered that reduced contamination could be achieved by catching the milk in the midstream of one continuous squirt rather than getting it right from the start.
6. Samples were packed in ice cooler boxes immediately following collection and stored at approximately 4 degrees centigrade.
7. No samples were collected from cows on or with a recent history of antibiotic therapy.

3.1.6.2 California Mastitis Test

The California Mastitis Test was used as screening test for the cows under study. The CMT estimates the somatic cell counts of milk samples. For reliable results the test was conducted just before milking after stimulating the cow and discarding the foremilk. An equal volume (approximately 2-3 ml) of milk was then mixed with the CMT reagent in the test paddle and swirled. The results of the reaction were interpreted as shown in table 3.2 below (Philpot and Nickerson, 1991).

Table 3.2 Interpretation of CMT findings (Philpot and Nickerson, 1991).

Score	Gelling	Interpretation	SCC mean score cells/ml	Range '000 cells/ml
0	none	negative	100 000	>200
trace	slight	suspicious	300 000	150-5 000
1	slight to moderate	positive	900 000	400-1 500
2	moderate	positive	2 700 000	800-5 000
3	heavy	positive	8 100 000	>5 000

Traces were considered as not abnormal reactions and thus as having normal somatic cell counts (de Graaf and Dwinger, 1996).

3.1.6.3 Microbiological culturing techniques

Microbiological culturing was performed on samples from suspected cases that had been identified by the CMT. For this, the following media were used for primary plating:
-Non selective blood agar (Columbia Blood agar),
-MacConkey agar,
-As a preservation means, 0.1 ml of milk was inoculated into 10 ml of nutrient broth and then incubated for 24 hours and stored in the refrigerator at 4°C.

Unchanged milk was subsequently be plated out on the above media followed by incubation at 37°C for 24 hours. In cases of insufficient or absence of growth the incubation was increased by a further 24 hours.

3.1.6.4 Interpretation of cultural results.

The following assumptions were made for the interpretation of the laboratory results:
1. It was assumed that a sample was positive on culture if an organism known to cause mastitis was present on culture,
2. It was assumed to be negative if there was no growth,
3. It was assumed to be contaminated if there was a profuse growth of a mixture of microbes of environmental origin, e.g. coliforms, environmental streptococci, pseudomonas spp.

For environmental microorganisms, confidence that they were the cause of mastitis was based on the following criteria:
1. If they were isolated in pure culture from a single sample,
2. When isolated from two to three consecutive samples obtained at no greater than a 30 day interval.

A resample was preferred whenever a sample was contaminated or suspected to be contaminated.

3.1.6.5 Differentiation of bacteria

The following flow diagram was used as the basis for the differentiation of microbial isolates.

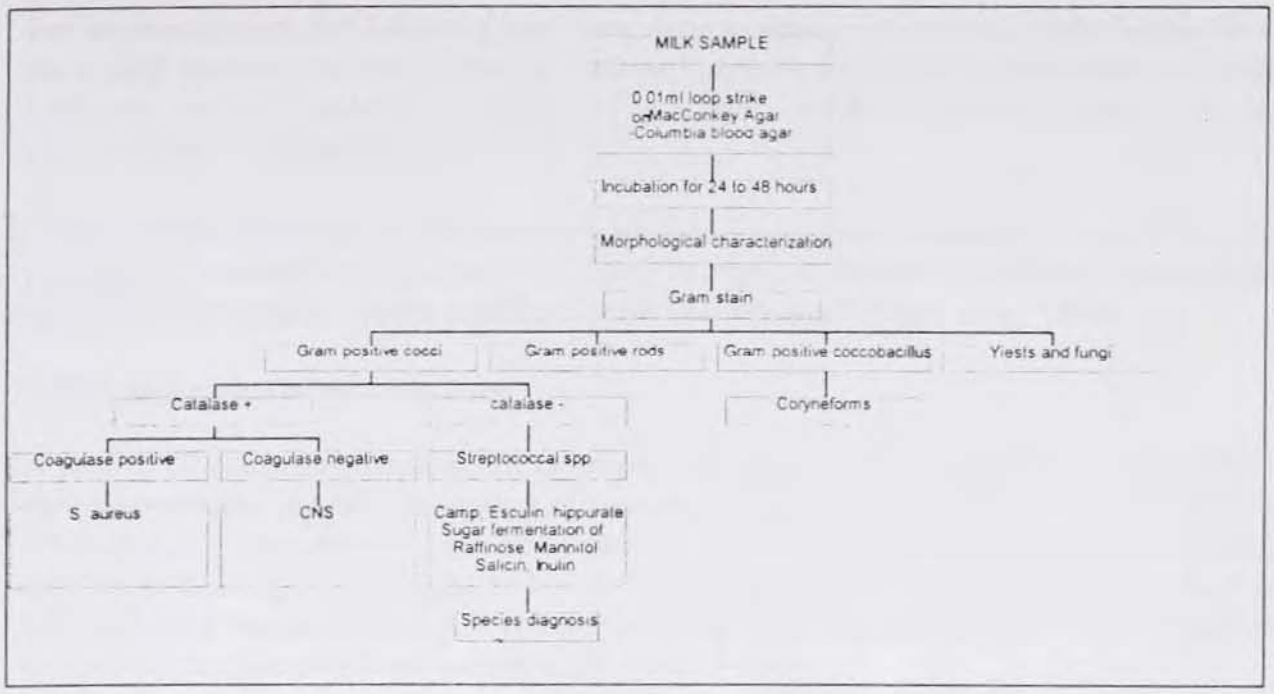

Fig 3.1 Diagnostic flow diagram for differentiation of the isolates obtained in the cross-sectional study.

Phenotypical methods

Growth pattern on culture media: differentiation was based on the following:

1. On the speed of growth as either fast or slow,
2. Morphology of colonies,
3. Haemolytic characteristics. Includes the CAMP test,
4. Microscopy-gram staining as either gram positive or gram negative,
5. Morphological appearance especially of yeasts, mold and fungi.

Microscopy

All colonies that grew on either MacConkey or Columbia blood agar were subjected to gram staining. It was at this stage that some pathogens were conclusively identified namely Gram positive bacilli and yeasts or fungi. The other organisms were presumptively identified as either gram positive cocci, gram positive pleomorphic rods or coccobacilli (coryneforms) or gram negative rods.

Biochemical tests

Biochemical tests tests were conducted following standards as outlined by Barnes-Pallesen *et al.*, (1985).

Organisms that were identified as gram positive cocci were then subjected to the catalase test. Those reacting positively were considered staphylococci whilst those reacting negatively were classified as streptococci.

Those classified as staphylococci were then subjected to the coagulase test and those that were positive were considered to be *Staphylococcus aureus*. Those that reacted negatively were assumed to be coagulase negative staphylococci (CNS). No further tests were carried out on this group of organisms.

For the streptococci, the following tests were done to achieve the species identification level: the CAMP reaction, esculin hydrolysis, sodium hippurate test and the fermentation of sugars (raffinose, mannitol, inulin and salicin). *Str. agalactiae*, *Str. uberis*, *Str. dysgalactiae*, and *Str. bovis* were identified in this manner.

Gram positive pleomorphic coccobacilli were identified to the species level as *Actinomyces pyogenes*, *Corynebacteria ulcerans* or *C. bovis* through the catalase test, haemolysis on blood agar and ability to grow on 9% sodium chloride nutrient broth (Sears *et al.*, 1993).

3.1.6.6 Antimicrobial resistance test

A total of 89 isolates obtained in the cross-sectional study were selected at random to assess their antimicrobial profiles. Seventeen of these were *Staphylococcus aureus*, 17 coryneforms, 20 streptococci other than *Str. agalactiae* and 35 CNS. The Mueller Hinton agar was prepared with the addition of 5% defibrinated blood. The isolates were streaked from broth culture (at 0.5 Mcfarland standard) onto a Mueller Hinton agar plate using a sterile swab. Antibiotic discs were then placed before incubation for 18 to 24 hours.

Evaluation of the antimicrobial resistance test results

The diameters of the zones of inhibition were measured in milliliters and the results were judged as resistant, medium sensitive or sensitive.

Table 3.3 Reference values for zones of growth inhibition for microbiological agents.

Antibiotic agent or group	Loading (micrograms/disc)	Judgement: Inhibitory zone diameter (mm)			Source
		Resistant	intermediate	sensitive	
Tetracycline	30	<16	17-21	>22	DIN
Ampicillin	10				
• Enterobacteriacae		<14	15-21	>21	DIN
• Streptococcus		<21	22-29	>30	DIN
• Staphylococcus		<28	-	>29	DIN
Gentamicin	10	<14	15-17	>18	DIN
Erythromycin	15	<16	17-20	>18	DIN
Penicillin G	10 I.U.				
Staphylococci		<28	-	>29	DIN
Others		<19	20-28	>29	DIN
Nitrofurantoin	100	<17	18-21	>22	VWL
Chloramphenicol	30	<20	-	>21	DIN
Cotrimoxazole	25	<13	13-16	>16	DIN
Polymyxin	30	<8	9-11	>12	WHO
Oxacillin	5	<15	-	>16	DIN
Streptomycin	10	<14	15-16	>17	NCCLS
Neomycin	30	<12	13-16	>17	WHO
Lincomycin	15	<18	19-23	>24	DIN
Sulphamethoxazole	300	<10	11-15	>16	WHO
Tylosin	30	<18	19-23	>24	VWL
Cephamandol	30	<18	-	>18	DIN

DIN = Deutsche Industrie Normung; CCLS = National Committee for Clinical Laboratory Standards; VWL = Vorlaeufiger Wert von Laborergebnissen; WHO = World Health Organisation.

3.2 Analytical steps

The singe factor analysis of variance (ANOVA) was used to detect significant differences between the large and smallholder farms at the error probability of 5 % ($p<0.05$) for the following parameters:

1. Mean prevalences of subclinical and clinical mastitis,
2. Mean incidence densities of subclinical and clinical mastitis.
3. Mean frequencies of pathogens isolated in subclinical mastitis cases.

4. RESULTS

4.1 Questionnaire survey and production system appraisal

This part of the study attempted to characterize and gain insight into the farming system, and the production conditions such as management, social conditions, farm structure and to focus attention on management factors that have an influence on the occurrence of mastitis.

To achieve these objectives a questionnaire was served to the farmers. It was divided into three parts for administrative reasons. Areas covered were: farm structure, hygiene and mastitis control measures and treatment procedures. A fourth component which was not served to the farmers directly focused on housing and prevailing environmental conditions. These factors were assessed by the principal investigator directly.

The results of the questionnaire survey are summarized in the tables below; and for ease of comparison the answers are categorized into three components: these were 19 smallholder farms in the secondary town of Debre Zeit, 12 Addis Ababa small holdings and 9 large scale farms in the peri-urban and urban locations.

4.1.1 Social aspects and farm structure

Table 4.1 summarizes the findings concerning social aspects and farm structure.

Table 4.1 Summery results of the survey on farm structure and social aspects.

Questions	Smallholder Debre zeit n=19	Smallholder Addis Ababa n=12	Large scale farms n=9
Sex of owner			
Female	3	4	7
Male	16	8	2
Average age (years)	51	37	49
Other Occupation/job			
Businessman	3	1	5
Professional	11	4	0
Full time farmer	5	3	4
Avg. years experience	7.2	11.8	15
Is a telephone available on the farm?			
Yes	10	1	9
No	9	11	0
Location			
Urban	19	12	3
Peri-urban	0	0	6
Avg. distance from town centre	2.4	8.8	15.5
Avg. farm size (square meters)	1625	406	97000
Is the farm fenced?			
Yes	18	12	8
No	1	0	1
Is contact allowed with animals outside the farm?			
Yes	3	4	2
No	16	8	7
Are you engaged in crop farming?			
Yes	3	0	3
No	16	12	6
Do you own land elsewhere?			
Yes	2	0	1
No	17	12	8
Do you have other animals on the farm?			
Yes	10	9	3
No	9	3	6

The most salient points to be noted from these data were:

1. Twenty-two percent of the farms were owned by women. The average age of the farmers was 45 years. Of the 40 farmers interviewed, only 12 were full time farmers. Other professionals (for example teachers and soldiers) predominated in the smallholder sector but were absent from the large scale farms, where full time farmers and businessman shared ownership almost equally. It was found that the most experienced farmers were in the large scale sector with an average of experience of 15 years compared to only 7 years for the smallholder farms.
2. All smallholder farms were intra-urban, with only two of the nine large scale farms being intra-urban and the rest being peri-urban. The large farms of necessity were much larger in

area averaging 97 000 m^2 . The smallholder holdings in Debre Zeit were much larger than those in Addis Ababa (1625 m^2 compared to only 406 m^2). All the farms except two were completely fenced, although 9 smallholders allowed their animals to graze outside on road verges and commonages. All smallholders had their dairy unit located very close to the living quarters and in some cases they had converted parts of their living quarters into animal houses.

3. Other farm activities: Only three of the farmers owned land in the rural areas where crop farming was practiced by two; and one farmer used rural land for pasture to supply cut grass for the animals in the town. The majority of the smallholders (19 out of 31) raised other livestock on their farms. Animals owned included sheep, chicken, zebu, cattle, bees, horses, and donkeys. These animals were raised primarily for home use and in most cases did not constitute an economic enterprise.

4.1.2 Hygiene and control measures

Table 4.2 summarizes the findings concerning management factors assumed to be of significance to the occurrence of mastitis.

Table 4.2 Results of the questionnaire survey on milk hygiene and control measures.

Questions	Smallholder Debre Zeit n=19	Smallholder Addis Ababa n=12	Large Scale farms n=9
Do you give excercise to the animals?			
Yes	11	6	5
No	8	6	4
Drying off method			
Abrupt	14	9	5
Intermittent	5	3	4
Do You administer dry cow therapy to the cows?			
Yes	0	0	1
No	19	12	8
Do you have knowledge of dry cow therapy?			
Yes	3	0	2
No	16	12	7
Which drug(s) do you use for dry cow therapy?			
Pen-strep	0	0	1
Do you use a teatdip?			
Yes	1	0	1
No	18	12	8
Do you have knowledge of teat dipping?			
Yes	1	0	4
No	18	12	5
Which chemical do you use for teat dipping?			
Iodine	1	0	0
Savlon	0	0	1
When are the teats dipped?			
Before milking	0	0	0
After milking	1	0	0
Both	0	0	1
Does the chemical require dilution?			
Yes	1	0	1
No	0	0	0
At what stage do you cull a cow?			
After the loss of 1 teat	0	1	0
After the loss of 2 teats	2	4	4
After the loss of 3 Teats	7	0	0
After the loss of 4 teats	0	0	2

Table 4.2 continued

Questions	smallholder Debre Zeit n=19	Smallholder Addis Ababa n=12	Large Scale farms n=9
Do you check milk for abnormality at each milking?			
Check	11	5	5
Do not check	7	7	4
Do not Know	1	0	1
What method do you use for checking?			
Floor or hand or milking container	10	4	6
CMT	1	0	0
pH-card	0	1	0
When do you milk cows with mastitis?			
First	1	0	0
Last	13	6	7
Without consideration to udder health status	0	3	2
What antiseptic material is used at milking?			
Soap	3	0	0
None	16	0	9
Are the teats washed before milking?			
Yes	18	12	9
No	1	1	0
Are teats dried before milking?			
Yes	18	11	8
No	0	1	1
What is used for drying the teats?			
Cloth	4	10	5
Air	12	2	3
Sponge	1	0	0
Is a separate towel used for each cow?			
Separate towel	3	3	0
Towel shared	2	7	6
Do you wash your hands between each cow?			
Yes	15	10	7
No	0	2	2
What do you use for washing your hands?			
Water only	13	12	7
Soap	6	0	1
Disinfectant	0	0	1

The most important findings of this survey part are (4.12.1 to 4.1.2.5):

4.1.2.1 General management.

Nearly half of the farmers reported that they did not exercise their animals and therefore kept them in total confinement in tied stalls. The contrary was true in the cases where the farmers responded that they allowed their animals to graze outside. All the farmers milked their cows by hand, twice a day and only from the right side. The majority also reported that they dried their cows abruptly after a 7 month pregnancy. Consultations made with the veterinarians responsible for these animals revealed, however, that the animals were dried in most cases only at approximately two weeks prior to calving, particularly when the milk changed to colostrum. The majority of the smallholders said they never had to cull a cow because of mastitis, and those that had done so would only cull after the loss of two or more teats. The tendency to keep cows with blind quarters was strongest in the large farms.

Only half of the farmers checked milk for abnormalities at each milking. The methods used in most cases were stripping the milk to the floor, in the hand or in the bucket. Only one smallholder used the California Mastitis Test. The rest only became aware of the problem when clinical signs appeared. Nearly all farmers (96 %) were aware of the importance of segregation to prevent the spreading of mastitis amongst cows and would milk mastitic cows last. All the cows were milked inside the barn where they stayed. None of the farms had a purpose built milking parlour at the time of the study. Only the largest farm was in the process of installing a purpose built milking parlour during the study period.

4.1.2.2 Dry cow therapy

Only five of the farmers were aware of dry cow therapy as a means for controlling mastitis. Two of these were large scale farmers. However, only one large farmer had practiced this control measure before but was unable to continue because the needed drugs could not be found in the country.

4.1.2.3 Teat dipping

Only one farmer in the smallholder knew of teat dipping. Knowledge for on this was better in the large farms where 4 out of the 9 farmers knew of this practice. however, only one practiced it.

4.1.2.4 Use of antiseptics

None of the farmers used any conventional antiseptics for washing hands, the udder or the milking utensils. Three smallholders just used soap for these purposes. Those who were aware of this practice said they were unable to do so because the chemicals were too expensive.

4.1.2.5 Udder preparation

All except for two smallholders did not wash the teats of the cows prior to milking. Almost 50 % of the farmers also used a towel, and was common practice by the smallholders in Addis Ababa. Those that did not dry the teats using a towel, cloth or sponge stated that they air dried which was in all cases not effective. All large farmers used a common cloth for several cows. On direct observation, the large farmers washed the udder using water from a common bucket for several cows until the water became evidently dirty. None of them used a hose-pipe. The

smallholders tended to have a bucket of stock water and by scooping some water from it with a smaller container washed the udder. This ensured that each animal got 'fresh' water. The washing procedure tended to cover much of the udder (about two thirds). This allowed filth from the upper part of the udder to drip to the teat end.

Although not quantifiable, it was apparent that the large farms all employed paid labour for milking, the ratio of milkers to cows was approximately 1 to 10. In only one big farm the milkers were allocated a fixed group of cows. The smallholders tended to use family labour or contract milkers. The contract milkers went from farm to farm milking cows. Other smallholders did have domestic employees who doubled up as cow attendants and milkers.

4.1.3 Treatment procedures

This part of the questionnaire aimed at establishing the usage of antimicrobials for treatment of mastitis. Also it aimed at assessing the proper utilization and administration of drugs. Table 4.3 below summarizes these findings.

Table 4.3 Results of the questionnaire survey on treatment procedures

Questions	Smallholders Debre Zeit n=19	Smallholders Addis n=12	Large scale farms n=9
Is mastitis a problem in your herd?			
Yes	13	7	9
No	6	5	0
Who advises on products to use for treatment?			
Veterinarian	16	4	7
Vet assistant	1	0	4
Myself	2	0	1
Which drugs do you use for treating mastitis?			
Penstrep	7	0	6
Gentamycin	3	0	4
Neomycin	3	0	1
Tetracycline	3	0	0
Multiject ®	1	0	1
Mastiject ®	0	1	1
*Some combinations of the above	6	0	3
Do not know	4	3	1
Never treated before	4	8	0
Who treats the animals ?			
Veterinarian	7	2	0
Myself	12	2	9
Used for Preparation for intramammary treatment?			
Antiseptic	2	0	2
Soap	1	0	1
Water only	3	1	4
No preparation	2	0	2
Insertion of intramammary antibiotic canula is by			
Full insertion?	13	4	9
Partial insertion?	0	0	0

*Combinations=some of farmers used some combinations of the drugs listed above

Almost a third of the smallholder farmers maintained that they had not encountered mastitis in their herds and therefore never had treated their cows for this disease. All the large scale holders reported had mastitis problems in their herds.

Of those who had treated their cows for mastitis almost 80% consulted a veterinarian for advice and only 4 consulted a veterinary assistant. The most commonly used drugs were the

penicillins/streptomycin combinations followed by gentamycin, tetracycline, "Multiject ®", and "Mastiject ®" The use of antibiotics was low in the Addis Ababa smallholder farms where only one respondent remembered a product he had used before. Eight out of the twelve of the Addis farmers interviewed had never treated their cows for mastitis.

More than 70 % of the farmers administered the drugs themselves. For intramammary treatment all farmers and veterinarians inserted the canula fully into the teat canal.

4.1.4 Housing and environmental management survey

Table 4.4 below summarizes the findings of the housing and environmental management survey.

Table 4.4 Results of the survey on housing and environmental management factors

Questions	Smallholder DZ n=19	Smallholder Addis Ababa n=12	Large scale farms n=9
Building material used:			
Modern	8	2	9
Local	4	1	0
Mix(1&2)	5	9	0
Outdoor	1	0	0
Housing Type:			
Barn/stanchion	16	12	9
Open Pen/stanchion	2	0	0
Outdoor/stanchion	1	0	0
Floor type:			
Concrete	14	3	9
Arranged stones	3	9	0
Earth	2	0	0
Does sunshine enter Barn?			
Yes	7	7	1
No	12	5	8
Roof is rainproof?			
Yes	13	10	8
No	7	2	1
Free of hazards?			
Yes	12	2	6
No	6	10	3
Separate maternity pens?			
Yes	0	0	2
No	19	12	7
Maternity pens dry and well bedded?			
Yes			2
No			0
Dry cow facilities available?			
Yes	0	0	2
No	19	12	7
Dry cow facilities clean?			
Yes			2
No			0
Adequate space for the cows?			
Yes	17	10	5
No	2	2	4

Table 4.4 continued.

Questions	smallholder DZ n=19	Smallholder Addis n=12	Large scale farms n=9
Drainage system is:			
Excellent	3	1	2
Satisfactory	5	3	5
Poor	11	8	2
Ventilation is:			
Excellent	5	1	1
Satisfactory	4	6	2
Poor	9	5	5
Soiling of udders is:			
Inapparent	3	4	1
Moderate	15	8	8
Serious	1	0	0
Flies on the cows are:			
Negligible	9	9	6
Moderate	9	3	3
Too many	1	0	0
General farm hygiene?			
Excellent	3	2	2
Satisfactory	14	3	4
Poor	2	7	3

4.1.4.1 Building structures

Nearly 50% of the housing materials used were modern (cement, corrugated iron sheets, timber planks). Local materials were used mostly by the small farms but nearly all of these had used iron roofs on pole and mud structures. All large farms had modern structures with concrete walls and floors and three used iron sheets for the walls. Only one smallholder had an open air system with his animals tied to trees and posts. Some smallholder farmers' cow houses in Addis Ababa were completely enclosed with hardly any natural light entering and artificial lighting having to be used inside the houses even during the day.

4.1.4.2 Floor types.

All large farm barns had concrete floors. The use of stones arranged on the floor was a common feature of the Addis Ababa smallholder farms. The surface of these houses were uneven with prominent crevices between the stones where urine, dung and water could easily collect and stagnate.

4.1.4 3 Hazards.

Hazards such as protruding objects, cracked or cratered floors, sharp edges etc., were found in two thirds of the houses. The situation was worse in Addis Ababa because of the use of uneven stone floors.

4.1.4.4 Dry cow facilities/calving pens.

Only two large scale farms had maternity pens which were also used as dry cow facilities. In both cases the housing was adequate, dry and well bedded.

4.1.4.5 Space/ventilation/drainage

Eighty percent of the houses provided adequate space for the animals and overcrowding was not a serious problem. There were ventilation problems in half of the houses with some houses completely sealed, resulting in humid hot conditions inside with the strong smell of ammonia in some cases. Also, more than 50 % of the structures had poor drainage systems which resulted in the pooling of urine and fecal matter cleaning was also difficult. This was particularly the structure with stone floors.

An assessment of the cows' cleanliness was also done. See Appendix 3 for details on procedures. Most cows had moderately soiled udders and this seemed to correlate with the not so well cleaned houses or those having drainage problems. The fly challenge was also looked at and almost the farms did not suffer seriously from this problem. The fly challenge seemed to be more prominent in the more open well ventilated barns. Flies seem not to like houses that are sealed and dark inside.

A subjective assessment was also made of the general farm hygiene and orderliness only 30% were classified as of poor hygienic standards.

4.2 Prevalence study

4.2.1 Prevalence of subclinical mastitis in smallholder farms

The prevalence was determined by the California Mastitis Test and by microbiological culture. Four hundred cows had their quarters tested using the California Mastitis Test. Forty-eight of these quarters (2.8 %) were blind at the time of collection and therefore 1688 quarters were screened. Of these 267 or 15.9 % of the quarters were CMT positive. Twenty seven had clinical mastitis. Therefore the prevalence for subclinical mastitis was based on 1653 quarters. Two hundred and forty quarters (14.5 %) were positive on the California Mastitis Test. From the 240 CMT positive quarters 205 (85.4 %) yielded growth of a known causative agent for mastitis.

4.2.1.1 Prevalence of subclinical mastitis in smallholder farms

Table 4.5 summarizes the results of investigation the prevalence of clinical and subclinical mastitis in smallholder farms of Addis Ababa (AA) and Debre Zeit (DZ), as determined by the California Mastitis Test and by microbiological methods.

Table 4.5 Prevalences of subclinical mastitis in smallholder dairy farms in Debre Zeit and Addis Ababa

Area	Farm no.	Number of cows	CMT+	culture +	Clinical cases	CMT positive prevalence %	Culture positive prevalence %	Clinical cases prevalence %
*AA	4	2	0	0	0	0	0	0
AA	5	2	1	1	0	50	50	0
AA	6	1	1	1	0	100	100	0
AA	7	2	0	0	0	0	0	0
AA	9	1	0	0	0	0	0	0
AA	10	2	2	1	0	100	50	0
AA	11	1	1	1	0	100	100	0
AA	12	1	1	1	0	100	100	0
AA	13	3	2	1	0	67	33	0
AA	14	1	0	0	0	0	0	0
AA	15	2	0	0	0	0	0	0
AA	16	6	0	0	0	0	0	0
AA	17	6	3	1	0	50	17	0
#DZ	2	2	2	2	0	100	100	0
DZ	3	5	2	2	0	40	40	0
DZ	4	4	0	0	0	0	0	0
DZ	5	1	1	1	0	100	100	0
DZ	6	1	0	0	0	0	0	0
DZ	9	1	0	0	0	0	0	0
DZ	10	0	0	0	0	0	0	0
DZ	11	5	1	1	1	20	20	20
DZ	12	2	0	0	0	0	0	0
DZ	13	3	0	0	0	0	0	0
DZ	15	2	0	0	0	0	0	0
DZ	16	1	0	0	0	0	0	0
DZ	19	3	1	1	0	33	33	0
DZ	20	1	1	1	0	100	100	0
DZ	21	3	1	1	1	33	33	33.3
DZ	22	1	1	1	0	100	100	0
DZ	24	2	1	1	0	50	50	0
DZ	25	1	1	1	1	100	100	100
Total		68	23	19	3	33.8	27.9	4.4

* AA=Addis Ababa; # DZ=Debre Zeit

Of the 13 Addis Ababa smallholder farms, 6 farms (=46%) with a total population of 14 cows had no cases of subclinical mastitis (see Table 4.5). In the Debre Zeit area 8 (=44%) of the 18

smallholder farms with a total population of 10 cows also had no cases of subclinical mastitis. The overall prevalence of subclinical mastitis in the smallholder group farms was 33.8 % based on the CMT and 27.9 % based on microbiology.

4.2.1.2 The prevalence of subclinical mastitis in large herds

Table 4.6 Prevalence of subclinical and clinical mastitis in large farms in and around Addis Ababa and Debre Zeit.

Area	farm no.	Number of cows	CMT+	Culture +	Clinical cases	CMT positive prevalence %	Culture positive prevalence %	Clinical cases prevalence %
AA	1	53	18	17	0	34.0	32.1	0.0
AA	2	28	12	9	7	42.9	32.1	25.0
DZ	1	20	8	8	1	40.0	40.0	5.0
kaliti	1	33	7	7	0	21.2	21.2	0.0
kaliti	2	34	15	14	1	44.1	41.2	2.9
kaliti	3	22	6	5	0	27.3	22.7	0.0
sebeta	1	23	10	8	2	43.5	34.8	8.7
sebeta	2	22	4	4	1	18.2	18.2	4.5
sebeta	3	131	45	40	6	34.4	30.5	4.6
Total		**366**	**125**	**112**	**18**	**34.2**	**30.6**	**4.9**

The highest prevalence of subclinical mastitis was recorded on Farm 2 in Kaliti with 44.1 % and 41.2 % as determined by the CMT and microbiology respectively, almost equally high prevalences were found in three other farms, Debre Zeit farm 1, 40.0 % (40.0 %), Sebeta farm 1, 43.0% (35 %) and Addis Ababa farm 2, 43.0% (32 %), for CMT and microbiological results respectively.

The mean farm prevalences of subclinical mastitis were significantly different between large and small farms, large farms showing a higher level of subclinical mastitis than small farms.

Table 4.7 summarizes the results of prevalence investigations of subclinical mastitis at the cow level. The farms are subdivided into two categories, (smallholders and large farms).

Table 4.7 Prevalences of subclinical mastitis as determined by the CMT and microbiology in 41 large and small farms in and around Addis Ababa and Debre Zeit, Ethiopia. (cow level)

Farm category	# of farms	# of lactating cows	CMT positive cows	Prevalence based on CMT	microbiology positive cows	Prevalence based on Microbiology
Smallholders	31	68	24	35.3	19	27.9
Large farms	9	366	125	34.2	112	30.6
Overall	40	434	149	34.3	131	30.2

The overall prevalence of subclinical mastitis at the cow level and as determined by microbiological methods was 30.2 % from a total population of 434 cows. Based on CMT, prevalence was 34.3 %. Prevalence was higher in the large farms (30.6 %) than in the small farms (27.9 %) based microbiological results. Using CMT results the difference between the two groups of farms was small. It was higher in the smallholder farms at 35.3 % as compared 34.2 % in the large farms. All these differences were not significantly different when the 95 % confidence intervals were compared. Thus, no differences in subclinical mastitis prevalence were found between between small and large farms at the cow level.

Figure 4.1 illustrates the prevalences of subclinical mastitis as determined by the CMT and by microbiological isolatios.

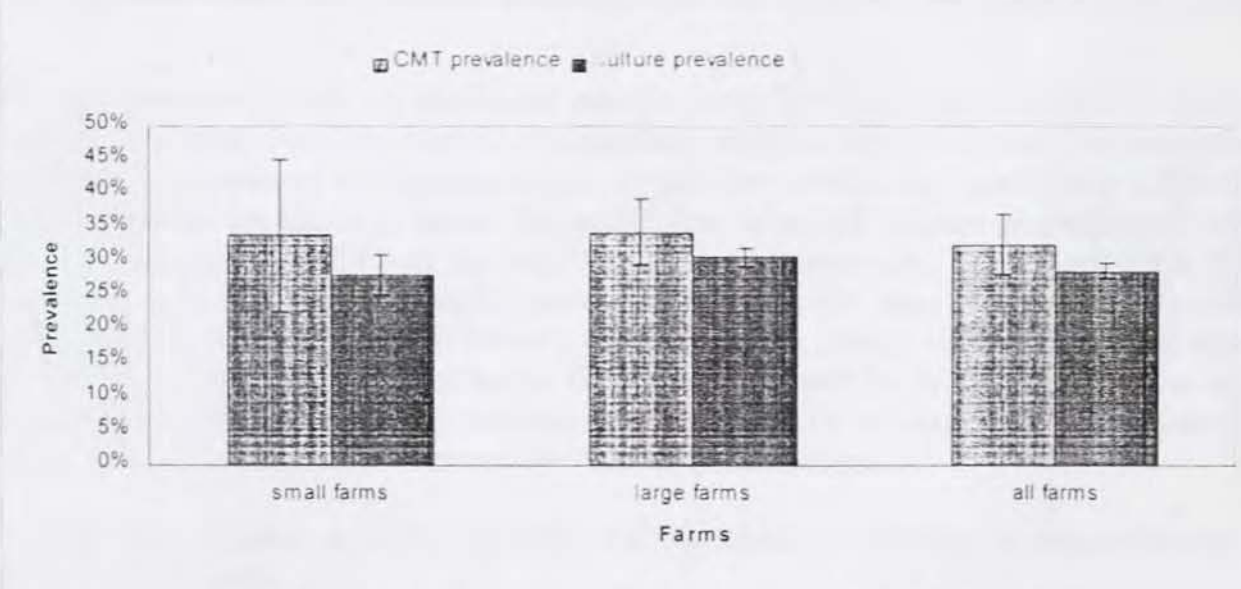

Fig. 4.1. Prevalences of subclinical mastitis in 40 farms in the Addis Ababa region

4.2.1.3 Microbiological isolates from mastitis cases from the cross-sectional study

A total of 211 isolates were cultured from samples collected in the prevalence study. Thirty-five milk samples (14.2 %) were negative at culture although they had been positive in the CMT.

Table 4.8 summarizes the findings of the microbiological cultures for subclinical cases of mastitis in the small large farms.

Table 4.8 Microbiological isolates of subclinical mastitis cases from large and small farms in and around Addis Ababa and Debre Zeit, Ethiopia.

Isolate	Total no isolated	% of all isolates	Large farms, # of isolates	% of all isolates (large farms)	Smallholder farms, # of isolates	% of all isolates (smallholder farms)
CNS	114	54.0	93	51.4	21	70.0
S. aureus	19	9.0	17	9.4	3	10.0
Str. agalactiae	57	27.0	56	30.9	1	3.3
Coryneforms	11	5.2	6	3.3	5	16.7
Str. dysgalactiae	1	0.5	1	0.6	0	0
Str. uberis	4	1.9	4	2.2	0	0
Str faecalis	3	1.4	3	1.7	0	0
Gram + Bacilli	2	0.9	1	0.6	0	0

The most common causes of subclinical mastitis were CNS accounting for 54.0% of the isolates. The mean farm frequencies of coagulase negative staphylococcus, *Streptococcus agalactiae*, coryneforms and *Staphylococcus aureus* were statistically significantly different between smallholder and large farms. The smallholder farms had a higher proportion of CNS (70%) as compared to 51.4% in the large farms. The difference was more marked for *Str. agalactiae* with 3.3% in smallholder cattle and 30.9% in the large farms. *Streptococcus agalactiae* was absent from Kaliti Farms 1 and 3 as well as Farm 2 in Sebeta This was also the case with coryneforms. In smallholder farms they accounted for 16.7% of the isolates, for large farms the value was 6%. Environmental streptococci were isolated from the large farms only, where they accounted collectively for 3.9% of the infections.

The pie diagrams below depict the distribution of microbiological isolates, in the smallholder, large and in all farms.

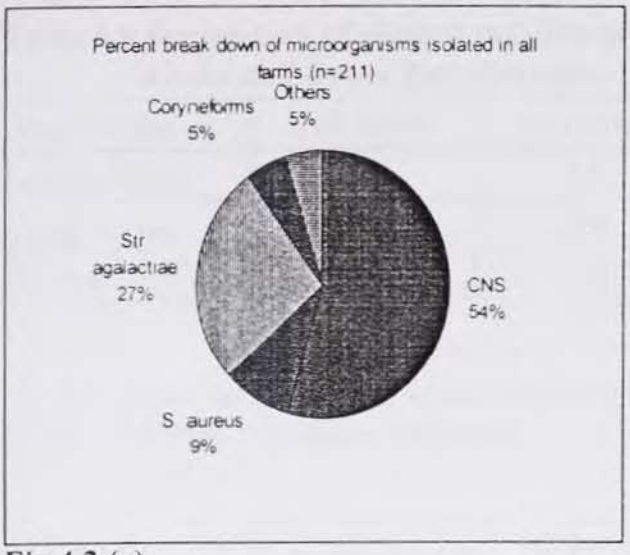

Fig 4.2 (a)

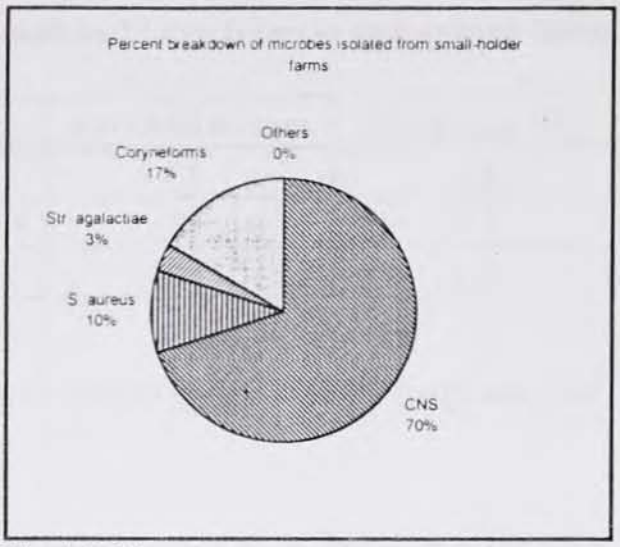

Fig 4.2 (b)

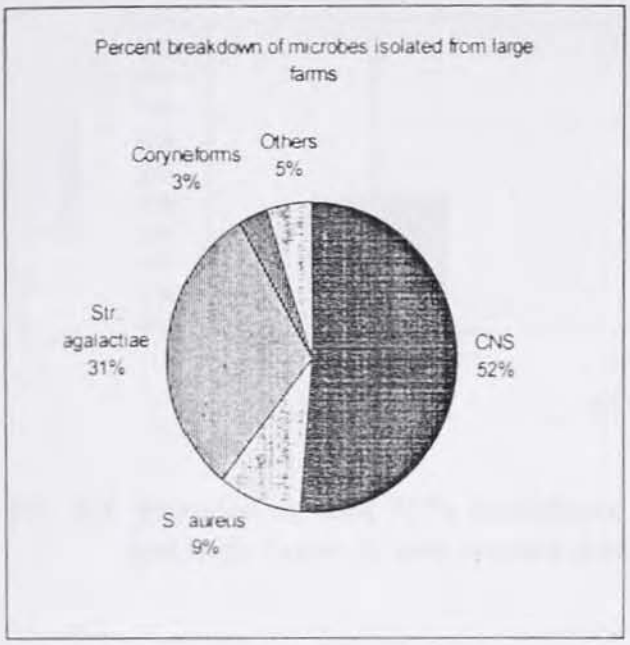

Fig 4.2 (c)

Fig. 4.2 (a, b, c): Percent breakdown of microbiological isolates of subclinical mastitis cases from large and small farms in and around Addis Ababa and Debre Zeit, Ethiopia.

4.2.2 The prevalence of clinical mastitis

The prevalence of clinical mastitis was determined by clinical examination. This was done by the detection of clinical signs such as heat, pain and swelling of the udder as well as changes in the consistency of the milk such as the presence of pus, clots, watery milk or blood. The California Mastitis Test was also used. It became apparent during the investigation that there was no difference between the CMT score of 3 and the presence of clinical mastitis. Therefore, essentially all CMT 3 were classified as clinical mastitis.

Table 4.9 summarizes the number of clinical mastitis. The farms are divided into two groups, that is the smallholder farms numbering 31 and 9 large farms.

Table 4.9 Prevalences of clinical mastitis in small and large farms in and around Addis Ababa and Debre Zeit, Ethiopia.

Farm category	# of farms	# of cows	# of clinical cases	Prevalence (%)
Smallholders	31	68	3	4.4
Large farms	9	366	21	5.7
Overall	40	434	24	5.5

Fig 4.3 shows the prevalence of clinical mastitis in the two groups of farms (large and small farms) with 95% confidence intervals.

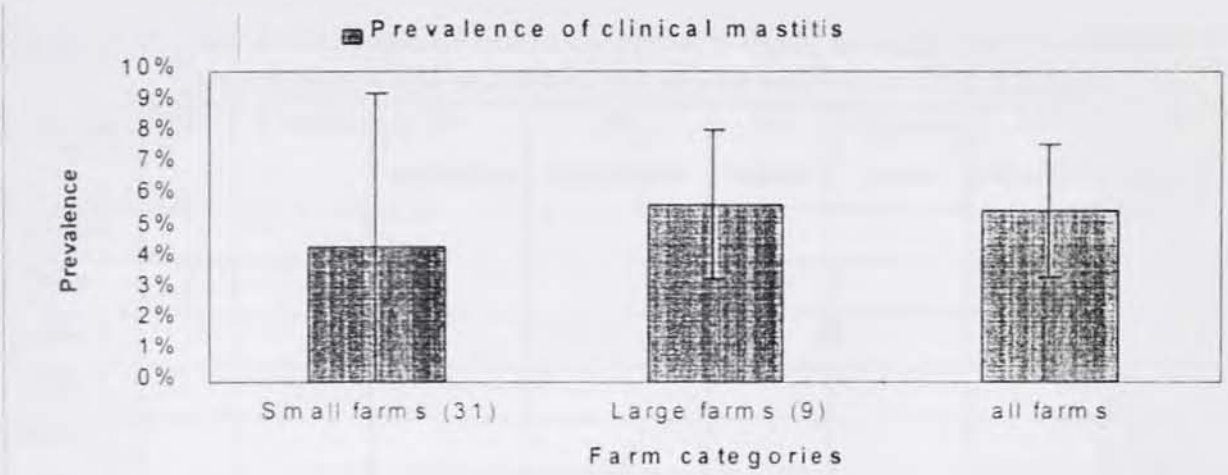

Fig. 4.3. Prevalences (and 95% confidence intervals) of clinical mastitis in smallholder and large farms in and around Addis Ababa and Debre Zeit, Ethiopia.

The mean farm prevalence rates were significantly different between the large and small farms (see Tables 4.5 and 4.6). The prevalence was higher in the large farms with 5.7% as compared to 4.4% in the small farms.

The overall prevalence of clinical mastitis in all farms was 5.5% from a total population of 434 lactating cows. Twenty-seven quarters had clinical mastitis.

4.2.2.1 Prevalence of clinical mastitis in smallholder farms

In the smallholder farms of Debre Zeit and Addis Ababa, where lactating herd sizes were 2 cows on average, the prevalence was 4.4%. The 13 smallholder farms in Addis Ababa had no clinical cases of mastitis recorded. The 3 identified clinical cases were from three different farms in Debre Zeit. The corresponding incidence rate for clinical mastitis in Debre Zeit was 7.9%.

4.2.2.2 Prevalence of clinical mastitis in large scale farms

The highest prevalence among the large farms was in Farm 2 of Addis Ababa with 25.0%. Zero-prevalence was recorded from two farms (farms 1 and 3), in Kaliti, where collectively the lowest prevalence of clinical mastitis (1.1%) was found. In these two farms no *Str.*

agalactiae were isolated. Overall the highest prevalence was found in the Addis Ababa farms with 8.6%.

4.2.2.3 Microbiological isolates from clinical cases.

A total of 25 isolates from all farms were cultured at microbiology. From three quarters combinations of microorganisms were found. Two of these were infections by coagulase negative staphylococcus (CNS) and *Str. agalactiae* and one was a combination of *S. aureus* and *Str. agalactiae*. Three cows had clinical mastitis in two quarters.

Table 4.10 summarizes the distribution of organisms isolated according to farms or groups of farms.

Table 4.10. The distribution of microbiological isolates in large and smallholder and large farms in and around Addis Ababa and Debre Zeit, Ethiopia.

Farms	CNS	*S aureus*	*Str. agalactiae*	*Str. dysgalactiae*	*Str. uberis*	Coryne-forms	G+ Bacillus	No growth
DZ-S.H.		1		1	1			
Large								
Addis 1			1					
Addis 2	1	1	3					1
Sebeta 1						1		1
Sebeta 2	1							
Sebeta 3	4	2	6				1	3
DZ 1	1							
Kaliti 2								1
Total	7	4	10	1	1	1	1	6
%	22.6	12.9	32.3	3.2	3.2	3.2	3.2	19.4

The most frequently identified organism of clinical mastitis was *Str. agalactiae* (32.3%) of all clinical cases followed by CNS, (22.6%) and *S. aureus* (12.9%). Six cases or (19.4%) of the quarters yielded no growth. From the results it can be importantly seen that no *Str. agalactiae* occurred in smallholder farms.

4.2.4 Anti-microbial susceptibility test results

A total of 67 isolates were subjected to the in-vitro antimicrobial susceptibility test. The bacteria were in four groups; *Staphyloccocus aureus*, *Streptococcus* spp., coryneforms, and coagulase negative *Staphylococcus* spp.. The isolates were selected by simple random sampling from isolates obtained in the cross-sectional study. In cases where the isolates were small in number some isolates from the longitudinal study were also used. The total number of *Staphylococcus aureus* isolates from the cross-sectional study was 23, *Streptococcus* species 12, coryneforms 12 and coagulase negative *Staphylococcus* 121.

Table 4.11 summarizes the results of antimicrobial susceptibility tests. The table contains the antibiotics that were tested, and the percentage of susceptible isolates for the respective antibiotic.

Table 4.11 Antimicrobial susceptibility profiles of sixty-seven bacteria isolates from mastitis cases.

Microbe	*Staphylococcus* aureus n=17	*Streptococcus* spp n=19	Coryneforms n=10	Coagulase negative *Staphylococcus* n=21
Antibiotics	Susceptibility (%)			
Ampicillin	12	68	0	71
Chloramphenicol	18	37	0	29
Gentamycin	100	63	60	86
Erythromycin	100	89	80	86
Nitrofurantoin	0	37	20	38
Cefamandole	94	63	60	71
Lincomycin	94	53	60	67
Neomycin	100	42	20	90
Oxacillin	100	53	50	86
Penicillin	18	53	10	67
Polymixin B	53	42	70	76
Sulphamethoxazole	0	5	20	0
Streptomycin	18	11	10	48
Tetracycline	12	37	50	57

4.2.4.1 Staphylococcus areus.

Staphylococcus aureus isolates were found to be 100% susceptible to gentamycin, erythromycin, neomycin, and oxacillin and 100% resistant to nitrofurantoin and sulphamethoxazole. They were highly susceptible to cefamandole and lincomycin (94% for each).

4.2.4.2 Streptococcus spp.

Only *Streptococcus* spp. other than *Streptococcus agalactiae* were tested; and these included *Streptococcus dysgalactiae, Sreptococcus uberis, Streptococcus faecalis* and *Streptococcus bovis*.

Erythromycin was the most effective antibiotic against the *Streptococcus* spp. with 89% of the isolates being susceptible, followed by ampicillin (68%), erythromycin and cefamandole (63%).

4.2.4.3 Coryneforms

Erythromycin was the most effective anti-microbial with 80% of the isolates being sensitive, followed by polymixin B (70%) and 60% to gentamycin, cefamandole, and lincomycin.

4.2.4.4 Coagulase negative Staphylococcus spp.

Coagulase negative *Staphylococcus* isolates were found to be highly susceptible to neomycin (90%), gentamycin, erythromycin and oxacillin (86%) respectively. Seventy-six percent of the isolates were susceptible to polymixin B.

4.2.5 Economic losses due to clinical and subclinical mastitis

Economic losses due to clinical and subclinical mastitis were calculated based on the findings of the prevalence study. Losses were estimated to be approximately 270 Ethiopian Birr (=40US$) per cow per lactation or 0.55 litres of milk per cow per day during the lactation period. The total percentage volumetric production loss was 6.9%. All calculations were based on volumetric losses.

4.3 Longitudinal study

4.3.1 Incidence of subclinical mastitis

Table 4.12 summarizes the breakdown of the incidence rates in the three week intervals investigated. Overall incidence and cumulative incidence for the 9 week period are displayed as are incidence rates based on the one cow-month at risk. The farms are divided into two groups (smallholder and large scale farms).

Table 4.12 Nine week incidence densities and cumulative incidence ratesof subclinical mastitis in smallholder and large farms in and around Addis Ababa and Debre Zeit, Ethiopia.

Farm group	cows at risk at the beginning	cows at risk at end of period	# of new cases over 9 weeks	incidence density in the 9 week period (%)	cumulative incidence	Incidence per cow-month at risk (%)
Smallholders	27	17	6	27.3	22.2	12.1
Large farms	147	72	46	42.0	31.3	18.7
All Farms	174	89	52	39.5	29.9	19.0

Although there were 31 farms under investigation for the incidence study, only 23 farms supplied new cases for the calculations. These consisted of 8 large-holder farms and fifteen smallholders. The number of cows at risk and the respective incidences calculated for each of the three week intervals are shown in Annex 4. Overall there were 174 cows at risk of developing subclinical mastitis at the beginning of the study period out of a total population of 309 cows (56.3%). At the end of the period 89 cows were still at risk. The overall incidence for the 9 week period was 39.5%.

Mean farm incidence rates between small and large farms were significantly different. Incidence was higher in large farms with 42% of the cows developing subclinical mastitis in the 9 week period as compared to 27.3% in the smallholder farms. There was, however, no significant difference at the cow level (Fig 4.4).

Fig. 4.4 depicts the trends in the incidence rates for subclinical mastitis over the 9 week investigation period. Ninety-five per cent confidence intervals are shown.

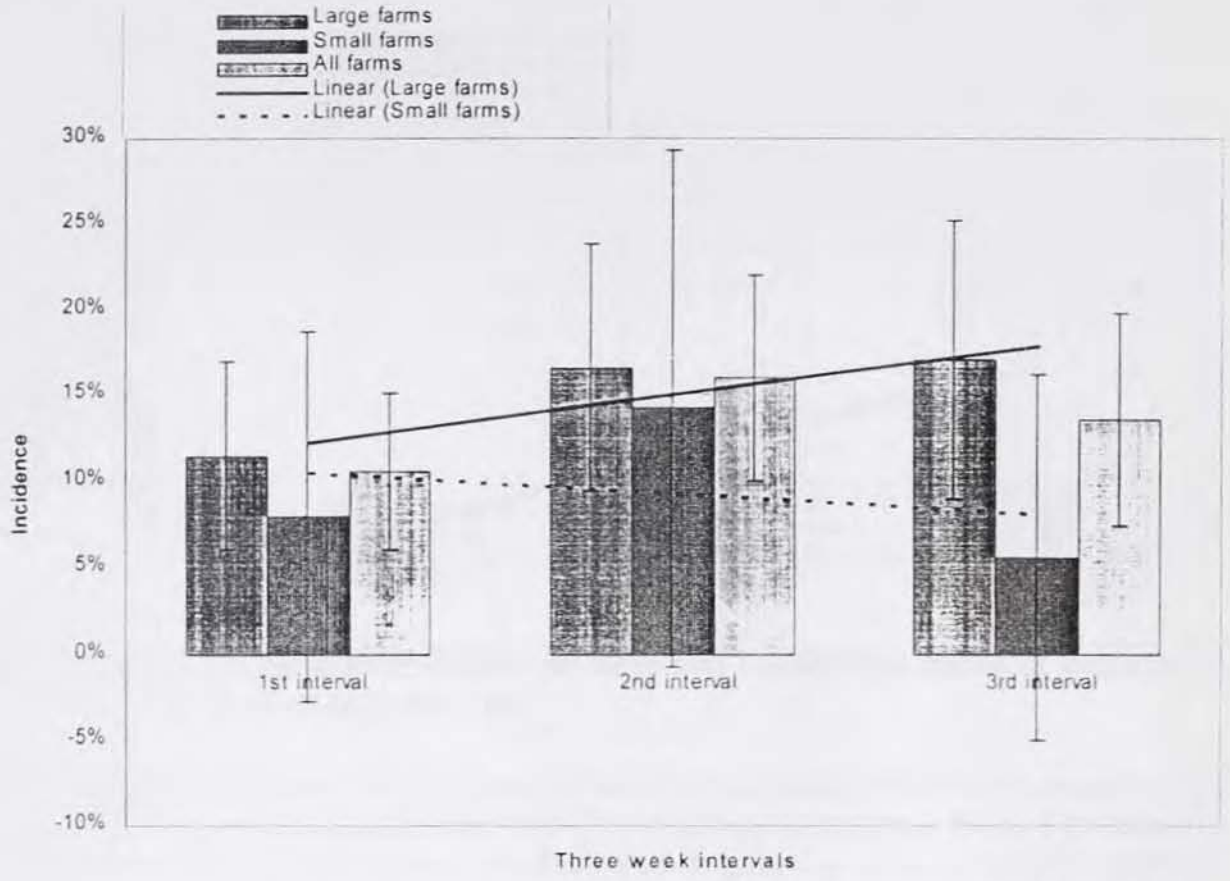

Fig 4.4 Incidence of subclinical mastitis in three week intervals in large- and smallholder farms in and around Addis Ababa and Debre Zeit, Ethiopia.

Generally, incidence rates increased in the large farms as time progressed, while it was on the decline in the smallholder farms.

4.3.2 Incidence of clinical mastitis

Table 4.13 summarizes of the results of calculations of the incidence of clinical mastitis in the smallholder and large farms.

Table 4.13 A summary of the incidence of clinical mastitis in small and large farms in and around Addis Ababa and Debre Zeit, Ethiopia.

Farm group	# at risk at the beginning	# at risk at the end of 9 weeks	# of new cases over 9 weeks	incidence in the 9 week period (%)	cumulative incidence	Incidence per cow month at risk (%)
Small farms	42	36	6	15.4	14.3	6.8
Large farms	219	212	18	8.4	8.2	3.7
All farms	261	248	24	9.4	9.2	4.2

The overall incidence for the nine week period was 9.4% and the cumulative incidence was 9.2% . Calculated to the month the incidence was 4.2 per cow month at risk. The mean farm incidence rates between large and small farms were significantly different. The incidence was higher in small (15.3%) and lower in large farms (8.4%).

Figure 4.5 below illustrates the trends of the occurrence of clinical mastitis in the two farm categories over the 9 week period.

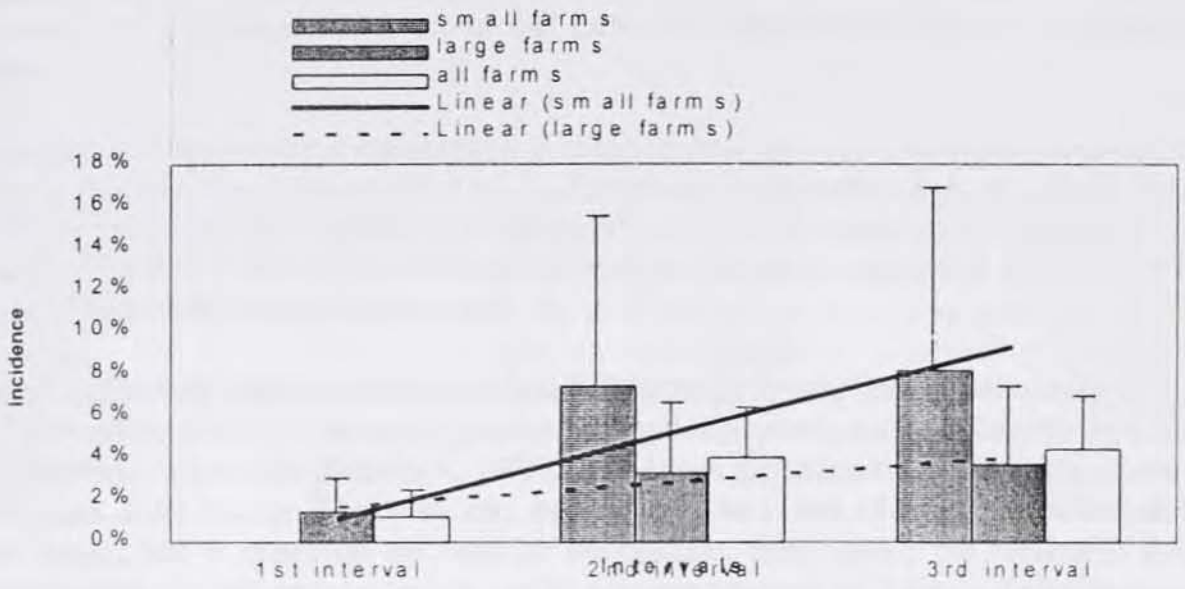

Fig. 4.5. Incidences of clinical mastitis in large and smallholder farms in and around Addis Ababa and Debre Zeit.

The graph shows that there was an upward trend in the incidence in both farm categories. The increase was larger in the smallholder farms as compared to the large farms. Details of the incidence of clinical mastitis for the individual farms or groups of farms is shown in Annex 4.

6. DISCUSSION

The results of the questionnaire survey revealed that 22% of the farms were owned by women, including the largest and most intensively used farm in the study. This confirms the important role women play in the dairy industry in Ethiopia. Only 30% of the farmers, however, were full time dairy farmers. This may have meant that the majority were in the business to add to their income from their main occupation. The average number of years of experience with dairy was lower in the smallholder sector, many had just recently started. All these information point out, that the dairy business is fairly young, but obviously an attractive investment. Large deficits, however, exist in knowledge of handling a dairy. This may therefore imply the need for more intensive extension in the smallholder sector.

Farm sizes were extremely small in the smallholder sector, particularly in the Addis Ababa area where holdings averaged 406 square meters. This small area was mostly taken up by the farmers' living quarters. Although in most cases the animals in this situation were not physically overcrowded, problems of for example effluent disposal were obvious and made living conditions for both man and animals difficult. It also meant that should the farmer

intend to exercise his or her animals they had to be taken out of the farm premises making it difficult to maintain closed herd systems. Land holdings were better in the large farms where on average they were 97.000 square meters. This ensured easier effluent disposal although this still remained a problem in the intra-urban farms.

Only 3 of the farmers owned land elsewhere, where two of them grew crops and the other was producing hay for the animals in town. Farming activities thus largely were not in a position to benefit from advantages of crop-livestock interaction. Roughage was in most cases purchased from rural farmers. Although 55% of the farmers kept other livestock this activity was mainly for subsistence. This meant that there was little spread of risk in the farming systems.

The majority of the farmers were engaged in other income generating activities not directly related to farming. For instance, 15 of the smallholders were professionals in various fields and five of the large scale farmers were successful business entrepreneurs. It would lead one to postulate that the risks in the farming enterprises were to be cushioned by these other sources of income (or the other way round).

Eighteen of the forty farmers interviewed kept their animals in total confinement inside barns. There was no possibility of them to exercise. Animals thus were unable to benefit from the positive effects of exercise (Gustafson, 1993). In addition, the animals that were kept in total confinement under zero-grazing conditions were likely to be at risk of nutritional deficiencies as the owners had to constitute the diets for the animals. Only freshly cut forages in their vegetative state, for instance, are able to supply adequate amounts of Vitamin E (Smith *et al.*, 1984). In most cases, however, old sun bleached hay was fed to the animals.

Five of the large farmers provided exercise to the animals during the dry season but confined the animals throughout the rainy season when tick challenge and the risk of tick-borne diseases were at their peak. During the day the animals were allowed to roam in an open field and were housed at night. The benefits of this were that the barn could be adequately cleaned when the animals were outside and the barn would dry adequately before the animals came in for the evening. This must have been an effective way of controlling environmental mastitis pathogens. The effect of total confinement during the wet season ought to be assessed in these herds as far as the udder health status is concerned.

Seventy per cent of the farmers dried their cows abruptly usually 2 weeks prior to calving. Among cows not receiving dry-cow therapy new infections at calving are more numerous with abrupt cessation of milking than with intermittent milking (Philpot and Nickerson 1991). The practice of intermittent milking combined with feeding only free choice hay during the last three weeks of lactation will increase protective factors such as white blood cells and antibodies, although the effect on the rate of new infections is questionable (Philpot and Nickerson 1991). It would appear that the widespread use of the abrupt method of drying off, compounded by the lack of dry-cow therapy could constitute a risk factor for mastitis in these herds.

The knowledge and use of dry-cow therapy, teat dipping, culling and the use of conventional disinfectants were generally absent from all farms. This was also found to be the case in the smallholder farmers of Kiambu district of Kenya (Omore *et al.*, 1996). It would be particularly important that these measures are introduced in the large scale farms where the

reservoir pool of infection and cow-to-cow transmission is most present. The smallholders should be encouraged to take up these measures.

There was a reluctance among the farmers to cull their cows in chronic cases of mastitis. For instance, 7 out of the 19 respondents in Debre Zeit would only cull a cow after it had lost three quarters of its udder whilst two of the large farmers would cull a cow only after it had lost all its quarters. Continuing with this practice not only would the farmers be losing on milk production but this practice also poses a serious risk of infection for the clean cows particularly with regard to contagious pathogens (Smith, 1983; Radostits *et al.*, 1996).

Fifty percent of the farmers did not check the milk by any means such as strip cup and/or the California Mastitis Test. Therefore, no subclinical mastitis were detected at all. This fact would also mean that there are no means of monitoring the udder health status. Without this, however, the farmers are unable to know what is happening in their herds at any given time. Of those that reported to check milk for abnormality most would use the floor, the hand or in the milking container. The effectiveness of these methods is totally inefficient.
Generally, most of the farmers (65 %) said that they would milk those cows with mastitis last. This practice has been advocated by many authors as an effective means of segregation between infected and clean cows and an effective means of limiting cow-to-cow transmission. Twenty-five out of 28 respondents said they did not use any antiseptics at milking for udder preparation, hands or utensils. Only three used ordinary soap. The reason for not doing so was the high cost of the chemicals.

After the washing of the udder and teats 37 of the respondents reported to dry the teats prior to milking. Seventeen farmers allow the teats to dry on their own. This method cannot be considered as drying at all. Only 6 of the 19 farmers who dried the teats used a separate towel or cloth for each cow. The use of a single cloth, however, is an important risk factor for the transmission of the contagious pathogens, particularly in large herds. Apparently none of the large farmers used a separate cloth for each cow.

The washing of hands before milking each cow was practised by all farmers except four. However, since most of these farmers used pure water, a reduction in the amount of pathogens may have not been effective. It has been shown that even disinfectants were unable to eliminate all the pathogens from the milkers' hands.

Twenty-nine (73%) of the farmers admitted that mastitis was a problem in their herds. The remainder who were all smallholders maintained that they had never encountered a case of mastitis in their herds. This finding perhaps helps to support the hypothesis that mastitis becomes of greater importance with increasing herd sizes.

The fact that all except three farmers consulted a veterinarian or a veterinary assistant was an encouraging finding; by this farmers are able to benefit from expert advice. However, only nine of the smallholders had their animals treated by a veterinarian. Provided the farmers are well trained to administer treatment themselves, there should not be a problem. Improper administration of intramammary treatments or the use of badly constituted home-made remedies, however, poses a potential hazard in that pathogens such as *Nocardia asteroides*, *Pseudomonas aeruginosa*, yeasts and fungi (Radostits *et al.*, 1996) may be introduced into the udder this way. Philpot and Nickerson (1996) report that partial insertion of the mastitis syringe results in a 50% reduction in new infections by major mastitis causing microorganisms where dry-cow therapy is practiced. Full insertion of the conventional

mastitis syringe canula can result in the temporary dilatation of the teat sphincter muscle and the keratin plug that normally occludes the teat canal is sometimes removed allowing entry of microorganisms (Philpot and Nickerson, 1991).

Considering the housing situation, 26 of the housings examined had concrete floors. This was a good attribute as it allows adequate cleaning of the floor. Wherever stones had been used (particularly in the Addis Ababa area), the cleaning of floors was difficult. The stones themselves were also a traumatic hazard to the cows and perhaps a stressing factor because of the discomfort they gave to the animals when they lay down.

The cleansing effect of sun or radiation has been shown to have a beneficial effect against environmental mastitis (Smith *et al.*, 1985; Schukken *et al.*, 1988). The lack of entry of sunlight into half of the barns meant that no benefit derived from this natural resource.

Only ten of the barns had roofs that were not rain-proof. This meant that during the wet season the amount of moisture would increase. Ventilation was a problem in 50% of the barns and drainage problems were encountered in more than fifty per cent. The existence of organic materials (urine, dung, milk, etc.), moisture and temperature encourage the growth of environmental organisms (Zehner and Farnsworth, 1986).

Flies did not appear to be a problem in the herds studied. Flies were found to be associated with the more open housing systems than with those that were sealed. Flies have, for instance, been incriminated in the transmission of *Actinomyces pyogenes* (Radostits *et al.*, 1996).

The prevalence of subclinical mastitis reported here is lower than that reported for Ethiopia by other workers. Prevalence of subclinical mastitis in previous studies ranged from 37.6% to as high as 68.1% (Biru, 1989; Abdella, 1996, Zerihun, 1996; Gezahegn, 1996; Tegegne 1997, personal communication). The prevalence of subclinical mastitis was also found to be particularly high in smallholder dairy herds in Kenya. Omore *et al.* (1996) reported a prevalence of 71% . However, in Malawi, Klapstrup and Halliwell (1977) in contrast found a low prevalence of subclinical mastitis in smallholder dairy herds (ranging from 17 to 19%). They attributed this low frequency of subclinical mastitis to hand milking and open housing systems in that country.

Clinical mastitis with an average prevalence of 5.5% is on the high side. It certainly is higher than what was established in an extensive survey by the International Livestock Research Institute, Ethiopia, which found a prevalence of 1.2% in the 725 farms surveyed (Tegegne, 1997 personal communication).

The mean prevalence rates of subclinical and clinical mastitis between large and small farms were significantly different. Prevalences were higher in the large farms. This supports the hypothesis that the cows in the larger more intensive farms were at a greater risk of mastitis than those in the smaller herds.

The main microorganisms isolated were coagulase negative staphylococcal species which accounted for 54%, *Streptococcus agalactiae* (27%), *Staphylococcus aureus* (9%), and coryneforms (10%). Environmental streptococci played a minor role. The mean frequency rates of these organisms differed significantly between the large and small farms.
The distribution of the microorganisms showed that CNS predominated in both groups of farms although they were more prominent in the smallholder farms (70% of all isolates). The

high frequency of this minor pathogen exert had a protective effect against major pathogens (Lam *et al.*, 1997). It may be hypothesized that this was the case in the large farms.

Streptococcus agalactiae was almost absent from the smallholder sector. It was also found to be absent in three of the large farms. This may be an important finding in that a policy could be devised to maintain such herds *Str. agalactiae*-free and to try and eliminate this pathogen in herds where it is present. The practice of a closed herd system for instance or the introduction of only confirmed *Str. agalactiae* free cows would be beneficial. The findings, however, show that the larger farms were more susceptible to this pathogen because of the greater possibility of cow-to-cow transmission during milking. This transmission possibility is limited in the small herds where sometimes there was only one cow; still, contract milkers go from house to house milking cows and this may be a risk of spreading *Str. agalactiae* that way. The lack of most essential control measures such as absence of adequate milking hygiene, dry cow therapy and teat dipping may account for the high prevalence of this organism in the large herds.

Most reports have incriminated *S. aureus* as one of the most frequent causes of subclinical mastitis in smallholder dairy herds (Kinabo and Assey, 1983; Biru, 1989; Abdella, 1996). In this study *S. aureus* was much less frequently isolated than in other studies.

In clinical mastitis cases, *Str. agalactiae* and CNS were the most frequently isolated microorganisms. *S. aureus* again did not feature prominently as a causal agent for clinical mastitis. *S. aureus* and *Str. agalactiae* have generally been implicated as the causes of most intramammary infections (Erskine *et al.*, 1987; Sischo *et al.*, 1993).

Environmental streptococci did not appear to be of much significance in both groups of farms. Also no gram negative bacteria were isolated in the cross-sectional study. This is in agreement with previous studies by Biru (1989) and Abdella (1996).
The number of isolates that were subjected to antimicrobial susceptibility testing was small. Still the results give some hints on the trends concerning antimicrobial resistance as well as for therapeutic decisions.

The mean incidence rates for subclinical mastitis between large and small farms were significantly different. In the smallholder sector the disease was on the decline while it was on the increase in the large farms. This decline was realized despite of the fact that the study started at the beginning of the rainy season. If this decline was a time effect or a confounding result of the study itself remains undecided. The farmers may have responded positively to the advice given during the study period. It was unavoidable to do so. Published information on the incidence of subclinical mastitis is not available for Ethiopia to make comparisons. The inherent difficulties in the implementation of this kind of study also were experienced in this investigation; its applicability under field investigative conditions remains a constant challenge (Radostits *et al.*, 1996).

The mean incidence rates of clinical mastitis were also significantly different between the large and smallholder farms and were found to be higher in the smaller farms. This finding may point against the hypothesis that larger farms are at greater risk than the smaller farms. However, the knowledge and more widespread use of antibiotics by the large farms may have meant that cases were identified earlier and were treated. In this investigation, although an attempt was made to record cases that have occurred between consecutive visits, information

given mostly was not sufficiently accurate. Therefore, intermittent therapy may have confounded the results.

In general the results of this study differ from those of other workers where the annual incidence was found to be lower than 10% in smallholder dairy production systems in the East African region (Hamir *et al.*, 1978; Kinabo and Assey 1983; Okello *et al.*, 1996).

The incidence of clinical mastitis rather was found to be similar to that found in the western hemisphere were annual incidences are in the 30 to 55% range per annum (Wilesmith *et al.* 1986; Bartlett *et al.*, 1992; Miller *et al.*, 1993). The projected annual incidence in this study was slightly over 50% for both farm groups.

7. CONCLUSIONS AND RECOMMENDATIONS

This study was able to verify that there was a significant difference in the occurrence (prevalence and incidence) of mastitis and the concerned etiologic agents between large and smallholder farms in and around Addis Ababa and Debre Zeit, Ethiopia.

Prevalence rates of clinical mastitis, however, were higher than reported elsewhere for East African smallholder systems.

The most important etiologic agents that cause mastitis in the farms studied were coagulase negative staphylococcus, *Streptococcus agalactiae*, *Staphylococcus aureus* and coryneforms. Environmental streptococci played a less significant role.

In contrast prevalences of subclinical mastitis were lower than those found in other studies. Prevalences of subclinical mastitis were generally within acceptable limits.

The incidence of subclinical mastitis was higher and on the increase in the large farms. At the same time there was a decrease in the smallholder farms as the rainy season progressed. The incidence of subclinical mastitis, although it could not be compared to results of other studies due to lack of information, with an overall projected annual incidence of 228 cases per hundred cows at risk should warrant closer attention. Extension services should emphasize on educating farmers on methods of limiting the occurrence of new infection through control measures such as teat dipping, dry cow therapy, good milking hygiene, culling of chronically infected cows, and above all to have some means of monitoring the level of infection in their herds. These measures should be implemented in the large farms at least initially.

The mean incidence rates of clinical mastitis were also significantly different between the two groups of farms. The overall projected annual incidence of 50.4 cases per hundred cows at risk was comparable to findings in the western hemisphere. The rate was much higher in smallholder farms where the projected annual incidence was 81.6 cases per hundred cows at risk. This higher incidence in the smallholder sector may be attributed to the lower antibiotic usage, particularly in the Addis Ababa area.

The barn structures of farms in many cases were structurally unsuited to the keeping of dairy livestock under tropical conditions. The farmers are spending considerable amounts of money to put up expensive structures that however adversely affect the living conditions of the

animals. Thus farmers should be made to realise that their crossbred animals are well adapted to cold conditions and that it is unnecessary to construct animal houses that completely enclose the animals. Attention ought to be focused on those that may be starting dairy farming to be given adequate professional advice on this issue. Indeed, some structures violated animal welfare considerations.

A closer look at host and environmental risk factors for mastitis is recommended; it includes, for example milk yield, anatomical considerations such as pendulous udders, teat end shape and hind-limb conformation, teat lesions, age or parity, nutritional status and hygiene. Establishment of seasonal trends may also be of interest.

More refined microbiological techniques to enable the isolation of pathogens such as *Mycoplasma* and *Nocardia* species are further recommended. The use of augmented cultural techniques to improve the isolation of pathogens and reduce the frequency of samples yielding no growth is also recommended.

Somatic cell counts by either the direct microscopic technique or by electronic cell counting methods should also be done to establish a practical threshold for the infection status in these herds.

A more thorough and objective assessment of the economic losses due to mastitis should be done. This will help in the assessment of the feasibility of implementing certain control measures and practices. The formulation of suitable, effective, practical and farmer tailored extension strategies ought to be designed and implemented.

The antimicrobial susceptibility tests finally also need to be done more extensively than was done in this study. Coagulase negative staphylococci were found to be the most predominant isolates causing subclinical mastitis. The impact of these organisms as mastitogenic agents and as protection against the major pathogens warrants further elucidation.

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Annex 1

The map showing the study area

Annex 2.

CROSS- SECTIONAL AND LONGITUDINAL PROSPECTIVE STUDY OF BOVINE CLINICAL AND SUBCLINICAL MASTITIS, IN INTRA-URBAN AND PERI-URBAN DAIRY PRODUCTION SYSTEMS IN AND AROUND ADDIS ABABA, ETHIOPIA.

Date_____

GENERAL INFORMATION

Farm Structure

Farm owner _____ sex_____ age_____ yrs
Occupation _____
Farm physical address_____
_____ Tel._____
Year dairy farming started_____
Location intra-urban/secondary town_____
intra-urban _____
peri-urban _____
Distance from town center_____km.
Farm size_____Ha.
Is the farm fenced? yes no
Is there frequent contact between your animals with other herds?
yes no
Crop livestock farming: Types of crops grown and quantity.

Type of crop livestock farming	Quantity

Do you own land elsewhere e.g. rural area? yes no
What do you do on this land? _____
_____
_____
_____

Social Factors

What was the last school you attended?
(1) elementary
(2) junior high school
(3) college university
Professional qualifications _____
How did you acquire skills to raise dairy cattle?
(1) agricultural training (level _____)
(2) from extension agents (which ones _____)

What do you do after administration of the drug into the udder? _____

_____

_____

dry cow treatment

At which month do you dry off your cows? _____

How do you dry off your cows?

(1) by intermittent milking

(2) abruptly

Do you practice dry cow treatment? _____

Which drugs do you use for dry cow treatment? _____

_____

What do you do with animals that remain infected with mastitis for a long time?

milking procedure

How many times do you milk your cows in a day?

(1) once

(2) twice

(3) three or more times

Is the udder cleaned before milking?

yes no

Do you use a teat dip antiseptic?

yes no

When do you dip the teats?

(1) before milking

(2) after milking

(3) both

Which chemicals do you use for teat dipping? _____

_____

Does the chemical require dilution?

yes no

When do you milk cows with mastitis?

(1) first

(2) last

(3) at any stage

hygiene management

Milking hygiene management?	yes	no
Teats are washed before milking?	yes	no
Teats are dried before milking?	yes	no
Is a separate towel used for each cow?	yes	no
Are hands washed between cows?	yes	no
Is a disinfectant used for washing hands?	yes	no
Is the teat dip used for each cow?	yes	no
Does the teat dip applicator appear clean?	yes	no

Farm inspection report

housing

Building material used is
- (1) traditional
- (2) modern
- (3) mixture

Housing type
- (1) pen
- (2) barn
- (3) stanchion
- (4) freestall

Floor type
- (1) earth
- (2) concrete
- (3) stone
- (4) other

Does the building structure allow sunshine into the building	yes	no
Is the roof structure rain proof	yes	no
Cow stalls are free of hazards	yes	no
Parturition pens are clean and dry and well bedded?	yes	no
Dry cow facilities are clean	yes	no
Cow stalls are of adequate size (220cm × 110cm)?	yes	no

The drainage system
- (1) excellent
- (2) satisfactory
- (3) poor

Ventilation in the building
- (1) excellent
- (2) satisfactory
- (3) poor

General farm hygiene and orderliness
- (1) excellent
- (2) satisfactory
- (3) poor

cow cleanliness

Soiling of the udders
- (1) not apparent
- (2) moderate
- (3) serious

Flies on the cows
- (1) negligible
- (2) moderate
- (3) too much

Is hair clipped from the udder

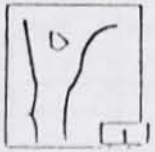
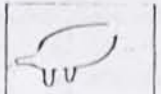
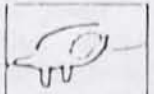
yes no

Annex 3

Cow cleanliness

TARJETA PARA LA VALIDACION DE LA HIGIENE DE LA PARTE POSTERIOR Y DE LA UBRE DE LA VACA

Antes de empezar con la valorizacion de la higiene de las vacas, busca las que se encuentren en riesgo, como por ejemplo las recién paridas, las novillas recien entradas en el hato o de las vacas diagnosticadas con mastitis clínica o subclínica. Hay que valorar el lado con mayor suciedad de la vaca. Luego sume los valores y calcule el promedio.

Valorizacion / Numero vacas	 0	 1	 2	 3	 4	 0	 1	 2	 3	 4
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20										
Total valorizacion										
Promedio										

Interpretacion del valor promedio:
0 a 2 : vacas limpias
3 a 5 : se puede mejorar la higiene al controlar algunos factores ambientales
> 5 : se puede mejorar la higiene al controlar el conjunto de los factores ambientales

Conclusion:

Annex 4

									incidence of subclinical mastitis in all farms									
Area	Farm No.	riskat beg (1st visit)	# at risk on 2nd visit	cases at 2nd visit	incidence on 2nd visit	# at risk on 3rd visit	cases on 3rd visit	ce on 2nd interval	# at risk on 4th visit	# new cases on 4th visit	incidence on 3rd interval	# of cows on the farm	incidence for 9weeks	cumul incid	weekly incid	monthly incid	annual incid
DZ	1	14	10	4	0.16667	6	3	0.1875	6	0	0	21	0.700	0.700	0.078	0.337	4.044
DZ	2	0	0	0	#DIV/0!	0	0	#DIV/0!	0	0	#DIV/0!	2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
DZ	4	4	2	2	0.33333	2	0	0	2	0	0	4	0.667	1.000	0.074	0.321	3.852
DZ	5	0	0	0	#DIV/0!	0	0	#DIV/0!	0	0	#DIV/0!	1	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
DZ	6	1	0	0	0	1	0	0	1	0	0	1	0.000	#DIV/0!	0.000	0.000	0.000
DZ	10	0	1	0	0	1	1	0.5	1	0	0	2	2.000	1.000	0.222	0.963	11.556
DZ	11	4	2	0	0	1	0	0	2	0	0	6	0.000	0.000	0.000	0.000	0.000
DZ	12	2	2	0	0	2	0	0	2	0	0	2	0.000	0.000	0.000	0.000	0.000
DZ	13	3	3	0	0	2	1	0.2	2	0	0	3	0.400	0.333	0.044	0.193	2.311
DZ	20	0	0	0	#DIV/0!	0	0	#DIV/0!	0	0	#DIV/0!	1	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
kali	2	17	16	1	0.0303	15	1	0.03226	13	1	0.03571	36	0.200	0.188	0.022	0.096	1.156
kali	1	29	23	0	0	16	5	0.12821	13	2	0.06897	41	0.333	0.304	0.037	0.160	1.926
kali	3	16	15	1	0.03226	13	0	0	10	1	0.04348	25	0.154	0.133	0.017	0.074	0.889
seb	1	12	9	3	0.14286	7	2	0.125	6	1	0.07692	31	0.667	0.667	0.074	0.321	3.852
seb	2	18	9	3	0.11111	7	2	0.125	5	1	0.08333	25	0.522	0.667	0.058	0.251	3.014
seb	3	27	24	2	0.03922	21	2	0.04444	14	7	0.2	48	0.537	0.458	0.060	0.258	3.100
aa	2	14	8	1	0.04545	6	2	0.14286	5	1	0.09091	33	0.421	0.500	0.047	0.203	2.433
aa	3	0	1	0	0	1	0	0	1	0	0	1	0.000	0.000	0.000	0.000	0.000
aa	4	2	1	0	0	1	0	0	1	0	0	4	0.000	0.000	0.000	0.000	0.000
aa	5	1	1	0	0	1	0	0	0	0	0	2	0.000	0.000	0.000	0.000	0.000
aa	6	0	0	0	#DIV/0!	0	0	#DIV/0!	0	0	#DIV/0!	1	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
aa	7	2	2	0	0	2	0	0	0	0	0	2	0.000	0.000	0.000	0.000	0.000
aa	9	1	1	0	0	1	0	0	1	0	0	1	0.000	0.000	0.000	0.000	0.000
aa	10	0	0	0	#DIV/0!	0	0	#DIV/0!	0	0	#DIV/0!	2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
aa	11	0	0	0	#DIV/0!	0	0	#DIV/0!	0	0	#DIV/0!	1	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
aa	12	0	0	0	#DIV/0!	0	0	#DIV/0!	0	0	#DIV/0!	1	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
aa	13	1	0	0	0	0	0	#DIV/0!	0	0	#DIV/0!	3	0.000	#DIV/0!	0.000	0.000	0.000
aa	14	1	1	0	0	1	0	0	0	1	1	1	2.000	1.000	0.222	0.963	11.556
aa	15	2	2	0	0	2	0	0	2	0	0	2	0.000	0.000	0.000	0.000	0.000
aa	17	3	3	0	0	1	1	0.25	2	0	0	6	0.400	0.333	0.044	0.193	2.311
farms		174	136	17	0.05484	110	20	0.0813	89	15	0.07538	309	0.395	0.382	0.044	0.190	2.285

Annex 5

Clinical mastitis incidence data

Area	Farm No.	#at risk first visit	#at risk 2nd visit	new cases 1st interval	# at risk 3rd visit	new cases 2nd	All farms- incidence of clinical mastitis: # at risk 4th visit	new cases 3rd interval	incid in first interval	incid in 2nd interval	incid in the 3rd interval	incid 9week period	weekly incid	monthly incid	annual incid
DZ	1	18	18	0	18	0	18	1	0.000	0.000	0.056	0.056	0.006	0.025	0.321
Kaliti	2	33	33	1	31	2	33	0	0.030	0.063	0.000	0.091	0.010	0.040	0.525
Kaliti	1	36	33	0	35	0	31	2	0.000	0.000	0.061	0.060	0.007	0.027	0.345
Kaliti	3	22	24	1	22	0	22	0	0.043	0.000	0.000	0.045	0.005	0.020	0.263
sebeta	1	22	22	1	23	3	26	0	0.045	0.133	0.000	0.167	0.019	0.074	0.963
sebeta	2	24	16	0	18	0	19	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sebeta	3	42	44	0	43	2	35	4	0.000	0.046	0.103	0.156	0.017	0.069	0.900
Addis	2	22	20	0	25	0	28	1	0.000	0.000	0.038	0.040	0.004	0.018	0.231
Addis	4	2	3	0	3	0	4	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Addis	5	2	2	0	2	0	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Addis	6	1	1	0	1	0	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Addis	7	2	2	0	2	0	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Addis	9	1	1	0	1	0	1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Addis	10	2	2	0	2	1	2	0	0.000	0.500	0.000	0.500	0.056	0.222	2.889
Addis	11	1	1	0	1	0	1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Addis	12	1	1	0	0	0	0	0	0.000	0.000	#DIV/0!	0.000	0.000	0.000	0.000
Addis	13	3	2	0	3	0	2	1	0.000	0.000	0.400	0.400	0.044	0.178	2.311
Addis	14	1	1	0	1	0	1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Addis	15	2	2	0	2	0	2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Addis	17	6	6	0	4	1	5	1	0.000	0.200	0.222	0.364	0.040	0.162	2.101
DZ	2	2	2	0	2	0	1	1	0.000	0.000	0.667	0.667	0.074	0.296	3.852
DZ	4	4	4	0	4	0	4	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DZ	5	1	1	0	1	0	1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DZ	6	1	1	0	1	0	1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DZ	10	0	1	0	1	1	2	0	0.000	1.000	0.000	1.000	0.111	0.444	5.778
DZ	11	4	4	0	1	0	4	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DZ	12	2	2	0	2	0	2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DZ	13	3	3	0	3	0	2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DZ	20	1	1	0	1	0	1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		261	253	3	253	10	248	11	0.012	0.040	0.044	0.094	0.010	0.042	0.545

CURRICULUM VITAE

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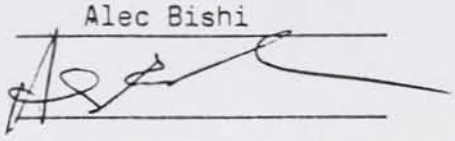
Hoyer M.J.; Codd R.; **Bishi A.S.**; Pawandiwa A.; Usenik E.A. (1991): The Prevalence of Subclinical Mastitis in Beef Herds in Zimbabwe. Zimbabwe Vet. J. Vol. 22, (1) 1-7.

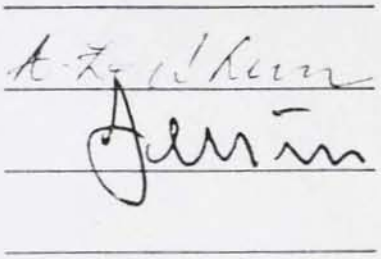
I the undersigned, declre that the thesis is my original work and has not been presented for a degree in any university.

Name Alec Bishi

Signature 

Date of submission 7th January 1998

This thesis has been submitted for examination with our approval as University advisors



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Prof. Zessin

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