ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SMALLSCALE FARMING PRACTICES AND CROSSSECTIONAL STUDY OF MASTITIS IN NAZARETH, EAST SHOA

BY
TESFAYE ALI

A Thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Masters of Science in the Department of Tropical Animal Health and Production.

June, 2007
Debre Zeit, Ethiopia

ACKNOWLEDGEMENTS

My deepest respect and thanks to my advisors, Dr. Mekonnen Hailemariam for his intellectual guidance, provision of material, unreserved effort for facilitating this research work and unreserved input to make this MSc. research work real.

It is a good opportunity to thank and appreciate all staffs of Microbiology laboratory department and those students that were in the laboratory specially for those MSc students for their support and smooth approach during my stay.

Last but not least, my families and friends, you did a lot to me. The day to day unweaving support, encouragement and whole support of my family has priceless value and no existing word express my love, respect and appreciation to you. Without your support and encouragement, things were not easy as passed. I really thank you As', You are so different!!!

I, above all would like to thank the heavenly ALLAH for his be my thought, word and deed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AI	Artificial Insemination
BCS	Body Condition Score
FAO	food and Agricultural Organization
IMI	Intramammary Infection
IMVC	Indole Methylblue Vogesprosker and Citrate test
l	liter
m.a.s.l.	meter above sea level
MOA	Ministry of Agriculture
NMC	National Matitic Council
O-F	Oxidative-fermentative test
SCC	Somatic Cell Count
SDP	Smallholder Dairy Development Project
TLU	Tropical Livestock Unit

ANNEXSES

ABSTRACT

The present study was conducted to asses the husbandry practices, identify major health constraints, establish mastitis prevalence, determine potential risk factor of mastitis and isolate responsible agents of mastitis in smallholder dairy farms in Nazareth, East Shoa. A total of 95 market oriented smallholder dairy farms comprising 234 cows were included in the study. Data were collected by using questionnaire survey, farm visit, animal examination, and California Mastitis Test (CMT). Microbiological assessment of milk samples was conducted at the Faculty's microbiology laboratory. Fifty nine percent of the farms were owned by women and the remaining by men. In most cases (74.7%) dairying was a side-line business while only 25.4% entirely earn their living from this activity. The majority (52.6%) of farm owners had high school and above level of education. The average herd size per farm was 5.23. There were 93.7% crossbred and 6.3% indigenous cattle. The breeding methods used were only AI in 64.2% of the farmers and both AI and natural services in the rest. About 21.1% of the herds were under poor housing conditions. Full time hired laborers perform routine farm activities in 19% of the farms and contract hired laborers practiced milking in 39% of the farms. The most encountered health problem, indicated as primary disease, was mastitis. There was no record that enables the owners to compare the performance of his/her cows with in a herd or with those of his/her community. Based on clinical examination and CMT the cow-level prevalence of clinical and subclinical mastitis was 6.3% and 41.4% respectively. Aseptic collections of milk samples from all clinical and subclinical (CMT+) mastitis positive lactating cows were performed. Of 91 cow milk samples 90.11% were positive for aerobic pathogenic bacteria; predominant isolates being of CNS 21.2%, *S. aureus* 14.7%, *S. agalactiae* 11.6% and *E. faecalis* 10.6%. Duration of farming, poor drainage/slope of stable area, feed provision before milking, milking of clinical cows at any stage, farming practice as a side business were highly associated with mastitis. Poor body condition, previous clinical mastitis problem and leaking teats were associated with mastitis. Although smallholder dairy farmers, were facing a lot of problems specially lack of technical know-how, there is great potential and opportunity for the development of the sector.

Key words: smallholder, dairy cow, husbandry practice, mastitis, prevalence, risk factors, and bacteriology.

1. INTRODUCTION

Ethiopia holds large potential for dairy development due to its large livestock population and the favorable climate for improved and high-yielding animal breeds. Given the considerable potential for generation of income and employment, the development of the smallholder dairy sector, in Ethiopia, has a promising feature and can contribute significantly to poverty alleviation and improved nutrition in the country. According to Ahmed *et al*. (2003) milk production during the 1990s expanded at an annual rate of 3.0% compared to 1.63-1.66% during the preceding three decades. The dairy sector in Ethiopia is expected to continue to grow over the next one to two decades given the large potential for dairy development in the country, the expected growth in income, increased urbanization, and improved policy environment (Kelay, 2002).

Livestock represent a major national resource and form an integral part of the agricultural production system. The country has the largest livestock population of any African country with an estimated 35 million Tropical Livestock Units (TLU); this includes 31 million cattle, 42 million sheep and goats, 7 million equines, 1.2 million camels, and more than 53 million chickens and immense bee and fishery resources (Minister of Agriculture (MOA), 1997). Cows represent the largest proportion of cattle population of the country. According to the Food and Agriculture Organization (FAO) (2001), 3.42% of the total cattle heads for the private holdings are milking cows. Milk produced from these animals provides an important dietary source for the majority of rural as well as a considerable number of the urban and peri-urban population. However, milk production often does not satisfy the country’s requirements due to a multitude of factors. Disease of the mammary glands known as mastitis is among the various factors contributing to reduced milk production (Fekadu, 1995).

Milk is a cash crop for smallholders, converting low value forages and crop residues and using family labor, in to valued market commodity. The ownership between 1 and 20 animals, with a small land area used for cropping or pasteur production is a characteristic feature of the market-oriented smallholder production system. Such production systems are common in urban and peri-urban areas (Moran, 2005).

Feeding and nutrition have been highlighted repeatedly as major constrains to animal production globally. The significance of improved nutrition is particularly important since feed cost make up 50% to 60% of total cost of milk production. In smallholder systems, inadequate land and size of operation are further production constraints (Moran, 2005). The limited availability of good quality forages combined with an intensive production system has forced daily farmers to rely heavily on concentrates (Devendra, 2001).

The optimal milk productivity of cattle in Ethiopia has not yet been realized due to several constraints that include poor animal management, poor feeding particularly during the dry season when pastures become scarce and the high prevalence of diseases as a result of poor disease control. The major health constraints include tick-borne diseases, infectious diseases, trypanosomosis, helminthosis and nutritional disorders (Ahmed *et al*., 2003).

Mastitis has been recorded as one of the major diseases of economic importance in the dairy industry worldwide (Radostits *et al.,* 1994b). It causes greater economic loss, much of the losses are related to production loss from inflammation of the infected quarter (Erskine, 2001). In Ethiopia, bovine mastitis is the most commonly encountered diseases in dairy cows. Generally the prevalence of mastitis (clinical and subclinical mastitis) in different parts of Ethiopia was reported by different authors that ranges from 1.2 to 46.6% (Abdella, 1996; Frese, 1999; Hussein, 1999; Lemma *et al*., 2001; Workineh, 2002; Kero and Tareke, 2003; Gizat, 2004 and Mungube *et al*., 2004).

The objectives of this study, therefore, were:

- To determine farm characteristics and present status of husbandry practices in smallholder dairy farms in Nazareth
- To identify major management constrains of production system of smallholders in Nazareth.
- To determine the prevalence of mastitis and the association of some risk factors of mastitis in smallholder dairy farms in Nazareth.
- To identify the major bacterial pathogens of mastitis in smallholder farms in Nazareth.

2. LITRATURE REVIEW

2.1. Background

The dairy sector is a large and dynamic segment of agricultural economy of many nations. In recent years there have been remarkable changes in the structure of dairy industry of developed countries. These changes include continued restructuring of the industry through reduced herd number, increased herd size and adoption of specialized management practices that encourage higher productivity. Most of these dramatic changes were the result of economic pressures produced by the high cost of labor, the rapid increase in interest rates on capital invested in dairy cattle, the large investment in facilities and equipment on a per cow basis, and the increased cost of feed (Radostits *et al*., 1994b; Reugg, 2001).

Dairy cattle are the most efficient of all farm livestock in converting feed protein and energy to human food (Reugg, 2001). As ruminant she can obtain 70% of her total feed intake from nonhuman food source such as forages and nonprotien nitrogen. This places the dairy cow in strong competitive position as the major supplier of high quality food now and in the future (Etgen and Reaves, 1978). The primary objective of production management program of dairy farm is to minimize productivity losses that are due to disease, nutritional disorders and management problems (Reugg, 2001).

The productivity of a herd or a farm is the sum of the productivity of individual cows in the farm. The total productivity of a cow is sum of the value of total milk production she produces, the value of its progeny kept as herd replacement or sold as culls, and its own value when it is eventually sold as a culled-age-cow (Radostits *et al*., 1994b; Reugg, 2001). According to Radostits *et al*. (1994b) factors affecting individual cow productivity are inheritance, age, nutrition, reproductive efficiency, lactation length, body condition score (BCS), mastitis, and other diseases. Profit is the primary goal of most dairy farms. Increased profit can be achieved by increasing income, decreasing production expenses, and increasing income and decreasing expenses concurrently. Income can be increased significantly by increasing milk production per cow, milking more cows, minimizing productivity losses and receiving higher price per unit of milk sold. Feed expense is the largest production expense on most dairy farms. Increasing milk production per cow by high quality forage can lower production expenses significantly. Feed usually accounts 45-60% of the total. Labor (10-25%), building and equipment annual expense of (10-25%), taxes, interest, supplies, livestock expenses, utilities etc accounts the remaining production expense per unit of milk produced. Careful management can decrease production expenses per unit of milk produced and production losses due to disease, nutritional disorders and management problems (Etgen and Reaves, 1978; Reugg, 2001).

Mastitis is an inflammation of the mammary glands, primarily results from invasion of pathogenic microorganisms through the teat canal results in physical, chemical, pathological, and bacteriological changes in glandular tissue and milk (Erskine, 2001; Oliveira *et al*., 2000; Radostits *et al*., 2000). Mastitis is usually classified as clinical and subclinical, based on aetio-pathological findings and observations. Clinical mastitis is further classified as peracute, acute, subacute, chronic and gangrenious mastitis. Mastitis is most often subclinical, but it can be manifested in clinical signs from mild to peracute (Radostits *et al*., 2000).

Mastitis can also be classified as contagious and environmental mastitis. Contagious pathogens spread from cow to cow during milking and it is typical by more than one quarter of the cow to be infected. The contagious mastitis organisms of primary concern in most dairies are *Streptococcus agalactiae* and *Staphylococcus aureus* that are usually the predominant intramammary pathogens in herds with identified subclinical mastitis problems. Environmental infections occur from ubiquities organisms that are commonly found in the housing environment. The predominant causative agents of environmental infections that cause clinical mastitis in the low SCC herds were coliforms and streptococcal pathogens other than *Streptococcus agalactiae* (Erskine, 2001).

Mastitis is common disease in dairy cows that causes significant losses to the dairy industry and it affects milk quality, milk production, milk composition and sanitary features (Cullor, 1990; Harmon, 1994). Mastitis is also of great nutritional and technological significance in milk processing, as valuable components like lactose; fat and casein are decreased while undesirable components like ions and enzymes are increased (Heeschen and Reichmuth, 1995).

2.2. Overview of the dairy sector in Ethiopia

Over the last decade the dairy sector in Ethiopia has shown considerable progress. Total milk production grew at an estimated rate of 3% as compared to 1.63-1.66% during the period of 1975-1992, thus ending the long-time trend of declining per capita milk production in the country (Ahmed *et al*., 2003). The progress achieved is mainly due to technological intervention, policy reforms and population growth. According to Kelay (2002) and Ahmed *et al*. (2003) the dairy sector in Ethiopia is expected to continue to grow over the next one to two decades. The large potential for dairy development in the country, the expected growth of income of the population, increased urbanization, and improved policy environment were the ones considered for the above indicated expectation. The shift towards market economy is creating large opportunity for private investment in urban and peri-urban dairying. However, the main source of growth is expected to be the growth in demand for dairy products (Ahmed *et al*., 2003).

Given the considerable potential for improving smallholder income and employment generation from high-value dairy products (Staal, 2001), development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country. Ethiopia with an average annual per capita income of less than $100 is among the poorest countries in sub-Saharan African (FAO, 2001). According to FAO (2001) there is high level of malnutrition with an estimation of about 51 percent of the population is undernourished and over two million people are considered to be chronically food insecure.

2.2.1. Dairy production systems in Ethiopia

Livestock is raised under all farming systems in Ethiopia. Pastoralists, agro-pastoralists, and crop-livestock farmers all raise livestock. Redda (2001) broadly categorized milk production systems into urban, peri-urban and rural milk production systems, based on location. Both the urban and peri-urban systems are located near or in proximity of Addis Ababa and regional towns and take the advantage of the urban markets. The urban milk system consists of 5,167 small, medium and large dairy farms producing about 35 million liters of milk annually. Of the total urban milk production, 73% is sold, 10% is left for household consumption, 9.4% goes to calves and 7.6% is processed into butter and cheese. In terms of marketing 71% of the producers sell milk directly to consumers (Redda, 2001). The peri-urban milk system includes smallholder and commercial dairy farmers in the proximity of Addis Ababa and other regional towns. The rural dairy system is part of the subsistence farming system and includes pastoralists, agropastoralist, and mixed crop-livestock producers mainly in the highland areas. The dairy production system is non-market-oriented and most of the milk produced in this system is retained for home consumption (Ahmed *et al*., 2003).

As per Gebre Wold *et al*. (2000) and Kelay (2002) major dairy production systems in Ethiopia are lowland pastoral dairy production systems, rural highland smallholder dairy production system, urban and peri-urban small and large scale dairy production systems.

Lowland pastoralist dairy farming

About 30% of the livestock population in Ethiopia is found in the pastoral areas. These areas comprise 50% of the total land area of the country and have altitudes below 1500m.a.s.l. Pastoralism is the major dairy production system in the lowland. Livestock does not provide inputs for crop production but they are the backbone of their owners by providing all of the consumable and saleable out puts and regarded as insurance against adversity. Milk production is dependent on season due to the rainfall pattern that influences feed availability (Ketema and Tsehay, 1995).

Rural highland smallholder dairy farming

There are two types of systems in the highland: traditional system that is based on indigenous breeds and market-oriented system that is based on crossbred dairy cattle (Redda, 2001). The average lactation yield for indigenous cows is 524 liters for 239 days and the average age at first calving is 53 months and the average calving interval is 25 months. The average milk yield and lactation length for crossbred dairy cows ranges from 518-1448 kg and 110-300 days, respectively, depending on the breed type. The household mainly consumes the milk produced in the traditional system while most of the milk is sold to generate income in the market-oriented system (Tesfaye, 1995).

Urban and peri-urban dairy farming

It includes small and large private and state farms in urban and peri-urban areas concentrated in the central highland plateaus (Felleke and Geda, 2001).

This sector is commercial and mainly based on the use of grade and crossbred animals that have the potential to produce 1120-2500 liters over 279-day lactation. This production system is now expanding in the highlands among mixed crop-livestock farmers, such as those found in Selale and Holetta, and serves as the major milk supplier to the urban market (Gebre Wold *et al*., 2000; Holloway *et al*., 2000).

2.2.2. The role of the dairy sector in the Ethiopian economy

At household level, dairying is important in one way or another in all the farming systems of Ethiopia. In pastoralist and the mixed crop-livestock farming system milk is the most important source of protein (Tilahun, 1995). In the mixed crop-livestock farming systems milk is also used mainly as food to the household and to a lesser extent as a source of income (FAO, 1999). In urban and peri-urban areas, dairy production is practiced mainly as a source of income. Ethiopians consume less dairy products (as per capita consumption is 17 kg per head) compared to the average 26 kg per head for Africa (Gebre Wold *et al*., 2000).

2.2.3. The prospects of dairy farming in Ethiopia

The existing excess demand for dairy products in the country, an increase in total human population and accelerated rate of urbanization with high purchasing power of urban community will characterize the Ethiopian society in the coming 25 years is expected to induce rapid growth in the dairy sector (Kelay, 2002; Ahmed *et al*., 2003). In the other hand the shift towards market economy and liberalization policies, private entrepreneurs are expected to respond to the increased demand through increased investment in dairying and milk processing. While the response of the private sector to the increased demand for dairy is expected to be significant, the small-scale household farms in the highlands seems hold most of the potential for the dairy development (Ahmed *et al*., 2003).

2.3. Husbandry practices

2.3.1. Dairy cattle housing and environmental management

Good dairy housing is important for quality milk production. A well-designed barn provides a clean and comfortable home for the herd; and a pleasant and efficient workplace for the operator. Careful planning for the storage and handling of milk, feed, bedding and manure is very important as these account for most of the labor. Creation of an environment that meets the needs of animals being housed is essential. An appropriate dairy housing husbandry system should meet the basic essential needs of cattle based on the principles of animal care (Bickert and Radostits, 2001).

Housing systems

The dairy building and equipment should provide an environment that gives cattle the opportunity to be housed, fed and milked appropriately, and to reproduce efficiently. The housing system is interrelated with almost every aspects of dairy management (Radostits *et al*., 1994b). The dairy housing system most frequently used include individual stanchion or tie-stall barns, free stall barns, loose-housing (loafing) sheds, corral or dry lot systems and pasture systems. Factors affecting choice of housing type include climate, number of cattle on a farm, changing economics, management style (milking and dry cow) and tradition (Bickert and Radostits, 2001). Housing design should provide an environment for an animal that has a positive influence on animals' health, welfare and productivity. Environmental considerations include ventilation, stalls and beds, access to feed and water and walking surface (Williams, 1992).

Stanchion or tie-stall barns: these refer to the housing in which the cows are confined together on a platform and secured at the neck by stanchion or neck chains (Bickert, 1992). It offers many advantages in the ease of animal handling, mechanized manure removal, low labor feeding and reasonably efficient in milking. Depending on the weather or season, the animals may be confined inside through out the day and night or cows may be kept outside in the pasture or paddock throughout the entire day and night. Problems in tie-stall barns include tying and untying of cows, distribution of feed and bedding and difficulty of detecting cows in heat (Radostits *et al*., 1994b).

Free stall barns: this type of barns usually consist of resting areas divided in to individual stalls with out ties on each side of a central feed bunk. Lower bedding requirement, fewer injuries to cows, more concentrated housing in terms of spatial area of building per animal are advantages of this system (Bickert and Radostits, 2001).

Loose-housing shed: this comprises keeping cows loose in an open paddock pasture throughout the day and night except at the milking time. High bedding requirement, inefficient space utilization and difficult to keep cow clean are disadvantages of the system. Loose housing uses a deep-bedded resting area plus separate feeding, holding and milking areas. Bedding requirements are very high, so it is seldom used now except where bedding is inexpensive and abundant (Radostits *et al*., 1994b).

Corral or Dry lot system: the cows are totally confined through out the year. A dirt lot is used for exercise outside. Some type of shade usually used to attempt protection from heat stress. The cows are fed in the bunk or fence line feeders, and milked in milking parlor (Bickert, 1992).

Pasture systems: there is a complete reliance on pasture as source of energy. From environmental management stand point, the only facility requirement is holding pen and milking parlor (Radostits *et al*., 1994b).

Floor

The floor surfaces in dairy barns range from old style wooden platforms to solid concrete. The floor of the barns should be built above ground level to keep out runoff water (Hurnik *et al*., 1990; Bickert and Radostits, 2001). The floor of the standing should be paved with the slope of 1 in 40 towards drain. Solid concrete or slatted floors are used in alleyways. Sand, rubber mats, and other materials are used for free-stall or tie-stall flooring. Concrete floors must have adequate texture for good footing (Bickert, 1992; Radostits *et al*., 1994b).

Concrete surface may have a texture that is too aggressive. Jagged edges, sharp points and protruding aggregate that resulted from improper finishing and texturing may be injurious to the sole of the claw. Frequent removal of manure (at least twice daily) greatly helps (enhance the foot floor-grip) to reduce the exposure of the claw horn to manure and wetness. Prolonged contact between the claw horn and manure leads to softening of the claw wall and increase the rate of abrasion (Bickert, 1992; Bickert and Radostits, 2001).

A reasonably clean, dry resilient bed on which to lie down provides the cow with relieve comfortable place to lie down for rest and to relieve stress on feet legs. Bedding materials for dairy cattle include cereal grains straw, wood shavings, sawdust, fresh topsoil, sand and shredded news papers (Radostits *et al*., 1994b).

Ventilation

Climate is a combination of several elements such as environmental temperature, humidity, precipitation, air movement and solar radiation that have great effect on animal husbandry (Bickert, 1992). In the best housing situations, environmental conditions can affect herd productivity. Weather conditions combine high ambient temperature and high humidity with out periods of cooling generally causes rise in rectal temperature, decline in dry matter intake, increase in water intake, change in milk composition and reduction in milk yield (Bickert and Radostits, 2001).

Ventilation permeates all aspects of the animal environment. Most, often ventilation is associated with respiratory health of animals. Good ventilation in the free stall area of the lactating cow helps to keep bedding dry, a factor in favor of good udder health. Good ventilation along alleys helps to keep walking surfaces dry, a condition that contributes to healthy feet. Animal health and disease are influenced by air quality. Air quality, in turn is related to ventilation and its impact on removing contaminant from the air. Excess moisture, gases and other contaminants in the air are considered to be problematic (Bickert and Radostits, 2001).

Relative humidity is the most important factor influencing pathogen survival, but the effect varies greatly between pathogens. Some survive in humid conditions, other survive best in dry air. Ventilation is truly a process of dilution. Air moved through a barn actually serves to dilute the inside air that reduces concentration of moisture, heat and air borne disease organisms (Radostits *et al*., 2000).

Access to feed and water

There are two forms of feed access developed by feed mangers and bunks. In the first form, the animal eats from a surface that is at or slightly above the floor on which stands and in the second form the bottom of the bunk substantially higher than where the animal is standing. The first type is associated with open-dry-lot systems in warmer climates while the second one is the choice of loose housing developed in colder climates. Suggested dimension for post and rail feeding manager fence will depend on age and body weight of the cow (Gerloff, 2001).

2.3.2. Nutritional management

Dairy farm feeding programs have direct effects on production and growth and set the stage future productive potentials. Small changes in feeding program may bring about large changes in productivity, health, feed costs, labor allocation and income (Radostits *et al*., 1994b). In most dairy herd, nutritional management is the most important determinant of dairy productivity. The relation between nutrition and productivity begins at birth (Reugg, 2001).

Feeding system of dairy cattle

There are several systems for feeding of dairy cattle with its' own advantages and disadvantages, but with excellent management any system can be effective. The challenge is to find systems that are effective and efficient for the management level on the farm (Gerloff, 2001).

Radostits *et al*. (1994b) and Gerloff (2001) classified the feeding systems in to pasture, stanchion and tie-stall barns, milking parlor grain feeding and free-stall barns.

Pasture: pasture based feeding system can generate excellent profit per cow, and in many cases are much less capital intensive than other systems. Pasture based systems usually require less labor, less manure management, and produce less milk per cow (Gerloff, 2001).

Stanchion and tie-stall barns: cows may be fed in their entire ration in the stanchions or may consume a variable amount of feed (particularly forages) free choice from separate feed bunks during exercise or turnout periods. It offers the advantage of allowing individual cow care and feeding, but requires more labor per cow than free-stall systems (Radostits *et al*., 1994b).

Milking parlor grain feeding: a once popular approach is to feeding grain to dairy cows was to provide grain feeders in milking parlor. These individual feeders could feed each cow to her individual grain ration to match her production. The disadvantage include high producing cows may simply not have enough time to eat their allotted grain during milking and timing of grain feeding may not match forage intake leading a twice risk of rumen acidosis (Gerloff, 2001).

Free-stall barns: cows are fed in bunks, with a variety of systems for feeding grains. The following describes typical feeding systems found in free stall operations (Radostits *et al*., 1994b; Gerloff, 2001).

Bunk forages with top-dressed grain: forages in the bunks, with the grain or concentrate portion (including trace minerals and vitamins) placed in the top of the forage. Hay might be fed separately in the racks and cows are commonly grouped by production so that low-producing cows can be fed less concentrate top dressed on their forages (Radostits *et al*., 1994b; Gerloff, 2001).

Bunk forage individual cow grain feeding: forages feed in the bunks. Part of the grain might be fed top dressed on the forage, with additional grain fed in relation to production in the milking parlor. Another method of individual feeding is by transponder, magnetic key, or computer feeders. Each of these systems attempts to allow access to extra concentrate for high-producing cows while denying access to others (Etgen and Reaves, 1978).

Blended ration and total mixed ration: in blended ration, the forage and concentrate portion of the ration are first mixed together and then delivered to the bunk to be eaten. They can be fed both in the free-stall and in stanchion barn system. Some total mixed rations include all portions of the ration, including the hay, whereas others blend the silages, concentrate, and byproduct feeds for bunk while feeding hay separately (Radostits *et al*., 1994b).

Feeds for dairy cattle

There are several classes of feeds tuffs fed to dairy cattle: forages, concentrates, byproducts, minerals vitamins and additives.

Forages: typically make up the majority of dairy cows diet. The majority of forages are hay, silage and haylages, green chop and pasture. Hay is a dried grass or legume (eg. alfalfa, clover) by drying to about 90% dry matters that provide storage for long periods. The quality of the hay depends significantly on storage, drying and stage of maturity during harvest. Silages are forages with somewhat higher water concentration than hays (25% -50% dry matters). Corn silage is an excellent dairy feed and must be supplemented with protein and minerals to balance ration for dairy cows (McCullough, 1989).

Concentrates: concentrates by nature are the part of the plant that has concentrated the most nutrients, typically the seed of the plant. There are two broad groups of concentrates: energy sources (grains) and protein sources (usually legumes or oil seeds) (Etgen and Reaves, 1978).

Grains: the major role of the grains is to provide energy in the diet. The major type of grains are corn, barely and wheat (Etgen and Reaves, 1978).

Protein supplements: are concentrated source of protein or nitrogen. They are derived from the four major sources: oil seeds (eg. Soyabeans, peanuts, sun flower seeds, flax, cotton seed), grain processing and fermentation byproducts (eg. distiller dried grains), animal products (eg meat and bone meal, Blood meal, fish meal and feather meal) and none protein nitrogen sources (eg urea) (Gerloff, 2001).

Water: is essential nutrient, although it is commonly over looked. Cows consume more than any other nutrient. The simplest and most effective approach to satisfying the cow water needs is to provide easy non-competitive free choice access to clean fresh water all the time (Beede, 1991).

2.3.3. Dairy cattle breeding systems

The present livestock breeds in the world are the results of both natural selection and human intervention. Natural selection operates to provide reproductive ability and continuity of the fittest while human intervention is to change one or more traits of animals that the owner thinks desirable under his environment. The genetic variations available both within breeds and between breeds, have been used to accommodate interests and farmers wishes to make livestock more efficient in using available resources to produce human food and other agricultural products (Rendel, 1991; Sendros and Tesfaye, 1997).

The two commonly used dairy cattle breeding methods are selection within breeds (pure breeding) and mating parents of two or more different breeds together (cross breeding). The goal of pure breeding is to build additive genetic gains into the population by identifying individual superior genotypes and preferentially multiplying them in the next generation (Payne and Hodges, 1997). The basic objective of crossbreeding of high performing cattle breeds with tropical breeds is to produce crossbreds with an expected additive genetic merit being the mean of both parental breeds. The non-additive effect, which is observed as heterosis or hybrid vigor, is the amount by which merit in crossbreds deviate from the additive component and is fully exploited only when non-related breeds are crossed (Kinghorn, 2000). The use of temperate dairy breeds for crossbreeding with indigenous breeds has been widely accepted and aims at combining the superior performance of specialized dairy breeds with the superior adaptability of the local stock (Peters, 1991). According to Cunningham (1991) the crosses between the temperate dairy cattle breeds and local cattle in the tropics combined the milk producing ability of the temperate breeds with the climatic adaptability of the tropical breeds.

Dairy cows are mammals and produce milk only after giving birth. Reproductive management of a dairy herd is central to a dairy farm’s over all success in producing milk. Because milk is the major source of income for dairy farm, the general goal of dairy farms is for cows to produce a maximum amount of milk per day of life at a minimum feed cost. To do so cows should calve at regular intervals and therefore must breed and become pregnant within a given period after calving (Radostitis, 2001).

Breeding systems use in the vast majority of commercial dairies, may be classified as exclusively an artificial insemination (AI), exclusively bull bred system and an AI–bull hybrid system (Farin and Sleenning, 2001).

AI: AI is an essential technique in breeding programs with progeny testing. AI provides the opportunity to choose sires that are proven to transmit desirable traits to the next generation and minimizes the risk of spreading sexually transmitted diseases and genetic defects (Wattiaux, 1998). Zumbach and Peters (2000) estimated that 1820 cows can be inseminated by a bull using deep frozen semen. For dairies opt to breed solely through genetic artificial means, the advantages are primarily gained through genetic progress and from not having bulls on site. AI is not free of problem. First and for most, AI depends on human to determine when a cow is receptive (estrus detection) and to deliver live semen to the cow during the short period in which the cow can conceive (breeding technique and timing) (Etgen and Reaves, 1978). The technical constraints are poor heat detection skills, communication and transport problems that hamper timely insemination, poor semen collection and storage technology and handling procedures that affect semen quality, and inefficiency of AI technicians. System related problems include small herds, dispersed locations, limited production intensity and unaffordable cost (FAO, 2000).

Bull breeding: The use of bulls for natural service remains widespread even in areas where artificial insemination has proven to be very efficient. Many farmers believe that pregnancy rates are higher when a bull is used. The use of natural service may be indicated when personnel are inefficient to perform the tasks associated with heat detection and the techniques of AI, when long term genetic gain is of minor importance and when local conditions do not provide the infrastructure necessary for successful AI (Wattiaux, 1998). The advantage for bull breeding primarily relate to the bulls ability to accurately detect cows in estrus better than any human based system and deposit semen at the most appropriate time in a cows estrous cycle. Bull bred herds have particular problems such as they do not record the breeding they accomplished and can spread venereal disease such as campylobacteriosis and tricichomoniasis (Farin and Sleenning, 2001).

AI/Bull-breeding hybrid system: performed by large dairy herds. Cows are usually exposed to AI based breeding during early lactation and then they are brought to “a clean up” bull pen which gives advantages of both the positive qualities of both AI and bull breeding (Farin and Sleenning, 2001).

2.3.4. Health and production management

Considerable variations exist in the approaches of dairy health and production management. The health management program should assure the optimum care and well-being of the dairy animals and therefore reduce losses in productivity caused by diseases and management errors. The veterinarian delivering health management program is expected to fill variety of rolls depending on the interest, expertise and availability of other local experts. Veterinarians are the well-regarded sources of information of health care decisions. Although health management program promoted for a number of years, the primary use of veterinarian by dairy producers years constitutes as individual animal health care and emergency service (Reugg, 2001).

The most common aspect of any herd health program is the regularly scheduled visit to the herd or flock. The frequency of visits depends on the class of livestock, the size of the herd, the prevalence of the disease, the existence of particular disease problem, the level of risk aversion of the producer and the length of time the herd has been on a program. The management information system that includes production, health and financial records, is the foundation of all food animal production oriented health management program (Reugg, 2001).

Specific health and management tasks include analysis of milk production data, reproductive performance, udder health, calf health management and replacement program, nutritional management, environmental management, genetic progress and culling and dairy cow quality, disease control and residue avoidance (Radostits *et al*., 1994b).

Udder health

Individual cows can be marked for drying off, treatment during the dry period, or culling on the basis of drying off examination by CMT or culture or, more commonly, on the basis of a summation of individual cow milk somatic cell count (SCC) during the lactation (Reugg, 2001).

The dry period is recognized as an important portion of the over all management of dairy cattle and is an important component of herd productivity. Most successful dairy producers recognize that the dry period is not the end of lactation cycle but the beginning of the next lactation. Risk factors for most postpartum diseases of dairy cows occur during the dry period with clinical signs of diseases becoming evident after calving (Radostits *et al*., 1994b).

The length of the dry period has influence on milk yield in the subsequent lactation. When milk yield ceases, non-secreted milk absorbed and under secretary cells are rapidly lost this process (termed, "involution") is usually complete within about a 2-week period after milking is terminated. The recommended dry period is 6-8 weeks. Dry periods that are too large often lead to excessive weight gain and reduce production efficiency. Cows should be dried of by abrupt termination of milking followed by appropriate intramammary treatment (Reugg, 2001).

Genetic progress and culling

Selection is the major tool in establishing the genetic progress to meet national production requirements of milk production. The use of AI with progeny tested sires is now second nature to the dairy industry. The primary objective of genetic improvement program is to increase the total economic merit of the dairy cow (Radostits *et al*., 1994b).

Culling describes the removal of a cow from a herd because of sale, slaughter or death. Reasons of culling classified to major categories of voluntary and involuntary. Voluntary reasons include dairy sales due to excess animals in herd in the absences of know disease and low production. The involuntary one includes reproduction problem mastitis, udder problem, disease, injury, death, feet and leg problems temperament and other miscellaneous cases (Radke and Lloyd, 2000). Examples of cows in involuntary cull include those with egg fractures, diseases such as brucellosis and death (Reugg, 2001).

Economic and biological culling is another alternative way of categorizing culling reasons. All living animals culled from dairy herds are removed for economic reason, but the disposal of diseased cows is typically considered to be forced replacement (Dijkhuizen *et al*., 1997). A repeat breeder cow is culled due to reproductive failure; on the assumption that continued attempts to achieve pregnancy will lead to an extended time on late lactation or dry period. Biological culls are defined as cows incapable of any reasonable level of production, so the producers have no sensible choice but to cull animal (Gröhn *et al*., 1998). Reproduction is the primary reason given for why cows are culled in a modern industry (Bascom and Young, 1998).

Disease control

A large number of infectious diseases affect dairy cows and their control must be considered. Infectious diseases are important for a number of different but related reasons such as loss of productivity, epidemics of disease, diagnosis, principles of control, potential drug residues, antimicrobial resistance, zoonoses, problems with importation and exportation (Radostits, 2001).

Infectious diseases commonly cause loss of productivity that generally leads to major economic losses. Some diseases have been controlled by vaccination or eliminated by eradication schemes (Radostits, 2001). Educating dairy owners about the importance of various risk factors associated with infectious disease and the strategies for control is a challenging responsibility for the veterinarian (Williamson *et al*., 1988).

Antimicrobials are used in dairy cattle production, primarily to treat or prevent disease and to a lesser extent to increase milk production or improve feed efficiency. The use of antimicrobials therapy to treat and prevent udder infections in cows is a key component of mastitis control in many countries. Due to the widespread use of antimicrobial for treatment of mastitis in dairy cows, much effort and concern has been directed towards the proper management and monitoring of antimicrobials used in such treatment in order to prevent milk drug residue. However, widespread use of antimicrobials has created potential residue problems in dairy products to be consumed by the general public. Because of the public health significance, meat and milk contaminated with antibiotics are considered unfit for human consumption (Hillerton *et al*., 1999).

There is a concern that the use of subthrapeutic levels of antimicrobials results developmet of antibiotic resistant bacteria that are then transferred to humans where they cause diseases that are consequently difficult to treat (Holmberg *et al*., 1984; Hinton *et al*., 1986; Spika *et al*., 1987).

2.4. Mastitis

2.4.1. Definition and terminology

Mastitis: is inflammation of the mammary gland and primarily results from invasion of pathogenic microorganisms through the teat canal. An intra-mammary infection (IMI) will occur if the invading pathogen causes an inflammatory response and loss of potential milk production in the affected quarter of the gland (Erskine, 2001).

Mastitis is characterized by physical, chemical, and usually bacteriological changes in the glandular tissue. The most important changes in the milk include discoloration, the presence of clot and the presence of large number of cells (Blood *et al*., 1983). Mastitis can occur with wide range of clinical signs, from subclinical to severe, which depends on the virulence of pathogen and the extent of response of the host immune system (Radostits *et al*., 1994a).

Subclinical mastitis is the presence of pathogenic organisms in the milk, and inflammatory response that can only be detected by screening tests or laboratory procedures. The signs of inflammation that are detected by visual observations or palpation such as swelling, heat, redness, pain or systemic responses such as fever are not observed (Erskine, 2001).

Clinical mastitis refers to the condition where the cow's immune system responds with enough intensity to an IMI to elicit signs of inflammation that are physically observable. The nearly universal observation of clinical mastitis is abnormal color or texture of milk. As the degree of local inflammation increases, swelling, discoloration, and pain may become evident (Radostits *et al*., 2000). Contagious mastitis occurs with organisms spread from cow to cow. Environmental infections occur from ubiquities organisms commonly present in the housing environment (Erskine, 2001).

2.4.2. Effects on milk quality

Good quality milk production is one of the main objectives in, either small or large-scale dairy farms. This is because milk of good quality is desirable and hence saleable to the processors and acceptable by the consumers. Good quality milk and milk products is wholesome and of good appearance, has good predictable taste and flavor, maintains original nutritional qualities, safe from harmful organisms and substances and has a long shelf-life. To produce good quality milk, the producers must be certain that milk comes out not only from the disease free animals but also from healthy udders. Unhealthy udders, which are mostly attributable to mastitis, regardless of the cause, definitely produce bad quality milk composition or bacterial contamination (Thirapatsakun, 1999).

2.4.3. Economics of mastitis

The dairy cow is the most efficient converter of forage to food for humans, and milk is one of the least expensive major sources of animal protein. Unfortunately, the enormous investment in providing this important set of healthful products from the dairy industry is inadequately protected against the devastating inroads of mammary gland disease (Thirapatsakun, 1999).

Although mastitis occurs sporadically in all species, it assumes major economic importance only in dairy cattle. In terms of economic loss it is undoubtedly the disease with which the dairy industry has to contend. The loss is occasioned much less by fatalities than from the reduction in milk production from the affected quarters (Radostits *et al*., 2000). The largest proportion of the losses results from a direct drop in milk revenues, the non–marketable milk contaminated with antibiotics, decreased milk production which invariably accompanies the infection and premature culling of the animal in many instances. Costs can be divided into those where there is a decrease in revenue and those where there is an increase in outlay (Thirapatsakun, 1999).

2.4.4. Contagious and environmental Mastitis

Contagious mastitis

Infection is spread on to the teats during milking practice with organisms transferred from one cow to another cow. The most important contagious organisms are *Staphylococcus aureus* and *Streptococcal aglactiae*. *Corynebacterioum bovis* is also common in many dairy herds. *Mycoplasma bovis* is highly contagious, but it is very rare. The primary reservoirs for organism are infected quarters. *Staphylococcus aureus* readily colonizes teat skin and teat canal, and has also been isolated from the udders of heifers that have never calved (Radostits *et al*., 2000).

Environmental mastitis

Infection is spread on to the teats between milkings with organisms being transferred up into the udder during the milking process itself. If cows go and lie down in contaminated cubicles or straw yards immediately after being milked, organisms can also penetrate into the udder and infections can result (Radostits *et al*., 1994a).

Environmental infections occur from ubiquities organisms commonly present in the housing environment, particularly numerous in the beddings and manure. Environmental mastitis is of considerable significance to today's dairy farmer. If herds continue to expand slowly using existing accommodation, the level of exposure to organisms will increase. *Streptococcus uberis* is the bacteria causing the majority of problems, due to its resistance to treatment and as chronic infections result in high cell count animals (Erskine, 2001). The predominant causes of clinical mastitis cases in low SCC herds were coliform and streptococcal pathogens other than *Streptococcus agalactiae* (Erskine, 2001).

2.5. Risk Factors of mastitis

2.5.1. Environmental factors

Season: most mastitis cases occur during the summer months. This is particularly true in housed cattle and commonly caused by environmental infections; especially if the season is wet (Radostits *et al*., 1994a). Seasonal difference determines the prevalence of individual bacteria, as an example Streptococcus species is common in all seasons except in winter while *Staphylococcus aureus* is seen through out the year (Erskine, 2001).

Milking practices: this includes efficiency of milking personnel, milking machine, too high milking speed and hygiene in the milking parlour (Radostits *et al*., 1994a).

Management system: if hygiene and bedding maintenance are neglected in the housing accommodation the prevalence of environmental forms of mastitis can be disastrous (Radostits *et al*., 2000).

2.5.2. Pathogenic factors

Bacterial viability: each species of bacteria differ in its ability to survive in the cow's immediate environment. That is its resistance to environmental influence including cleaning and disinfections procedures (Radostits *et al*., 2000).

Colonizing ability: refers to the ability of the organism to colonize the teat duct, then adhere to mammary epithelium and set up mastitis reaction (Radostits *et al*., 1994a).

Susceptibility to antibiotic: inherent or acquired resistance to antibiotic therapy is a potent reason why some mastitis control programs are unsuccessful (Erskine, 2001).

Prevalence of infection: the greater prevalence of the disease in the herd, the greater new infection rate (Radostits *et al*., 1994a).

2.5.3. Host factor

Host characteristics are important risk determinants in the pathogenesis of mastitis (Grommers, 1988). These factors are associated with the development of specific immunity and with non-specific host defense mechanisms (general resistance) (Erskine, 2001).

Genetic resistance: heritability of SCC allows for selection against susceptibility to mastitis. It is worth to mention here that selection for milk yield has been accompanied by increased susceptibility to disease (Shook, 1989).

Physical characteristics, teat lesions, and leaking teat: anatomical characteristics of the teat and udder affect general resistance to mastitis. Leaking of milk between milkings has been associated with risk of clinical mastitis (Schukken *et al*., 1989; Erskine, 2001).

Stage of lactation: it is a determinant for both clinical and subclinical mastitis. Mastitis caused by environmental organisms, such as coliform, is most common in first few days after calving, and occurs less as lactation progress, while *Actinomyces pyogens* commonest in dry cows (Erb *et al*., 1988; Erskine *et al*., 1988; Schukken *et al*., 1989).

Parity: increasing parity is risk factor for increased incidence of clinical mastitis as well as increased prevalence of subclinical infections (Dohoo and Martin, 1984; Schukken *et al*., 1989).

3. MATERIAL AND METHODS

3.1. Study area

The study was conducted in Nazereth (East Shoa) located in Oromia Region, Central Ethiopia from September 2006-June 2007. Nazereth is found in the Rift Valley about 95 Km southeast of Addis Ababa (39.17^{o}N and 8.33^{o}E) with an altitude of 1622m.a.s.l. Its annual temperature ranges from 13.9 to 27 ^{o}C. Livestock population is estimated to be 70,622 bovine, 36,142 ovine, 42,968 caprine, 3,193 equine, 42 camels and 193,155 poultry (Adama District Agriculture Office, 2003).

3.2. Study population

The target population for the study was all market-oriented smallholder dairy farms and their cows in Nazareth.

The sample size was determined with expected mastitis prevalence of 13.8% (reported in Debre Zeit by Mekonnen *et al*., 2006) at 95% confidence interval and 5% precision level. Between clusters variance was calculated from mastitis prevalence of 11.6% and 13.8% reported in Debre Zeit by Mekonnen *et al*. (2000) and (2006) respectively. According to Thrustfield (1995) one stage cluster sampling method with predicted number of animals per cluster two is used to determine number of clusters to be sampled.

$$g = \frac{1.96^2 (nVc + Pexp (1- Pexp)}{nd^2}$$

where

g = number of clusters to be sampled (households)

n = predicted number of animals per cluster

Pexp = expected prevalence

d = desired absolute precision

Vc = between cluster variance

Table 1. Variables used to calculate herd and animal sample size

Site	Prevalence	Mean prevalence	Standard deviation	Between cluster variance	Desired absolute precision	Predicted number of animals per cluster	Number of clusters to be sampled
Debre Zeit by Mekonnen *et al*. (2000)	11.6%	12.6%	0.105	0..011	0.05	2	102
Debre Zeit by Mekonnen *et al*. (2006)	13.8%						

Accordingly the total number of market-oriented computed was 102. The list of market-oriented smallholder farmers were taken from the Adama District Agriculture Office and considered as sampling frame. Then 102 smallholder dairy farms were randomly selected by using the random table number. Only 95 farms were investigated under study the rest 4 farms were unfunctional and 3 farms were not agreeable for the study.

3.3. Study design

3.3.1. Data collection

Questionnaire survey

In each farm questionnaire survey was carried out by personal interview to determine the demographic characteristics of farm owners and management practice (husbandry practices). In demographic characteristics of the farm owners' information collected were sex, age, level of education and activities of farm owners, duration of farming and work force. Information including age, parity, stage of lactation, previous history of mastitis, average daily milk production, leaking teat and antibiotic therapy of individual lactating cows was also collected as cow data.

Regarding husbandry practices the information included were types of breeding systems, feeding systems, health management practices (include treatment of animals when sick, pregnancy diagnosis, vaccination for different diseases and deworming practice), record keeping, and commonly encountered health problems. Information on milking practice encompassed activities conducted during milking including type of milking, activities before milking (such as concentrate provision, introducing calf, washing hands, application of lubricant, drying after washing), frequency of milking, stage of milking of mastitic cow and dry cow practice (dry cow therapy, dry off style and dry of time).

Farm inspection

During farm visit observational assessments were made on milking hygiene practice, feed storage, farm cleanness, and housing systems. The housing conditions were detailed examined in respect to type of house, type of floor, roofing material, drainage/slope of the stable area, space, ventilation, water and feed trough space to the animal and feed and water trough cleanness (Annex 2).

Cow examination

Animals were clinically examined for feet and legs, udder and teats, and general health conditions. Body condition scorings were also conducted. The feet and legs were examined for hoof overgrowth, lameness, abrasions and others. The udder were examined for blind teats; lesions; and indicators of clinical mastitis such as evidence of pain, swelling, hotness and changes in milk including presence of clot, change of color and consistency.

3.3.2. California Mastitis Test (CMT)

CMT was performed for each quarter of a lactating cow. CMT was used to determine the prevalence of subclinical mastitis and also as screening for selection of samples to be cultured for the cows under study. A small sample of milk (approximately ½ teaspoon) from each quarter is collected into a plastic paddle that has 4 shallow cups marked RR, RF, LF and LR. An equal amount of CMT reagent was added to the milk. The paddle was gently rotated to mix the contents. The CMT results were interpreted (Annex 3) as negative (0), trace (1+), positive (2+) and strong positive (3 +) as per the recommendations given by Quinn *et al.* (1994).

3.3.3. Milk sample collection

Strict aseptic procedures were used (NMC, 1999; Queen *et al.*, 1999) when collecting milk samples in order to prevent contamination with the many microorganisms present on the skin of cow's flanks, udder and teats, on the hands of the sampler, and in the barn environment.

According to NMC (1999) the following procedures were used to reduce contamination during sample collection.

- Equipment: Sterile universal bottle with tight fitting screw caps were used.
- Time of Sample Collection: Samples for culture were collected before milking, after milking or in the inter-milking interval usually between 3-5 hours after milking.
- Preparing Udders and Teats: Udder and teats were cleaned and dried before sample collection was attempted. First few streams of milk were removed and discarded to reduce the number of contaminating bacteria in the teat canal. The teats were washed with soap and water and disinfected with 70% ethanol.
- Collecting Samples: To reduce contamination of the teat ends during sample collection; the rear teats were sampled first, then the far ones. The cap was removed from the sample vial without touching its inner surface; was then handled in such a way that the inner surface faces down wards. The vial was held as near the horizontal as possible, and by turning the teat to a near horizontal position, the streams of milk were directed into the vial. Precaution was taken not to touch the teat end with the cap or the vial opening.
- Handling and Storing Samples: After samples have been collected they were held in an icebox. In the laboratory, samples were cultured immediately or stored at 4-5°C.

3.3.4. Isolation and identification of pathogenic bacteria

Isolation and identification of pathogenic bacteria was conducted in the microbiology laboratory of the Faculty of Veterinary Medicine, Debre Zeit. For frozen quarter milk samples, the samples were thawed at room temperature and were mixed with vortex shaker. The bacteriological culture was performed following the standard microbiological technique (Quinn *et al*., 1999) and Microbiological Procedures for the Diagnosis of Bovine Udder Infection (National Mastitis Council, 1990). A loopful of milk streaked on 5% sheep Blood agar for primary culture. Blood agar and MacConkey agar were used for subculture of different colonies to detect and isolate any Gram-negative bacteria that were able to grow in on the medium. Edwards Blood medium that were highly selective for streptococci and also act as an indicator medium for hemolysis, were used for the isolation of catalase negative streptococci. Mannitol salt agar was used for isolation of catalase positive staphylococci. The plates were incubated aerobically at $37^{o}C$ for 24, 48 and 72 hours to rule out slow growing bacteria such as *Cornybacterium* species. The plates were examined for growth, morphological features of the colonies and hemolytic characteristics. Accordig to NMC (1999) the following assumptions were considered for the results. A sample was considered positive if one of the known mastitis causing pathogenic bacteria were isolated. A sample was considered negative if there was no growth after 48 hours or 78 hours. A sample was considered contaminated if there was profuse growth of environmental bacteria. Characterizations of isolated mastitis causing bacteria were done by different methods such as; cell shape, cell grouping, Gram stain, O-F test, catalase, oxidase, motility, sugar test, urease test, and IMVC tests.

3.3.5. Data storage and analysis

The data collected through questionnaire survey, farm inspection, animal examination, CMT and pathogenic bacterial isolation results were entered into the database management software micro-soft-Excel computer program (Version 6.0, 2000) and analyzed using STATA (7.0, 2001), and SPSS (SPSS release 11.5, 2002) statistical computer soft ware programs.

4. RESULT

4.1. Descriptive statistics

4.1.1. Demographic characteristic

The general information of dairy farm owners in Nazareth district is presented in Table 2. From the study 58.9% smallholder owners were female. The age of farm owners were between 24 to 70 years. The majority proportion (68.4%) was between 31 and 50 years.

Table 2. Demographic characteristics with mastitis prevalence and average milk yield of 95 smallholder farms in Nazareth

Factor and category	No of Herds	%	No of Cows	%	Mastitis Prevalence (%)	Milk yield/cow/day (l)
Farm owners						
Male	39	41.1	95	40.6	53.4	9.84
Female	56	58.9	139	59.4	51.8	9.73
Farm owners education						
Illiterate	23	24.2	54	23.1	74.2	9.29
Elementary	22	23.2	40	17.1	57.6	9.77
High school and above	50	52.6	140	59.8	47.9	9.77
Farm owners activity						
Only dairy farming	24	25.3	65	27.8	41.6	9.90
Dairying plus other activities	71	74.7	169	72.2	58.0	9.68
Duration of farming						
≤ 4 years	18	20	27	11.5	64.3	9.89
5-8 years	46	51.1	113	48.3	40.7	10.30
≥ 9 years	26	28.9	79	33.8	42.6	9.07
Work force						
Hired labor	18	19.0	53	22.7	43.6	9.48
Family member	77	81.0	181	72.3	55.1	10.25

It was observed that 25.3% of the owners had taken dairying as a main business and the rest as side business. The educational level of the respondents ranged from illiterate to those with university education. The highest percentage (52.6%) of the smallholder owners had higher secondary level education and above. Out of 95 farms in the study 5 farms were not known when farming practice was started, so only 90 farms were included in the analysis of duration of farming. 71.1% of the farms were at operation for past 8 years. In most (77.9%) of the farms the sources of animal were private smallholder dairy farms in Nazareth and the rest were private dairy farms outside Nazareth and Government dairy farms from Arsi. Farms that had hired labor for all farm activities and contract laborer for milking activities were 19% and 39% respectively.

4.1.2. Husbandry practice

Housing management: Most of the dairy farms had permanent housing system for their animals. Table 3 shows a summary of housing situation. 83.2% of the farms provide half or full building and 87.4% of the farms used corrugated metallic sheet to the roof. On the basis of floor type, 62.1% of farmhouse was found with concrete. Besides the type of floor 30.6% of the farms had good drainage/slope for stables (floor). The cleanness of the farms was good, satisfactory and poor in 22.1%, 56.8% and 21.1% respectively. The space and ventilation were good in most of the farms.

Feeding management: There were two systems of feeding, which are practiced by the dairy owners to feed their cattle. Stall-feeding was practiced in almost all farms in which the farms totally depend on purchased fodder and only two farms followed both stall and grazing system. Summary of feeding management were shown in table 3. The space between the feeding trough and animal were poor in 80% of the farms and good and satisfactory in the rest 20%. The feeding and watering trough cleanness were good in 27.4%, satisfactory in 63.2% and poor in 9.5% farms. Most of the farms store concentrates in well-managed situation than forages; generally 67.4% of the farms had good storage for concentrate and hay.

Table 3. Variables inspected the housing condition at the farm levels with prevalence of mastitis and average milk yield

Factor and category	No of Herds	%	No of Cows	%	Mastitis Prevalence (%)	Milk Yield / cow/day (l)
Housing type						
Closed	37	39	85	36.3	44.2	9.94
Semi-closed	42	44.2	97	41.5	50.9	9.85
Open	16	16.8	52	22.2	45.8	9.39
Floor						
Concrete	59	62.1	148	63.2	39.4	9.91
Stone layer	24	25.3	45	19.2	56.8	9.78
Soil	12	12.6	41	17.6	38.2	9.21
Drainage/slope of the floor						
Good	29	30.6	66	28.2	40.1	10.23
Satisfactory	33	34.7	81	34.6	41.2	9.82
Poor	33	34.7	87	37.2	58.1	8.96
Farm cleanness						
Good	21	22.1	44	18.8	42.9	
Satisfactory	54	56.8	138	59.0	41.2	
Poor	20	21.1	45	19.2	42.0	
Hay and concentrate storage						
Good	64	67.4	162	69.2		
Satisfactory	28	29.5	64	27.4		
Poor	3	3.1	8	3.4		

Breeding, health and record management: Almost all farms practiced AI. 64.2% of farms used AI as the only means of breeding, 33.7% farms were using both natural mating and AI and only two farms were practicing natural mating as only means of breeding. Health services were provided in most of the farms by professionals when they were called. In most of the farms there was neither regular vaccination nor deworming practice. Eventhough there was no well-managed record keeping system 39% of the farms had some sort of breeding records.

Milking and dry cow practice: summary of milking and dry cow practice presented in table 4. All farms practiced hand milking twice per day except two farms where they experienced three times per day. Only 5.3% of the farms practice milking of cows in separate place.

Different farms had different stages of milking of mastitic cow. From 95 farms 24 farms were not included in the analysis either having single animal or absence of previous clinical mastitis problem in the farm. Generally from 71 farms the highest percentage (56.3%) practiced milking of clinical mastitic cow at first and any stage. All the farms were not practicing dry cow therapy. In 88.4% of the farms drying of cows were performed two or more months before subsequent calving. In 90.5% of the farms dry off style were gradual.

Table 4. Variables inspected or measured at farm and animal levels in 95 smallholder farms

Farm level			Animal level		
Factor and category	No of Herds	%	Factor and category	No of Cows	%
Place of milking			Breed		
Barn	90	94.7	Local	13	6.3
Other place	5	5.3	Crossbred	193	93.7
Frequency of milking			General health		
Two times	93	97.9	Satisfactory	197	95.6
Three times	2	2.1	Not satisfactory	9	4.4
Stage of mastitic cow milking			Feet and leg problem		
First	11	15.4	Satisfactory	194	94.2
Last	31	43.7	Not satisfactory	12	5.8
Any stage	29	40.9	PMP		
Dry off time			Yes	80	39
Between 1&2 months	9	11.6	No	126	61
2 or more months before	82	88.4	Blind teats		
Dry off style			Yes	16	7.8
Abrupt	7	9.5	No	190	92.2
Gradual	83	90.5	Udder		
			Satisfactory	182	88
			Not satisfactory	24	12

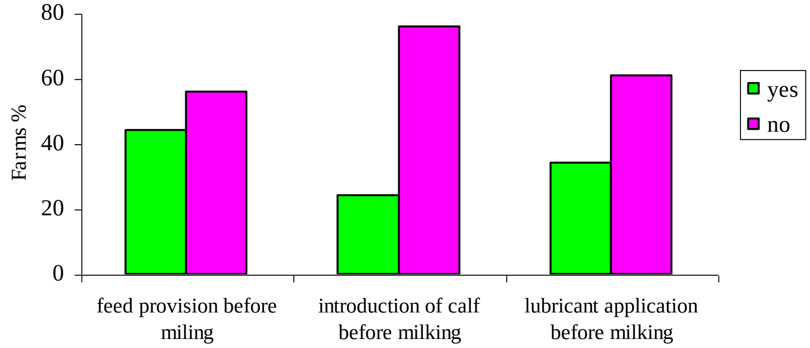


Figure 1. Activities applied by milkers in smallholders in Nazareth

Activities applied before milking were shown in figure 1. 24.2% of the farms were introduced calf to the dam and 35.8% were applied lubricants before milking. 44.2% of the farms provide feed usually concentrates before milking. All farms wash udder or teats with either cold or warm water and neither of the farms were used towel to dry the teat ends after washing.

Table 5. Major management and disease constraints as rated by smallholder dairy owners

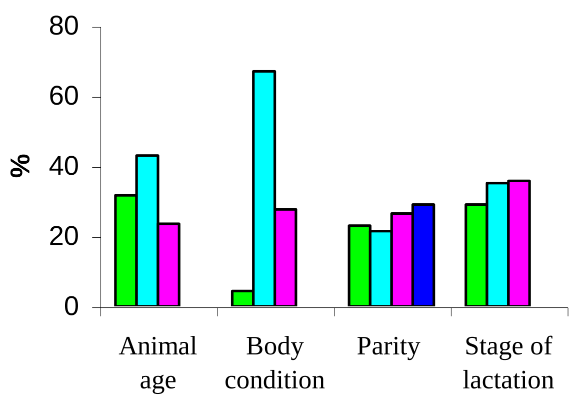
Factor and category	1(%)	2(%)	3(%)
Mastitis	35(43.21)	12(15.19)	7(10.61)
Repeat breeding	25(30.860	32(40.51)	8(12.12)
Systemic diseases	17(20.99)	21(26.58)	19(28.79)
Calf disease	2(2.467)	3(3.80)	9(13.64)
Feet and leg problem	-	4(5.06)	7(10.61)
Others	2(2.47)	7(8.86)	16(24.24)
Total	81(100)	79(100)	69(100)

Major problems: the major health problems encountered in dairy cows as per questionnaire survey were presented in table 5. The study noted that most encountered health problem was mastitis that was indicated as primary disease by 43.21% respondents followed by repeat breeding, systemic disease, calf disease and other health problems. Other health problems included in the study were respiratory problem, metabolic problem, dystocia, abortion, ectoparsite, bovine tuberculosis and meteritis.

4.1.3. Cow data

The dairy farms under study area consisted of different types of cattle, the percentage of which is present in figure 2. It was observed that the percentages of indigenous and crossbred cows were 6.3% and 93.7% respectively.

In 95 farms there were 497 animals consisting of 234 cows (206 were lactating), 173 up to 1-year calves (89 female and 84 male), 75 heifers and 5 breeding bulls. The average farm size was 5.23 (minimum 1 and maximum19) with average breeding cows 2.46 (minimum 1 and maximum 8). The age of breeding cows varied from 2 to15 years. The mean age of cows, parity number, body condition score and milk yield per day were 6.77, 2.99, 3.48 and 9.77 l respectively.



Legend	Animal age (Years)	BCS	Parity	Stage of Lactation (Months)
	2.5-5	≤ 2	1	1-3
	6-8	3	2	4-6
	≥ 9	4	3	>7
			≥ 4	

Figure 2. Variables measured or inspected at animal level for lactating cows

Summary of variables measured for individual lactating cow were presented in table 4 and figure 2. Physical examination of the cow revealed that 6.8% of the cows with feet and leg problem, 16.9% cows with udder problem and the rest 4.1% had other health problem including two cows having feet and leg problem and single animal that had both udder and feet and leg problem. From the total cows inspected 68.8% of cows had BCS of three. From the total lactating cows 36.2% of cows had previous history of clinical mastitis problem, 8.1% leaking teat and 7.2% had at least one blind teats.

4.2. Prevalence of mastitis

Table 6. Prevalence of mastitis

	Prevalence (%)		
	Clinical mastitis	Subclinical mastitis	overall
Farm level	13.7	61.1	62.1
Cow level	6.3	41.7	48.0
Quarter level	2.4	22.3	27.1

A total of 206 lactating cows from 95 market-oriented private smallholder dairy farms in Nazareth were investigated cross-sectionally to determine the magnitude of mastitis. Out of 824 quarters examined 30 (3.6%) were blind. Over prevalence of mastitis at farm level were 62.1% during the study period. Mastitis prevalence at cow level was 6.3% and 41.7% for clinical and subclinical respectively. Quarter level prevalence was 2.4% and 22.3% for clinical and subclinical respectively.

Table 7. Clinical, blind and CMT scores each quarter of lactating cow in 95 in smallholder

	Right rare	Right front	Left rare	Left front	Total
Negative (0)	140	151	160	147	598
Trace (1+)	5	9	5	14	33
Positive (2+)	26	20	18	14	78
Strong positive (3+)	13	16	18	18	65
Clinical	6	3	3	7	19
Blind	16	6	2	6	30
Total	206	206	206	206	824

4.3. Risk factors

4.3.1. Management and environmental factor

Association between husbandry practice and mastitis is shown in table 7. Prevalence of mastitis at cow level were significantly higher (p<0.05) in farms with $\leq$ 4 years duration of farming, poor drainage/slope for stable area, feed provision before milking and milking of clinical cows at any stage. Farm owners that practice dairy farming as side business had high prevalence of mastitis (p<0.05).

Table 8. Association of husbandry practices with mastitis

Factor and category	Mastitis %	χ^2	df	p
Farm owners activity				
Only dairy farming	41.6	5.51	1	0.021**
Dairy farming plus other	58.0			
Work force				
Hired labor	43.6	2.51	1	0.113
Family member	55.1			
Duration of farming				
$\leq$ 4 years	64.1	2.89	2	0.016**
5-8 years	40.7			
$\geq$ 9 years	42.6			
Floor				
Concrete	44.2	2.46	2	0.296
Stone layer	50.9			
Soil	45.8			
Stable drainage/slope				
Good	40.1	6.02	2	0.049**
Fair	41.2			
Poor	58.2			
Feed provision before milking				
Yes	58.2	6.77	1	0.009**
No	25.0			
Introduction of calf				
Yes	58.5	3.11	1	0.078
No	44.4			
Lubricant application				
Yes	53.2	1.83	1	0.177
No	43.8			
Stage of mastitic cow milking				
First	45.5	15.66	1	0.000**
Last	33.7			
Any stage	66.2			

** (p<0.05)

4.3.2. Animal factors

Cows with body condition score (BCS≤ 2), cows at parity of two, cows that had problem of leaking teat and cows with previous clinical mastitis problem were significantly (p<0.05) associated with mastitis with odds ratio greater than 1 and 95% confidence interval exclude 1. A good body condition (cows 3 and 4) and cows with ≤ 8 years had an odds ratio below 1 and 95% interval included 1.

Table 9. Summary of results of multivariate analysis between mastitis and risk factors

				Confidence interval (95%)	
Variable	Level	Odds ratio	*p*-value	lower	upper
Age	2.5-5 years	0.985	0.321	0.462	2.553
	6-8 years	0.129	0.720	0.462	2.333
	>8 years	2.378	0. 305		
Parity	1parity	1.798	0.180	0.773	3.933
	2 parity	3.887	0.049**	1.005	5.574
	3 parity	1.929	0.165	0.794	3.872
	≥4 parity	4.279	0.234		
BCS	≤ 2	6.285	0.039**	0.012	0.897
	3	0.473	0.937	0.529	1.799
	4	6.272	0.529		
Leaking teat		3.595	0.024**	1.197	2.093
PMP		5.109	0.050**	1.103	1.317
Udder health		1.397	0.237	0.472	3.056

**: p<0.05 (significant association at 95% confidence interval)

4.4. Bacteriology

From the total 99 cows that were positive either clinically or under screening test using CMT 91 pooled milk samples were collected. From 91 cultured samples 82 (90.11%) samples were positive for aerobic bacteria. The following bacteria were isolated with high prevalence of CNS 21.2%, *Micrococcus* species15.9%, *S. aureus* 14.7%, *St. agalctiae* 11.6%, *St. dysagalctiae* 6.3%, *St. uberis* 3.3%, *E. faecalis*10.6%. Figure 3 shows the percentage of bacteria in three different categories. The highes percentage (45.7%) were contagious pathogens.

Table 10. Frequency distribution of bacteria isolated from dairy cows in Nazareth

Bacterial isolates	Frequency	%
Staphylococcus aureus	14	14.7
Streptococcus agalctiae	11	11.6
CNS*	20	21.2
Streptococcus dysaglactiae	6	6.4
Streptococcus uberis	3	3.3
Enterococcus faecalis	10	10.6
E coli	7	7.5
Micrococcus species	15	15.9
Serratia marcescens	4	4.2
Arcanobacter pyogens	3	3.3
Bacillus cereus	2	2.2
Total	95	100

CNS*- Coagulase Negative Staphylococci

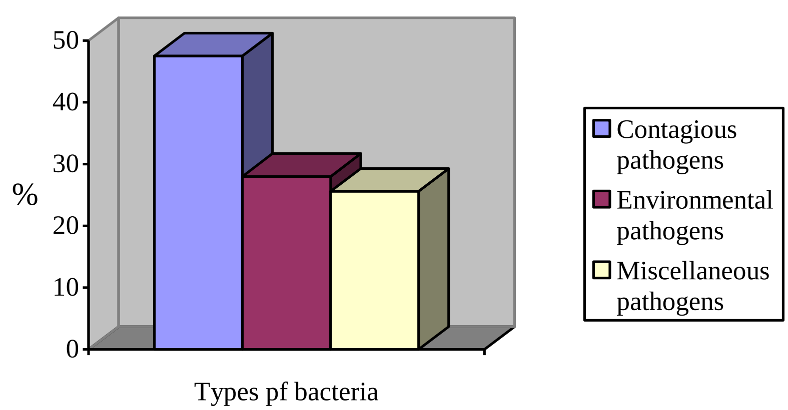


Figure 3. Isolated bacteria in three category

5. DISCUSSION

5.1. Descriptive statistics

A large portion of smallholder farms (58.9%) were owned by female that shows the role of women in smallholder dairying, which is higher than the 38% reported by Mekonnen *et al*. (2006) at Debre Zeit. In 81.0% of the farms farm activities, specially feeding of animals and cleaning of barns, were performed by family members. The findings of this study indicated that most of the responsibilities for dairy cattle management were that of family members, mostly children and women, were doing all the activities and were primarily responsible for herding of dairy cattle. It is to be noted that the responsibilities pertaining to dairy cattle management requires the attention of family members with a higher decision making status since crossbred dairy cattle are considered as high value assets. Thirty nine percent of the smallholders were using contract laborer for milking activities. In general, one contract laborer was performing milking activities in more than two farms. Moreover, in most cases, contract laborers were either not aware or giving mach attention to the importance of hygienic conditions during milking, proper milking practices and the necessary precautionary majors while milking mastitic cow.

The results showed that the highest percentage (74.7%) of the smallholders was practicing dairying as a sideline business and the rest as a main source of income. This finding is comparable to that of Mekonnen *et al*. (2006) who reported that in Debre Zeit 25.3% the smallholders were taking dairying as a main business, but much lower than that of Hossain *et al*. (2005) who reported 53% in Bangladesh. Highest percentage (52.6%) of the farmers had higher secondary level education that indicates the contribution of educated peoples in smallholder dairying. They make additional income from the farm.

Sources of animals for starting dairying were from private smallholder dairy farms in Nazareth (77.9%). The remaining were from Government breeding centers and smallholder dairies outside Nazareth. This shows the level of difficulty to get starter and replacer heifers from the government breeding centers. Transportation and other costs associated with purchases of heifers outside Nazareth, made smallholders rely on nearby sources. Previous works (Bebe *et al*., 2003) also reported similar observations.

Seventy one percent of the smallholder dairy farms had ≤ 8 years duration. This shows a dramatic increase in the number of farms within the last 8 years that indicates smallholder dairy activity becoming an attractive business within and around urban areas. The average 5.23 animals per farm was a characteristic feature of smallholder dairy farms and is in agreement with the existing reports by Devendra (2001) and Mekonnen *et al*. (2006) who reported average 4.56animals per farm.

Housing management: All respondents addressed no contact between cows from different herds and they practiced tie-stall farming system. This may help to control the spread of infectious diseases between herds. When mastitic cows mix with susceptible ones, it possible for the disease to be transmitted through flies (Fox and Gay, 1993). In 87.4% of the farms animals were protected from environmental stress such as heat stress from direct sunlight and heavy rainfalls by roofing with corrugate sheet materials. Sixty two percent of the cows' stables were concrete floor, which was consistent with 67% reported by Mekonnen *et al*. (2006). However, the farms had poor and unsatisfactory drainage that resulted accumulation of liquid such as urine and water used for cleaning of udders during milking. The liquid material mixed with the faeces of the cows that led to poor stable conditions, and poor dirty udder and body of the cow. Only 22.1% of the farms kept their farms clean and in good condition, a finding that is similar to the report made by Mekonnen *et al*. (2006) that 23% of the farm stables were clean. Almost all farms had good constructed houses for the storage of concentrate and 67.4% for storage of roughage (hay or teff straw). This observation diverge from what was reported by Mekonnen *et al*. (2006) that indicated only 20% of the farms had a well-managed feed storage situation. This might be due to the increased awareness of the farm owners on the spoilage of feed because of improper storage and alarmingly increasing feed cost.

Feeding management: Smallholders in Nazareth were characterized as market oriented urban small-scale dairying. Stall-feeding was practiced in almost all farms and they totally depended on purchased fodder. Only two farms with relatively large land areas were practicing grazing during rainy season to provide green feed for their cows. In highest percentage of the farms, the feeding trough was made from rubber like material. The feeding trough space per animal was poor in 80% of farms. Due to this fact part of the feed was wasted by being poured to the floor; increasing feed expense and decreasing profitability. Although the feeding trough space per animal were poor the feeding and watering trough cleanness were good in 27.4% of the farms especially in those farms where the feeding and watering trough were made from concrete. Satisfactory cleanness of the feeding tough were observed in 60% of the farms that washed the feeding tough within regular intervals

Milking and dry cow practice: In most cases (94.7%) cows were milked in the same barn where they stay. and the rest were milked in the open space. The indoor milking might have lessened stress conditions associated with disturbances like dog barking or other strange noises that are very frequent in Nazareth area. Stress conditions lead to the release of adrenalin that counteracts the effects of oxytocin for efficient milk ejection (Radostits *et al*., 1994a).

There were a number of cows that had extended duration of lactation (in their 20th months of lactation). This is much longer than the standard duration of lactation, which is 305 days and clearly shows decreased reproductive performance due to long calving interval. All the farms were not practicing dry cow therapy; a contributing factor for the high prevalence of mastitis

Milking of clinical mastitic cow at last stage reduce the transmission of contagious mastitis with in a herd (Radostits *et al*., 2000), but in 61.4% of the farms were practiced in first and last stage. Thus milking practices in Nazareth could be said partly responsible for the high prevalence of mastitis

Breeding, health and record management: Most of the smallholders at Nazareth were preferring AI than natural mating with crossbred bulls kept by some smallholder as a source of income through mating service. Eventhough almost all of the farms were using AI system; natural mating was also employed in good number of them (36%) for various resons. Similar findings were reported by Abdinasir (2000) who found AI being used by the majority of the farmers (87.5%) at Bilalo located relatively closer to Asela. 33.7% of smallholders that practice AI also practice natural mating in their farms due to some reasons such as failure to conception after repeated service, unavailability of AI service like at weekends, holidays and lack of communication to AI technicians. Azage *et al*. (1995) and FAO electronic conference (2000) on the appropriateness, significance and application of individual biotechnologies in developing countries indicated that poor heat detection skills of farmers, poor semen quality, inappropriate semen handling procedures, wrong timing of insemination and inefficiency of inseminators, insufficient means of communication and infrastructure, difficulty to bear costs for the production of liquid nitrogen and purchase of necessary equipment and poor management of AI operations are some of the problems associate with AI.

Health service were given mostly by the practitioner coming to the farm or some times by taking the animal to the near by clinics. Regular health program by professionals were not practiced but, in general, it can be said that all farms had access to health service by professionals when needed. This might be due to the income they get from the sale of milk that allows them to cover veterinary costs. In addition, almost all farms were not practicing regular deworming program because of lack of awareness about regular deworming. Deworming were practiced when prescribed by professionals at the times when animal were diseased or lost body conditions

The mean age of cows under study were 6.77 years. Although many of them were of single parity and some old ones with up to 7 parties, they had mean parity number of 2.9. There are old cows with single parity those had first calf late in their life or their calving interval longer than expected. Similar result was reported by Mungube (2004) in Addis Ababa and Mekonnen *et al*. (2006) in Debre Zeit. The 3.48 mean body condition score for smallholder dairy farms were similar those findings of Mekonnen *et al*. (2006) and Yoseph (1999) who reported 3.62 and 3.67 mean body condition score.

Record keeping was very poor in smallholders in Nazareth. Breeding record was practiced in 39% of the farms in the form of papers given by inseminators and a few farms record service date by some type of exercise book. Any of the farms were practicing either production or feeding record. All farmers were not record preventive or treatment status and did not keep their cattle isolated while sick. Since there is no health record vaccine programs were not well understood. In some farms there was vaccination against anthrax and blackleg diseases.

The major health problem encountered as questionnaire result was mastitis which is confirmed during physical examination of animal.

Cow data

It was observed that very little number (6.3%) of indigenous cattle found in the survey of private smallholder farms in Nazareth. Because, most of the farm owners are market-oriented they preferred crossbred cows for their higher production than local cattle and prefer AI for breeding purpose. Majority of smallholders were not started their dairy herds by upgrading Zebu cattle; instead, they procured dairy cattle of breeds of their choice from what was locally available and they maintained these by mating to dairy breeds, with the tendency towards the use of crossbreeding.

For 234 smallholders dairy cows with parity one and above there were 75 heifers that have the potential to replace 32.1% of the cows that was in agreement with the findings of 34.8% of Mekonnen *et al*. (2006). The space limitation, economic case and feed shortage might force some of the farms to sell the heifers which may serve as a potential source as starting animal for newly opening farms. 89 female calves that were in different herds can be used as replacer or as starter animal. According to Devendra (2001) the smallholders tend to keep older cows to maintain the existing number of animals due to space limitation.

5.2. Prevalence of mastitis

Many respondents can detect clinical form of mastitis but all subclinical mastitis cases went undetected due to lack of awareness on the disease. Subclinical mastitis were the major problem but incidentally it seemed it go unnoticed despite the fact that it causes great economical loss by decreasing milk production capacity. Usually Ethiopian farmers especially smallholders are not well informed about the invisible loss from subclinical mastitis since dairying is mostly a sideline business (Hussein, 1999). This is also true in the study area none of the respondents has knowledge about subclinical mastitis and none of the farms screened their cows for subclinical mastitis except seeking professional assistance at the time of clinical cases.

Clinical prevalence at cow level was 6.3% that is comparable with that of Bishi (1998) and Mungebe *et al*. (2004) who reported 5.3% and 6.6% respectively in Addis Ababa. However, the present study was higher than that reported by Gizat (2004) who reported 3.9%. The prevalence was much lower than Workineh *et al*. (2002) who reported 25.1% in Addis Ababa. Mastitis is a complex disease and the difference in results could be due to differences in management system between the farms.

Subclinical mastitis is defined as an inflammation of the udder that is not visible and requires a diagnostic test for detection, mostly on milk SCC. During subclinical mastitis microorganisms are usually present in the milk and SCC are elevated (Radostits *et al*., 2000). The CMT provides cheap and reliable method to estimate SCC of individual quarters..

This study has shown high prevalence (41.7%) of subclinical mastitis in smallholder dairy farms in Nazareth when compared with previous studies in smallholder Bishi (1998) who reported 34.3% in Addis Ababa and 34.4% reported by Gizat (2004) in Bahir Dar. The high prevalence of subclinical mastitis may be attributed to improper milking hygiene, poorhouse hygiene lack of use of postmilking teat dipping and practicing of milk by contract laborers. According to Radostits *et al*., (2000) contamination of milkers hands, washing the whole udder and absence of drying of the teats or udder have were increase the incidence of staphylococcus species that were common practice in the study area. The improper milking hygiene practiced by family members and contract laborers in the study area is due to poor or lack of knowledge about subclinical mastitis and its economic implications on milk production.

This study has revealed subclinical mastitis as an unfamiliar problem amongst smallholders in Nazareth. In this study as well as in other similar studies (Kassa *et al*., 1997; Hussein, 1999, Workineh, 2002; Kero and Tareke, 2003; Gizat 2004; Mungube *et al*., 2004) subclinical mastitis were overwhelming compared to clinical mastitis. In Ethiopia the subclinical mastitis received little attention and effects have been concentrated on the treatment of clinical mastitis while the high economic loss could come from subclinical mastitis (Hussein *et al*., 1997). Because of its insidious nature, the subclinical mastitis might be among the causes of sub optimal milk production that is evident in many smallholder farms. In most of the results indicated in table1 and table 2 the average milk production was lower in variables where mastitis prevalence was higher. According to Radostits *et al*. (1994b) an infected quarter showed 30% and a cow 15% reduction in milk yield. If milk production in the study area has to be improved, creation of awareness and control of mastitis through proper milking hygiene such as use of hot water to rinse the towels, individual towels for each cow and quarter, post-milking teat disinfection and proper treatment of mastitis cases is important.

5.3. Risk factor

Risk factors, which influence the prevalence of infection clinical mastitis were outlined as animal, pathogenic, and environment and management risk factor (Radostits *et al*., 2000). Prevalence of mastitis at cow level were higher in those farms ≤ 4 years duration of farming, poor drainage/slope for stable area, feed provision before milking and milking of clinical cows at any stage. Farms at operation for a short period of time (≤ 4 years) had no enough awareness on farming practices and also they usually buy a starter animal (cows with more than one parity) from smallholders which are usually as a cull due to different reasons. These cows may be positive for subclinical mastitis in the previous farm. Poor drainage/slope of the stable area results accumulation of liquid such as urine and water used for cleaning of udders during milking. The liquid material mixed with the faeces of the cows that led to dirty udder and teat. The environmental bacteria such as *E. coli* and other got acces to enter trough teat canal and result infection. In the study area where there is no awareness on standard procedure in milking practice, miliking of mastitic cow at any stage may result transmission of contagious bacteria with in a herd.In the other hand activities before milking like provision of feed usually concentrates before milking favor the mastitis problem. Animals that were given concentrate after milking will stay at stand that gives enough time for teat closure. Partly this problem may be associated with the milking hygiene because in most of the farms concentrate were provided after pre milking preparation such as washing of udder and teat. This may give a chance to transfer the bacteria from the feed to teat.

Poor body condition score increased the risk of mastitis. This is in line with the previous report on mastitis in Ethiopia (Hussein, 1999; Kero and Tareke, 2003; Mungube *et al*., 2004) and industralised countries (Dulin *et al*., 1998; Suriyasathaporn *et al*., 2000). Leaking teat ($P<0.05$) were increased the risk of mastitis, this may be partly associated with opened tat sphincter and wide teat canal. Leaking teat may result in increased levels of mastitis pathogens in the environment and an increased exposure of other cows mastitis pathogen, as bacteria from infected quarters may present in the milk (Peeler *et al*., 2000). Furthermore, cows that leak milk outside the milking parlor may themselves at high risk of mastitis, because they may have wide teat canals would facilitate the entry of mastitis pathogens in to the udder (Lacy-Hubert and Hillerton, 1995). Previous problem of clinical mastitis ($p<0.05$) were increased the risk of mastitis. The increase in risk for mastitis in quarters previously affected is well documented in industrialized countries (Eberhart 1986), but has not been reported in industrialized countries. Parity number two were significantly associated with mastitis.

5.4. Bacteriology

In this study CNS was the predominant pathogen that comprises 21.2% of all isolates. The high level of isolation of CNS in this study lower than with the findings of Bishi (1998), Hussein (1999) and Gizat (2004) who reported 54%, 42% and 46% respectively. CNS regarded as minor pathogens and normally considered as normal inhabitants of bovine udder and usually mentioned in association with a slight increase in SCC (Rainard and Poutrel, 1988). However, recently CNS were isolated from bovine and other animals mastitic milk samples (Ameh *et al*., 1993; Boscos *et al*., 1996; Almaw and Molla, 2000). Some studies indicated that CNS could be pathogenic and even more mastitic cows than *S. aureus*. 834 cows with clinical mastitis cows in Ontario, Canada, 28.7% of the cases were due to staphylococcus species (other than *S. aureus*) where *S. aueus* was isolated from 6.7% of the cases (Sargeant *et al*., 1998). According to Pyröröllä (1999), over 30% of subclinical mastitis and nearly 20% of clinical mastitis were usually due to CNS. The high isolation of CNS in this study could be associated with lowered resistance of the cow due to teat injury. Staphylococci typically colonize a broken skin and hence abrasion of the teat ends increases the risk of staphylococcal organization at the end and subsequent transfer in the udder. CNS can be chronic and isolation from the same cow for longer period is possible for longer period (Pyröröllä, 1999). CNS were also credited for increasing the resistance of the colonizing quarter to invasion by major pathogens and hence lowering the isolation rate of major pathogens relatively to major pathogens like CNS (Rainard and Poutrel, 1988).

The isolation of *S. aureus* (14.7%) in this study was closely comparable to the findings of Bishi (1998) and Hussein (1999) in Addis Ababa and Gizat (2004) in Debre Zeit who reported 9%, 10.69% and 17.8% respectively. However, the present study is much lower than that of Workineh *et al*. (2002) and Kero and Tareke (2003) where *S. aureus* accounted for 39.24% and 40.5% of isolates respectively in their study at Addis Ababa and Southern Ethiopia. The relative high prevalence of *S. aureus* in this study could be associated with total absence of dry cow therapy, postmilking teat dipping, the invariably hand milking practice, low culling rate of chronically infected animals, limited knowledge of smallholders in segregation as control program and contract laborers that had poor milking hygiene.

Streptococci (31.9%) were the dominant bacterial pathogen population as mastitis pathogen *St. agalalctiae* (11.6%), *St. dysaglactiae* (6.4%) ad *St. uberis* (3.3%) and *E. faecalis* (10.6%) This finding was comparable to that of Kero and Tareke (2003) who reported higher isolation rate of 13.1% of *St. agalalctiae* and 5.6% *St. dysaglactiae* and 5.1% *St. uberis*. Bishi (1999) reported higher isolation rate (27%) for *St. agalalctiae* and lower isolation (0.5%) *St. dysaglactiae* compared to the current findings. Bishi (1999) in Addis Ababa and Gizat (2004) findings on *St. uberis* who rporeted (1.9%) and 1.48% were closely similar to the present findings. The environmental Streptococci were higher in prevalence due to the slope for stable area were poor in most of the farms. Housing and management practices on dairy farms contribute to the contamination of bedding materials and teats to environmental streptococci. Housing facilities, which dispose to the accumulation of faces on cows, will increase the rate of exposure of the teats and udder to the pathogen (Radostits *et al*., 2000). Further more the high prevalence of environmental streptococci were due to the absence of teat drying after washing and absence of teat dipping. Exposure to environmental streptococci may occur during milking, between milkings and during dry period (Radostits *et al*., 2000).

E. coli (7.5%) and *Serratia marcescens* (3.3%) were the two bacteria isolated from *Enterobactericae* group. The prevalence of high environmental *E. coli* may be associated with poor farm cleanness and poor slope of stable areas that result cow faces to stay in stable areas. Faces, which are a common source of *E. coli*, can contaminate the premium directly or indirectly through bedding, calving stalls, udder wash water and milkers hand (Radostits *et al*., 2000). The coliform bacteria (*E. coli*) are opportunists and contamination of the skin of the udder and teats occur primarily between milkings and when the cow in contact with contaminated bedding than between milkings (Radostits *et al*., 2000). *Serratia marcescens* causes mild chronic mastitis in which swelling of the quarters with clots in the milk appears periodically (Radostits *et al*., 2000).

6. CONCLUSION AND RECOMMENDATION

Market oriented smallholder dairy farms, which are increased in number at faster rate than commercial dairy farms, are the major suppliers of milk for urban population. Most newly established dairy farms have got their starter animals, mainly cows with parity number more than one, from other smallholders within and nearby Nazareth areas. The common observations in many of the farms studied were poor sanitary conditions and practices, confinement of animals in poorly designed house, poorly designed feeding trough and inadequate feeding trough space per the animal. In most of the farms studied, the hygienic condition was generally poor. Health problems related to the animal's environment, such as feet and leg problems and environmental mastitis were observed. In a good number of the farms milking practice were carried out by contract laborers. There was no performance record that enables the owners to compare the performance of his/her cows with in a herd or with those of his/her community. Both the family member and contract laborers had inadequate information/awareness regarding standard milking hygiene and subclinical mastitis. Preventive measures like the use of udder disinfectants, strip cup, postmilking teat dipping and dry cow therapy were not used in any of the farms. Subclinical mastitis, which has greatest detrimental effect on milk production was found much more prevalent compared to clinical mastitis. Both contagious and environmental pathogens were isolated. The pathogens involved were CNS, *S. aureus, St. agalactiae, St. dysagalctiae, St. uberis, E. faecalis,* Micrococcus species, *E. coli, Serratia marcens, A. pyogens* and *B. cerus*. Among these the most significant isolates were CNS, *S. aureus, St. agalactiae, E. faecalis, Micrococcus species* and *E.coli.*

For better exploitation of the potential of the smallholder dairy systems the following recommendations are forwarded

- The establishment of dairy organizations and cooperatives should be encouraged.

- Research institutes and government breeding centers should supply well-adopted pedigree starters to smallholders.

- Strong extension package that involves training on farm hygiene, health care and feeding record keeping, culling of chronically infected animals and dry cow therapy should be enforced. Specially training should be given to those who are in charge of the milking and other husbandry activities.

- Proper milking management methods such as premilking udder preparation, proper removal of the milk and postmilking teat dipping should be introduced and promoted.

- The effect of mastitis on milk production and its inter-relationship with management and other risk factors should be further investigated. The results of such studies should be well documented and provided to the responsible organizations such as district agricultural offices, dairy organizations and cooperative.

7. REFERENCE

Abdella, M. (1996): Bacterial causes of bovine mastitis in Wondogenet, Ethiopia. *J. Vet. Med.*, **43**(6): 379-384.

Abdinasir, I. B. (2000): Smallholder Dairy Production and Dairy Technology Adoption in the Mixed Farming System in Arsi Highlands, Ethiopia. PhD Thesis, Humboldt University of Berlin, Department of Animal Breeding in the Tropics and Subtropics, Germany.

Adama District Agriculture Office (2003): Document, Unpublished.

Ahmed, M., Bezabih, E., Mohammad, J. and Simeon, E. (2003): Analysis of economic and nutritional impacts of market-oriented dairy production in the Ethiopian highlands. In: Socio-economic and Policy Research Working Paper, Livestock Policy Analysis Program, International Livestock Research Institute, Addis Ababa, Ethiopia.

Almaw, G. and Molla, B. (2000): Prevalence and etiology of mastitis in camels (*Camelus Dromedaries*) in Eastern Ethiopia. *J. Camel. Pract. Res.*, **7**: 97-100.

Ameh, J. A. Addo, P. B., Adkeye, J. O. and Guang, E. O. (1995): Prevalence of clinical and intramammary infection in Nigerian goats. *Prev. vet. Med.*, **17**: 4-16.

Amstutz, H. E. (1998): Dental development. In: Aeilo, S. E. (eds.), The Merck Veterinary Manual. 8^{th} Edition, Merck & Co. Inc., Pp 131-132.

Azage, T., Lahlou-Kassi, A. and Mukassa-Mugrwa, E (1995): Biotechnology in animal production development opportunities in livestock agriculture. In: Proceedings of the Second Annual Conference of the Ethiopian Society for Animal Production, May 26-27, 1993, Addis Ababa, Ethiopia, Pp 49-80.

Bascom, S. S. and Young, A. J. (1998): A summary of reasons why farmers cull their cows. *J. Dairy Sci.*, **81**: 2299-2305.

Bebe, O. B., Udo, H. M. J., Rowlands, G. J. and Thorpe, W. (2003): Smallholder dairy systems in the Kenya highlands: Breed preferences and breeding practices. *Livest. Prod. Sci.*, **82**: 117-127.

Beede, D. K. (1991): Mineral and water nutrition. *Vet. Clin. North Am. Food Anim. Prac.*, **7**: 439-464.

Bickert, G. B. and Radostits, O. M. (2001): Housing and environment in dairy cattle. In: Radostits, O.M. (eds.), Herd health, Food Animal Production Medicine. 3rd Edition, W.B. Saunders Company, Philadelphia, Pennsylvania, Pp 475-508.

Bickert, W. G. (1992): Dairy facilities for management. In: Proceedings of the Ontario Large Herd Operators' Symposium, Pp 12-16.

Bishi, A. S. (1998): 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. M.Sc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University, School of Graduate Studies and Free University, Berlin Germany, Joint Program.

Boscos, C., Stenfanakis, A., Alexopoula, C. and Samartgi, C. (1996): Prevalence of subclinical mastitis and influence of breed, parity, stage of lactation and mammary bacteriological status on Coulter counts and CMT in milk of saan and autochtnous Greek goats. *Small Rumin. Res.*, **21**: 139-147.

Cullor, S. (1990): Mastitis and its influence upon reproductive performance in dairy cattle. In: Proceedings of the International Symposium on Bovine Mastitis, Indianapolis, Pp 176-180.

Cunningham, E. P. (1991): Breeding programs for improved dairy production in tropical climates. In: Proceedings of the International Symposium on Animal Husbandry in Warm Climates, October, 1990, Viterbo, Italy, *EAAP publications*, **55**: 25-27

Devendra, C. (2001): Smallholder dairy production systems in the tropics: Characteristics, potential and opportunists for development. *Rev. Aust. J. Anim. Sci*., **14**(1): 104-113.

Dijkhuizen, A. A., Huirn, R. B. M., Jalvingh, A. W. (1997): Economic analysis of animal disease and their control. *Prev. Vet. Med*., **25**: 135-149.

Dohoo, I. R. and Martin, S. W. (1984): Disease, production and culling Holstein Friesian cows**.** **III.** Disease and production determinants of disease. *Pre. Vet. Med*., **2**: 671-668.

Dulin, A., Paape, M. J. and Nickerson, S. C. (1988): Comparison of phagocytosis and chemiluminescense by blood and mammary gland neutrophils from multiparous and nulliparious cows. *Am. J. Vet. Res*., **49**: 172-177.

Eberhart, R. J. (1986): Management of dairy cows to reduce mastitis. *J. Dairy Sci*., **69:** 1721-1732.

Erb, H. N., Smith, R. D. and Hilman, R. B. (1988): Rates of the diagnosis of six diseases of Holstein Friesian cows during 15 day and 21 day interval. *Am. J. Vet. Res.*, **45**: 333-335.

Erskine, R. J. (2001): Mastitis control in dairy farms. In: Radostits, O.M. (eds.), Herd health, Food Animal Production Medicine. 3rdEdition, W.B. Saunders Company, Philadelphia, Pennsylvania, Pp 397-432.

Erskine, R. J., Ebrehat, R. J. and Hutchinson, S. B. (1988): Incidence and type of clinical mastitis in dairy herds with high and low somatic cell count. *Am. J. Vet. Med. Assoc.,* **192**: 761.

Etgen, M. W. and Reaves, M. P. (1978): Dairy Cattle Feeding and Management. 6th Edition, Jhon Wiley & Sons Inc., USA.

FAO (1999): Livestock, environment and development (LEAD) initiative. Livestock and environment toolbox. http://www.fao.org/lead/toolbox/homepage.htm.

FAO (2000): The appropriateness, significance and application of biotechnology options in the animal agriculture of the developing countries., In: Electronics Forum on Biotechnology in Food and Agriculture, June 12-August 25, http://www.fao.org/biotech/C3doc.htm.

FAO (2001): FAO/WFP crop and food supply assessment mission to Ethiopia. Special Report.

Farin, W. B. and Sleenning, D. B. (2001): Maintaining reproductive efficiency in dairy cattle. In: Radostits, O.M. (eds.), Herd health, Food animal Production Medicine, 3rd Edition. W.B. Saunders Company, Philadelphia, Pennsylvania. Pp 325-370.

Fekadu, K. (1995). Survey on the prevalence of bovine mastitis and the predominant causative agents in Chaffa Valley. In: Proceedings of the Ninth Conference of Ethiopian Veterinary Association, Addis Ababa, Ethiopia, Pp 101–111.

Felleke, G and Geda, G. (2001): The Ethiopian dairy development policy: A draft policy document. MOA/AFRDRD/AFRDT Food and Agriculture Organization/SSFF, Addis Ababa, Ethiopia.

Fox, K. F. and Gay, M. J. (1993): Contagious mastitis. In: Hunt, E. (eds.), the Veterinary Clinics of North America, Updated on Bovine Mastitis. W.B. Saunders Company, USA. Pp 475-488.

Frese, M. (1999): Cross-site and Cross-location on Farm Investigation on the Epidemiology of Mastitis in Market-oriented Urban/Peri-urban Production Systems in the Regions of Addis Ababa and Debre Zeit, Ethiopia. Diploma Thesis, Free University, Germany.

Gebre Wold, A., Alemayehu, M., Demeke, S., Dediye, S., Tadesse, A. (2000): Status of dairy research in Ethiopia. In: The Role of Village Dairy Co-operatives in Dairy Development. In: Proceeding of SDDP, MOA, Addis Ababa, Ethiopia.

Gerloff, B. (2001): Dairy cattle and nutrition. In: Radostits, O.M. (eds.), Herd health, Food Animal Production Medicine, 3rd Edition, W.B. Saunders Company, Philadelphia, Pennsylvania, Pp 435-476.

Gizat, A. (2004): A Cross-sectional Study of Bovine Mastitis in and around Bahir Dar and Antibiotic Resistance Patterns for Major Pathogens. M.Sc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Gröhn, Y. T., Eiker, S. W., Duerock, V. and Hertle, J. A. (1998): Milk yield and disease in New York state dairy cows. *J. Dairy Sci*., **78**: 1693-1702.

Grommers, F. J. (1988): Host resistant mechanism of the bovine mammary gland: An analysis and discussion. *Neth. Milk Dairy J*., **42**: 43.

Harmon, R. (1994): Mastitis and genetic evaluation for somatic cell count. *J. Dairy Sci.*, **77**: 2103 –2112.

Heeschen, W. and Reichmuth, J. (1995): Mastitis influence on qualitative and hygienic properties of milk. In: Proceedings of the Third International Mastitis Seminar, Tel Aviv, Israel, **3**: 33-43.

Hillerton, J. E., Morgsan, W. F., Farnsworth, R., Neijenhucs, R. F., Mein, G. A., Ohnstad, I., Reinemam, D. J. and Timms, L. (2001): Evaluation of bovine teat condition in commercial dairy herds: Infectious and non-infectious. In: Proceedings of Second International Symposium on Mastitic Milk Quality, Compton, UK, Pp 352-356.

Hinton, M., Kaukas, A. and Linton, A. H. (1986): The ecology of drug resistance in enteric bacteria. *J. Appl. Bac*., **61**: 775-925.

Holloway, G., Nicholson, C., Delgado, C., Staal, S. and Ehui, S. (2000): How to make a milk market: A case Study from the Ethiopian highlands. Socio-economic and Policy Research Working Paper, International Livestock Research Institute, Addis Ababa, Ethiopia, Pp 28.

Holmberg, S. D., Osterholm, M. T, Senger, K. A. and Cohen, M. L. (1984): Drug resistant salmonella from animals fed antimicrobial. *N. Eng. J. Med*., **311**: 617-622.

Hossain, M. M., Alam, M. M., Rashid, M. M., Asaduzzaman, M. and Rahman, M. M. (2005): Small-scale dairy farming practice in a selective area of Bangladesh. *Pakistan J. of Nutrition*, **4** (4): 215-221.

Hurnik, F., Luescher, U. A. and Coukell, G. (1990): Recommended code of practice for dairy cattle. Agriculture Canada publication, 1853E, Ottawa, Canada.

Hussein, N. (1999): Cross-sectional and Longitudinal Prospective Study of Bovine Clinical and Subclinical Mastitis in the Peri-urban and Urban Production Systems in Addis Ababa Region.

M.Sc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University, School of Graduate Studies and Free University, Berlin Germany, Joint Program.

Hussein, N., Yehualashet, T., and Tilahun, G. (1997): Prevalence of mastitis in different local and exotic breeds of milking cows. *Eth. J. Agr. Sci*., **16**: 53-60.

Kassa, T., Wirtu, G. and Tegegne, F. (1999): Survey of mastitis in dairy herds in Ethiopian central highlands. *Eth. J. Sci.*, **22**: 291-301.

Kelay, B. (2002): Analyses of Dairy Cattle Breeding Practices in Selected Areas of Ethiopia. zur wrlangung des akademischen, Grades doctor rerum agriculturarum (Dr. rer. agr.), eingerichtet an der Landwirtschaft-Gärtenerischen Fakultät, der Humboldt-Universität ,zu Berlin

Kero, D. O. and Tareke, F. (2003): Bovine mastitis in selected area of Ethiopia. *Trop. Anim. Hlth. and Prod*., **35**: 197-205.

Ketema, H. and Tsehay, R. (1995): Dairy production systems in Ethiopia. In: Proceedings of a Workshop on Strategies for Market Orientation of Small-Scale Milk Producers and their Organizations, March 20-24, 1995, Morongo, Tanzania, Pp 50-56.

Kinghorn, B. (2000): Tactical approaches to implementing breeding design. In: Animal breeding, Use of New Technologies, Pp 291-308.

Lacy-Hubert, S. T. and Hillerton, J. E. (1995): Physical characteristics of bovine teat canal and their influence in streptococcal infection. *J. Dairy Sci*., **62**: 395-404.

Lemma, M., Kassa, T. and Tegeghe, A. (2001): Clinically manifested major health problems of crossbred dairy herds in urban and peri-urban production in the highlands of Ethiopia. *Trop. Anim. Hlth. and Prod.*, **33**: 85-93.

Matthewman, R. W. (1993): Dairying. In: Center for Tropical Veterinary Medicine. University of Edinberg, McMillan Press Ltd, Scotland, UK, Pp 67-68.

McCullough, M. E. (1989): Roughage for dairy cattle. Hoards' dairyman, Fort Atkinson, WI.

Mekonnen, H. M., Asmamaw, K. and Courreau, J. F. (2006): Husbandry practice and health in smallholder dairy farms near Addis Ababa. *Prev. Vet. Med*., **74**: 99-107.

Mekonnen, H., Tesfu, K., Azage, T. (2000): Major health problems of dairy cattle in market-oriented urban and peri-urban production system in central highlands of Ethiopia. In: Proceeding of Seventh Annual Conference of Ethiopian Society of Animal Production, Addis Ababa, Ethiopia, Pp 353-364.

MOA (1997): Ministry of Agriculture (MOA): National livestock development program. Proposed January 1997, Addis Ababa, Ethiopia, Pp 121.

Moran, J. (2005): Smallholder dairying. In: Tropical dairy farming, Feeding Management of Smallholder Dairy Farmers in Humid Tropics. Landlinks Press, Pp 19-25.

Mungube, E. O., Tenhgen, B. A., Kassa, T., Regassa, F., Kyule, M. N., Griener, M. and Baumann, M. P. O. (2004): Risk factor for dairy cow mastitis in the central high lands of Ethiopia. *Trop. Anim. Hlth. and Prod*., **36**: 463-467.

NMC (1990): National Mastitis Council (NMC) Microbiological Procedures for the Diagnosis of Bovine Udder Infection. 3rd Edition, NMC, Madison, USA.

NMC (1999): Natonal Mastitis Council (NMC): Laboratory and Handbook of Mastitis. NMC, Madison, USA.

Oliveira, P., Watts, J., Salmon, S. and Aarestrup, M. (2000): Antimicrobial susceptibility of S*taphylococcus aureus* isolated from bovine mastitis in the Europe and the United States. *J. Dairy Sci.*, **83**: 855-862.

Payne, W. J. A. and Hodges, J. (1997): Tropical Cattle: Origins, Breeds and Breeding Policies. 1stEdition, Blackwell Science, UK, Pp 319.

Peeler, E. J., Green, M. J., Fitzpatrick, J. L., Morgans, J. L. and Green, L. E. (2000): Risk factors associated with clinical mastitis in low cell count British dairy herds. *J. Dairy Sci*., **83**: 2404-2472.

Peters, K. J. (1991): Selection and breeding strategies for production in warm climates. In: Proceedings of the International Symposium on Animal Husbandry in Warm Climates. October 25-27, 1990, Viterbo, Italy, *EAAP publications*, **55**: 44-50.

Pyröröllä, M. (1999): Staphylococcal and streptococcal mastitis. In: Sandhol, M., Hankanan-Buzaski, T. S., Kaartinen, L. and Pyröröllä, M. (eds.), Bovine Udder and Mastitis. Gummeres kirjapaions 04, Jyraskyle, Finland, Pp 141-150.

Quinn, P. J.; Markey, B. K.; Carter, M. E; Donelly, W. J. C.; Leonard, F. C. and Maghire, D. (1999): Veterinary Microbiology and Microbial Diseases. Blackwell Science Ltd., Oxford, Uk, Pp 97-105.

Radke, R. R. and Lloyd, J. N. (2000): The Canadian rural practitionaraie market. *Can. Vet. J.*, **37**: 281-296.

Radostits, O. M. (2001): Control of infectious disease of food producing animal. In: Radostits, O.M. (eds.), Herd health, Food Animal Production Medicine, 3rd Edition, W.B. Saunders Company, Philadelphia, Pennsylvania, Pp 147-188.

Radostits, O. M., Blood, D. C., Gay, G. C and Hinchcliff, K. W. (2000): Mastitis. In: Veterinary Medicine, a Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horse. 9th Edition, W.B. Saunders Company Ltd., London, Pp 603-7000.

Radostits, O. M., Blood, D. C. and Gay, G. C. (1994a). Mastitis. In: Veterinary Medicine, a Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horse. 8th Edition, Bittler, Tindal, London, Pp 787-812.

Radostits, O. M., Lesilie, K. E. and Fetrow, J. (1994b): Herd health, food animal production medicine. 2ndEdition, W.B. Saunders Company, Philadelphia, Pennsylvania.

Rainard, C. and Poutrel, J. (1988): Effects of naturally occurring intramammary infections by major pathogens on a new infections by major pathogens in cattle. *Am. J. Vet. Res.*, **49**: 327-329.

Redda, T. (2001): Small-scale milk marketing and processing in Ethiopia. In: Proceedings of the Seventh Workshop on Smallholder Dairy Production and Marketing–Constraints and Opportunities, March 12-16, 2001, Anand, India.

Rendel, J. (1991): Animal breeding and genetics. Part **IV**. A lecture note. Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, *S-750 07, Uppsal*a, Sweden.

Reugg, L. P. (2001): Health and production management in dairy herds. In: Radostits, O.M. (eds.), Herd health, Food Animal Production Medicine. 3rd Edition, W.B. Saunders Company, Philadelphia, Pennsylvania, Pp 211-244.

Sargeant, J. M., Scott, M. M., Leslie, K.I, Ireland, D. and Bashiri, M. J. A. (1998): Clinical mastitis in dairy cattle in Ontario: Frequency occurrence of bacteriological isolates. *Can. Vet. J.*, **39**: 33-38.

Schukken, Y. H., Grommers, D., Van de Geer, A. and Brand, M. (1989): Incidence of clinical mastitis on farms with low somatic cell counts in bulk milk. *Vet. Rec*., **125**: 60-64.

Sendros, D. and Tesfaye, K. (1997): Factors to be considered in the formulation of livestock breeding policy. In: Proceedings of the Fifth National Conference of Ethiopian Society of Animal Production, 15-17 May 1997, Addis Ababa, Ethiopia, Pp 13-27.

Shook, G. E. (1989): Selection for disease resistance dairy cows. *J. Dairy Sci.,* **72:** 1349-1359.

Spika, J. S., Waterman, S. H. and Hoo, G. W. S. (1987): Chloramphenicol resistance Salmonella Newport traced through hamburger to dairy farm: A major persisting source of human salmonellosis in California. *Neth. Engl. J. Med.,* **316**: 565-570.

Staal, S. J., Owango, M., Muruiki, H., Kenyanjui, M., Lukuyu, B., Njoroge, L., Njubi, D., Baltenweck, I., Musembi, F., Bwana, O., Muruiki, K., Gichungu, G., Omore, A., and Thorpe, W. (2001): Dairy systems characterization of greater Nairobi milk shed. SDP (Smallholder Dairy (R&D) Project) Research Report, Ministry of agriculture, Kenya Agricultural Research Institute and International Livestock Research Institute, Nairobi, Kenya, **73**: 55-80.

Suriyasathaporn, W., Schukken, Y. H., Nielen, M. and Brands, A. (2000): Low somatic cell count: A risk factor for subsequent clinical mastitis in dairy herd. *J. Dairy Sci*., **83**: 1248-1255.

Tesfaye, K. (1995): Smallholder dairy in Ethiopia. In: Future of livestock Industries in East and Southern Africa. In: Proceedings of a Workshop July 20-23, 1992, KadomaRanch Hotel, Zimababwe.

Thirapatsakun, T. (1999): Mastitis management. In: Falvey, L., and Chantalakhana, C. (eds.), Smallholder Dairying in the Tropics, International Livestock Research Institute, Nairobi, Kenya, Pp 299-326.

Thrusfield, M. (1995): Veterinary Epidemiology. 2nd Edition, Blackwell Science Ltd., Oxford, UK, Pp 187-189.

Tilahun, F. (1995): The livestock sector: Potential and constraints. In: Proceedings of the Fourth Annual Conference on the Ethiopian Economy, Addis Ababa, Ethiopia, Pp 271-292.

Wattiaux, M. A. (1998): Heat detection, natural service and artificial insemination. Reproduction and Genetic selection, Babcock institute for International Dairy Research and Development.

Williams, L .A. (1992): Effect of wet weather on lameness in dairy cattle. *Vet. Rec.,* **118**: 259-264.

Williamson, W. B., Burton, M. J., Brown, W. B., Baumann, L. E. and Farnsworth, R. J. (1988): Changes in mastitis practice associated with client education and the effect of adopting recommended mastitis control herd procedures on production. *Prev. Vet. Med*., **5**: 213-223.

Workineh, S., Bayelegn, M. Mekonnen, H. and Potigietr, L.N.D. (2002): Prevalence and etiology of mastitis in cows from two major Ethiopian dairies. *Trop. Anim. Hlth. and Prod.,* **34**: 19-25.

Yoseph, S. (1999): Fertility Status of Crossbred Dairy Cows Under Different Production Systems in Holeta, Central Highland of Ethiopia. M.Sc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University, School of Graduate Studies and Free University, Berlin Germany, Joint Program.

Zumbach, B. and Peters, K. J. (2000): Sustainable breeding methods for smallholder dairy production under unfavorable conditions in the tropics. In: Proceeding of, a contribution to crisis prevention. October 11-12, 2000, International agricultural research, Hohenheim, Pp 246-247.

8. ANNEX

Annex 1. Data Collection Format

Farm number __________
Date ________________

I. FARM IDENTIFICATION

Owners Name_________________________ Sex _____ Age _____Profession ________________ Level of Education Address Other Activities_____________

Illiterate________ Woreda _________

Elementary_______ Keble_________

High School ______ House No. _________

College________ Tel No. _________

II. DISCUSSION WITH THE FARM

1. Date of farm started_________________________ Origin of animals ____________________
2. Number of animal started ____________________ Number of animal at present____________

- Calf: Male ______ Female ____
- Heifers: _________
- Breeding Bull: ____
- Cow: ______

3. Breeding system used: AI ☐ Natural ☐ Both ☐
4. Feeding System: Grazing ☐ Stall-feeding ☐ Both ☐
   - Roughage: Farm produced ☐ Purchased ☐ Both ☐
   - Concentrate: Farm produced ☐ Purchased ☐ Both ☐
4. Health service provided: Vet ☐ AHA ☐ Traditional Healers ☐ Owner ☐

   If by Vet or AHA: How often: When called ☐ Periodically ☐
5. Who takes care of the animal?
   - Feeding: Owner ☐ Family worker ☐ Laborer ☐
   - Cleaning barns: Owner ☐ Family worker ☐ Laborer ☐
   - Milking: Owner ☐ Family worker ☐ Laborer ☐
6. Is there any practice of record keeping: Yes ☐ No ☐

If Yes: Breeding record ☐ Health record ☐ Feeding record ☐
Production record ☐ Financial record ☐ Others_____________

7. Routine prophylactic and deworming practice: Yes ☐ ☐ No

If yes: Vaccination against__________________________________

Deworming : as per established schedule ☐ as needed ☐

10. Commonly encountered health problems: indicate the degree and extent of each problem by putting them in rank order 1 to 10 where 1 indicates the most significant and 10 shows the least important.

_______Foot and leg problems
_______Metabolic problem
_______ Dystocia
_______Abortion
_______Bovine TB
_______Mastitis
_______Exoparsite(Tick and others)
_______Calf disease
_______Endoparsite
_______Meteritis
_______Retained fetal membrane
______Others(____)

11. Culling practice: Yes ☐ No ☐

If yes;

- Common causes of culling: Health problem ☐ Space shortage ☐ feed shortage ☐
  Reproductive problem ☐ Low production level ☐ ☐
  Others____
- Method of Removal: Selling Death ☐ Slaughter ☐

III. MILKING PRACTICE AND MANAGEMENT

A. Milking practice

1. Is milking practice manual? Yes ☐ ☐ No
   - Is there concentrate provision before milking: Yes ☐ No ☐
   - Is introducing calf before milking: Yes ☐ No ☐
   - Is there application of lubricants before milking: Yes ☐ No ☐

- Do you wash udder before milking: Yes ☐ No ☐
- Do you wash hand between milkings: Yes ☐ No ☐

2. Do you dry after milking: Yes ☐ No ☐
3. Do you use the same cloth for both teats: Yes ☐ No ☐
4. Frequency of Milking: Once ☐ Twice ☐ Three/ more times ☐
5. When due milk cow with mastitis: first ☐ Last ☐ Any stage ☐
6. Milking conducted: Barn ☐ Separate Parlor ☐
7. Performing CMT regularly: yes ☐ No ☐

B. Dry cow practice

1. Due practice dry cow therapy: Yes ☐ No ☐
2. Dry off time: one month before ☐ two month before☐ when milk turns yellow ☐
3. Dry off style: Abrupt ☐ Gradual ☐

C. Drug usage

1. Drugs used for treatment of any disease ____
2. Drugs used for treatment of mastitis ____
3. Is there a problem of cure after therapy: Yes ☐ No ☐

IV. FARM INSPECTION AND ANIMAL IDENTIFICATION

1. Housing
   - 1.1. Housing: closed type ☐ Semi open ☐ Open ☐
     Animals tied☐ loose☐
   - 1.2. Floors: concrete ☐ Stone layer ☐ Soil ☐
   - 1.3. Roof: Metal sheet ☐ Grass ☐ Others (specify) __________________
   - 1.4. Drainage/slope: Good ☐ Satisfactory ☐ Poor ☐
   - 1.5. Ventilation: Good ☐ Good ☐
   - 1.6. Space: Adequate ☐ Inadequate ☐
2. Farm cleanness: Good Satisfactory Poor ☐
3. Feeding trough space/Animal: Good ☐ Satisfactory ☐ Poor ☐
4. Water trough space/Animal: Good ☐ Satisfactory ☐ Poor ☐
5. Feed and water trough cleanness: Good ☐ Satisfactory ☐ Poor ☐
6. Hay and concentrate storage condition: Good ☐ Satisfactory ☐ Poor ☐

	Breed	Age	BCS	Parity	ST	AMY	F & L	General health	PMP	Udder	LT	Blind Teats	CMT Score		Sample	
Cow1													R	L	R	L
													R	L	R	L
Cow2													R	L	R	L
													R	L	R	L
Cow3													R	L	R	L
													R	L	R	L
Cow4													R	L	R	L
													R	L	R	L
Cow5													R	L	R	L
													R	L	R	L
Cow6													R	L	R	L
													R	L	R	L
Cow7													R	L	R	L
													R	L	R	L
Cow8													R	L	R	L

BCS: Body condition score ST: Stage of lactation AMY: Average milk yield F & L: Feet and legs PMP: previous mastitis problem

LT: Leaking teat RF: Right front LF: Left front RR: Right rare LR: Left rare Lactation stage: 1-3 months: Early
4-8 months: Middle $\geq$ 9 months: late Sat: Satisfactory Unsat: Unsatisfactory

Annex 2. Variables observed and measured during farm visit

Variables	*Category*	*Indicators or methods applied*
Housing condition		
housing type	Closed	Observation
	Semi-open	
	Open	
Space	Sufficient	Available space per animal
	Inadequate	
Ventilation	Good	Position and size of air inlets
	Poor	and outlets
Farm cleanness	Good	Drainage/slope of stable,
	Fair	cleanness of stable and animal
	Poor	
Feed storage	Good	Presence or absence of separate shade for feed
	Fair	storage and the condition under which feed is
	Poor	stored
Animal examination		
Age	dentition	Based on Amstutz (1998) (Annex_)
Body condition score	0-5 scale	Procedure described by Mathewman (1993)
		Annex-
Health status	Feet and leg	Visual inspection and palpation
	Udder and teat	Visual inspection and palpation
	General health	As described by Radostits *et al*. (2000)
	Milk	Visual inspection and CMT

The age of animals which were determined by the method described by Amstutz (1998) were further sustained by farm owners information

Annex 3. Interpretation of CMT result

Score	*Interpretation*	*Visible reaction*
0	Negative	Milk fluid and normal
1+	Trace (Weak positive)	Distinct precipitation but no gel formation
2+	Distinct positive	Mixture thickens with gel formation
3+	Strong positive	Viscosity greatly increased. Strong gel formation i.e. cohesive with convex surface

Source: Quinn *et al*. (1999)

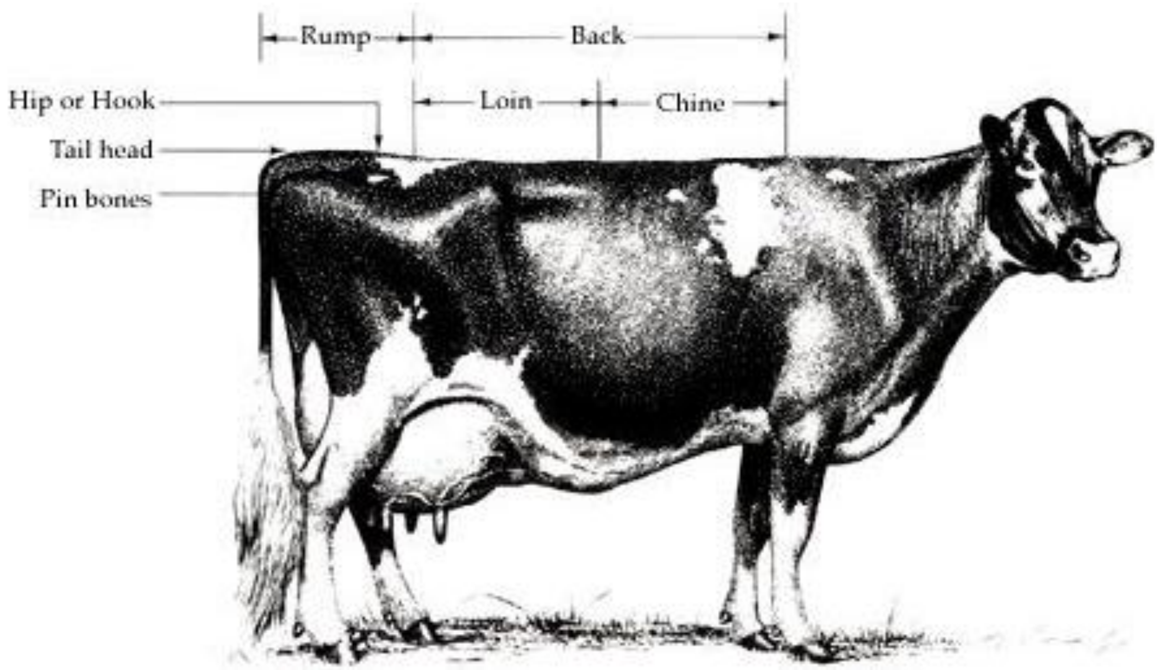
Annex 4. Age determination based on teeth eruption

Year	*Characteristic change*
1 ½-2	I_1 erupts
2-2 1/2	I_2 erupts
3	I_3 erupts
3 ½-4	C erupts
5	All incisors and canine in wear
6	I_1 is level and the neck has emerged from the gum
7	I_2 is level and the neck is visible
8	I_3 is level, neck is visible and C may be level
9	C is level and neck is visible
15	The teeth that have not fallen out are reduced to small round pegs

Source: Amstutz (1998)

Annex 5. Body Condition Scoring System

Fig 1. Anatomical regions used for determining BCS



Score of 1 (very poor body condition)

- Individual short ribs have a thin covering of flesh.
- Bones of the chine, loin, and rump regions are prominent.
- Hook and pin bones protrude sharply, with a very thin covering of flesh and deep depressions between bones.
- Deep cavity under tail and around tail head (between pin bones)
- Bony structure protrudes sharply, and ligaments and vulva are prominent.

Body condition score 2 (poor body condition)

- Individual short ribs can be felt but are not prominent.
- Ends of ribs are sharp to the touch but have a thicker covering of flesh.
- Short ribs do not have as distinct an "overhanging shelf" effect.
- Individual bones in the chine, loin, and rump regions are not visually distinct but are easily distinguished by touch.
- Hook and pin bones are prominent, but the depression between them is less severe.

- Area below tail head and between pin bones is somewhat depressed, but the bony structure has some covering of flesh.

Body condition score 3 (good body condition)

- Ends of short ribs can be felt by applying slight pressure.
- Short ribs appear smooth and the overhanging shelf effect is not so noticeable.
- The backbone appears as a rounded ridge; firm pressure is necessary to feel individual bones.
- Hook and pin bones are rounded and smooth.
- Area between pin bones and around tail head appears smooth, without signs of fat deposit.

Body condition score 4 (fat)

- Individual short ribs are distinguishable only by firm palpation.
- Short ribs appear flat or rounded, with no overhanging shelf effect.
- Ridge formed by backbone in chine region is rounded and smooth.
- Loin and rump regions appear flat.
- Hooks are rounded and the span between them is flat.
- Area of tail head and pin bones is rounded, with evidence of fat deposit.

Score of 5 (very fat)

- Bony structures of backbone, short ribs, and hook and pin bones are not apparent; subcutaneous fat deposit very evident.
- Tail head appears to be buried in fatty tissue.

Source: Mathewman (1993)

Annex 6. Composition and preparation of bacteriological medias used for culture

A) Blood Agar Base (BHIWADI-301019 Raj, INDIA)

Ingredients

Nutritive substrate (heart extract and peptone)	20g
Sodium chloride	5g
Agar-Agar	15.0g
pH	6.8±0.2 at 25 ^{0}C.

Instruction:

40g were suspended in 1 liters of deminralized water by heating in a boiling water bath and autoclaved at 121 ^{0}C for 15 minutes. Cooled to 45-50 ^{0}C and 5-7% sterile defibrinated sheep blood was added and mixed taking care to avoid bubble formation poured to plates.

B) Buffered Peptone Water (BHIWADI-301019 Raj, INDIA)

Ingredients

Peptone from casein	10.0 g
Sodium chloride	5.0g
Di-sodium hydrogen phosphate	3 g
Potassium dihydrogen phosphate	1.5 g

Instruction

Dissolve 20 g in 1 liter distill water, and sterilize by autoclaving at 121^{0}C for 15 minutes.

C) Nutrient Agar (BHIWADI-301019 Raj, INDIA)

Ingredients

Lab-Lemo powder	1.0 g
Yeast extracts	2.0 g
Sodium chloride	5.0 g
Agar	15.0 g
pH	7.4±0.2 at 25 ^{0}C

Instruction

20 g of media were suspended in 1 liter of distilled water brought to boil to dissolve completely. Sterilize by autoclaving at 121^{0}Cfor 15 minutes.

D) Manitol Salt Agar

Ingredients per liters

Protease peptone No. 3	10.0g
Bacto- Beef Extract	1g
D-manitol	10.0g
Sodium chloride	75g

Phenol Red	0.025g
pH	7.4±0.2 at 25^{0}C.

Instruction

To rehydrate, suspend 111 g in 1 liter distilled or deionized water and heat to boiling to dissolve completely. Sterilize in the autoclave for 15 minutes at 121^{0}C. Cool to 45-50 ^{0}C and dispense into sterile Petri dishes.

E) Brain Heart Infusion (BHIWADI-301019 Raj, INDIA)

Ingredients

Pancreatic digest casein	14.5g
Agar	5g
Brain Heart Solids from infusion	8g
Peptic digest of Animal Tissue	5g
Sodium chloride	5g
Dextrose	2g
Sodium Phosphate Dibasic	2.5g
Distilled water	1liter
pH 7.4 ± 0.2 at 25 ^{0}C	

Instruction

Dissolve 52 g in 1000 ml distilled water Stir and dissolve completely sterilized by autoclaving for 15 minutes at 121^{0}C .Cool to room temperature before use.

F) MacConkey Agar (BHIWADI-301019 Raj, INDIA)

Ingredients

Peptone	17g
Protease peptone	3g
Lactose	10g
Bile slat	1.5g
Sodium chloride	5g
Agar	15g
pH	7.1±0.3 at 25 ^{0}C
Distilled water	1 liter

Instruction

Dissolve 51.5 g in 1000 ml of distilled water, stir and dissolve completely, sterilize by autoclaving at 121 ^{0}C for 15 minute. Cool to room temperature before use.

G) MR-VP Medium (BHIWADI-301019 Raj, INDIA)

Ingredients

Peptone	10g
Dextrose	5g

Di potassium phosphate	5g
Distilled water	1liter
pH 6.9 ± 0.2 at 25 ^{0} C	

Instruction

Dissolve 17 g in 1000 ml distilled water. Gently heat to dissolve the medium completely. Distribute 10 ml amounts in each tube. Sterilize by autoclaving at 121 ^{0}C for 15 minutes.

H) SIM Medium

Ingredients

Peptone from casein	20g
Peptone from meat	6.6g
Ammonium iron (11) citrate	0.2g
Sodium thio sulphate	0.2g
Agar	0.3g

Instruction

Dissolve 30 g in 1000 ml, dispense into tubes to give a depth of 5 cm, autoclave at 121^{0}C for 15 minutes, allow solidifying in a vertical position.

I) Edwards Medium (BHIWADI-301019 Raj, INDIA)

Ingredients

Agar	15g
Peptic digest of animal tissue	10g
Beef extract	10g
NaCl	5g
Esculin	1g
Thallous sulphate	0.33g
Distilled water	1 liter
pH	7.4±0.2 at 25 ^{0}C

Instruction

Dissolve 41.33 g in 1000 ml of distilled water. Gently heat to dissolve the medium completely, sterilize by autoclaving at 121 ^{0}C for 15 minutes. Cool to 50 ^{0}C and aseptically add 5-7% v/v sterile bovine or sheep blood. Mix well before use.

J) Nutrient broth (BHIWADI-301019 Raj, INDIA)

Ingredients

Peptone	5.00g
Sodium chloride	5.00g
Beef extract	1.5g
Yeast extracts	1.5g
Distilled water	1 liter
pH	7.4 ± 0.2 at 25 0C

Instruction

Dissolve 13 g in 1000 ml distilled water. Gently heat to dissolve the medium completely. Distribute into tubes and sterilize by autoclaving at 121 ^{0}C for 15 minutes. Cool to room temperature before use.

K) OF Basal Medium (BHIWADI-301019 Raj, INDIA)

Ingredients

Sodium chloride	5g
Casein enzymatic hydrolysate	2g
Agar	2g
Dipotassium phosphate	0.3g
Bromothymol blue	0.08g
Distilled water	1 liter

Instruction

Dissolve 9.4 g in 1000 ml distilled water. Gently heat to dissolve the medium completely. Dispense in 100 ml quantities and sterilize by autoclaving at 121 ^{0}C for 15 minutes. To first 100 ml of sterile medium aseptically add 10 ml of sterile 10% Dextrose solution. To second 100 ml add 10 ml sterile 10% lactose solution. To third 100 ml add 10 ml sterile 10% Sacoharose solution. Mix and dispense in 5 ml amounts in sterile tubes in duplicate for aerobic and anaerobic fermentation.

Annex 7. Bacteria capable of causing mastitis showing some of their main characteristics leading to a presumptive identification.

Mastitis causing bacteria	Gram reaction	Shape	Catalase	Oxidase	Haemlysis	Growth on MacConkey agar	Aesculin hydrolysis	CAMP test	Other characteristics and confirmatory test
Streptococcus agalactiae	+	C	-	-	ß, γ, α	-	-	+	CAMP test positive
S. dysgalactiae	+	C	-	-	α	-	-	-	Alpha haemolytic, CAMP negative
S. uberis	+	C	-	-	γ, α	-	+	-	Aesculin–splitter, no growth on MacConkey agar
Entrococcus faecalis	+	C	-	-	γ, α	+	+	-	Pinpoint red colonies on MacConkey agar aesculin hydrolysis
S. pyogenes	+	C	-	-	ß	-	-	-	Susceptible to bacitracin (0.04 unit disc)
S. pneumoniae	+	C	-	-	α	-	±	-	Susceptible to optochin, often mucoid
Staphylococcus aureus	+	C	+	-	+	-	Golden yellow pigment; double zone haemolysis; coagulase positive and ferments maltose on purple agar base plus 1% maltose.		
Escherichia coli	-	R	+	-	±	+	'IMViC' test +/+/-/-. Metallic sheen on E MB agar. Occasionally mucoid, quit often haemolytic.		
Klebsiella pneumoniae	-	R	+	-	-	+	Mucoid colonies, non motile		
Enterobacter aerogenes	-	R	+	-	-	+	Mucoid colonies, motile ('IMViC' test -/-/+/+)		
Serratia marcescens	-	R	+	-	-	+	Red pigment at 25^{o}C, some strains at 37^{o}C		
Pseudomonas aeruginosa	-	R	+	+	±	+	Greenish-blue pigment, fruity smell		
Actinomyces pyogenes	+	R	-	-	+	-	Small colonies, hazy haemolysis.		
Pasteurella multocida	-	R	+	+	-	-	Colonies with sweetish smell. Non haemolytic, indole + and no growth on MacConkey agar		
P. haemolytica	-	R	V	+	+	+	No smell. Haemolytic, indole – and red, pinpoint colonies on MacConkey agar.		
Bacillus cereus	+	R	+	-	+	-	Forms endospores. Wide zone of haemolysis. large, flat, dry and granular colonies		

C= coccus, R= rod, + = positive reaction, ± = most strains positive, V = strains vary, - = negative reaction. 'IMViC' = indole, methylered, Voges- Proskauer and citrate tests.

Source: Quinn *et al*. (1994); NMC (1990).

Annex 8. Summary of the characteristics of the streptococci species that cause mastitis

	Heamolysis	Aesculin hydrolysis	Growth on MacConkey agar	CAMP TEST	Optochin susceptibility	Bacitracin susceptibility
Streptococcus agalactiae	ß, γ or α	-	-	+	•	R
S. dysgalactiae	α	-	-	-	R	•
S. uberis	α or γ	+	-	-	R	•
Entrococcus faecalis	α or γ	+	+	-	R	•
S. Pyogenes	ß	-	-	-	•	S
S. pneumoniae	α	-	-	-	S	•

+ = Positive reaction, **-** = negative reaction, **.** = not applicable, R = resistant, S= susceptible.

Source: Quinn *et al*. (1994); NMC (1990

Annex 9. Summary of the characteristics of the mastitis- causing coliform bacteria

	Esherichia coli	**Klebsiella pneumoniae**	*Serratia marcescens*
Motility	+(-)	-	+
Indole production	+	-	-
Methyle red (MR) test	+	- (+)	-
Voges-Proskaur (VP) test	-	+	+
Citrate utilization	-	+	+
Mucoid colonies	-(+)	+	+
Haemolysis	V	-	-

+ = Positive reaction, (+) = some strains positive, - = negative reaction, (-) = some strains negative, v = variable reaction.

Source: Quinn *et al* . (1994); NMC (1990).

Annex 10. Map of study area