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FACULTY OF VETERINARY MEDICINE

BACTERIOLOGICAL STUDY OF RAW MILK OF DAIRY GOATS WITH SPECIAL REFERENCE TO MASTITIS IN ADAMI-TULU JIDĐO-KOMBOLCHA DISTRICT, OROMIA REGIONAL STATE, ETHIOPIA

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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Medicine

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LIST OF ABBREVIATIONS

CMT	California Mastitis Test
CNS	Coagulase Negative Staphylococci
DMSCC	Direct Microscopic Cell Count
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization
IDF	International Dairy Federation
NCCLS	National Committee For Clinical Laboratory Standards
NMC	National Mastitis Council
RNA	Ribonucleic Acid
SCC	Somatic Cell Count

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ABSTRACT

A cross-sectional bacteriological study of goat milk with special reference to mastitis was carried out from September 2004 to March 2005 in Adami-Tulu Jiddo Kombolcha district. A total of 680 milk samples were collected from 30 flocks having 340 lactating goats.The prevalence of mastitis was 69.4% at goat level and 55% at halve level. The prevalence of clinical mastitis at animal level and halve level was 2.4% and 2.1% respectively, whereas the prevalence of subclinical mastitis at animal level and halve level was 67.1% and 53% respectively. Of all the flocks examined 30(100%) at least one animal was found infected with a mastitis pathogen. The flock prevalence ranged from 17.5% to100% of animals and from 11.8 % to 95% of the halves.

Two hundred seventy eight (40.9%) milk samples were CMT positive. Considering culture as a gold standard the sensitivity, specificity and agreement were 66.9%, 90.9% and 0.56 respectively. This indicates that CMT can be used for detection of subclinical mastitis in indigenous goats.

A total of 405 isolates were identified from 374 halves of 236 goats and they were diversified in species. The major organisms isolated were *Staphylococcus* spp. representing (22.5%) of the total isolates. *Staphylococcus aureus* was the dominant bacteria representing 12.8% of the total isolates and 57.1% of the *Staphylococcus* species. *Staphylococcus epidermidis* accounted for 9.6% of the total isolates and 42.9% of the *Staphylococcus* spp. *Bacillus* spp. were the second frequently isolated bacterial spp accounting for 10.4% of the total isolates. Other bacteria isolated were *E. coli* (7.9%), *P. aeuroginosa* (7.9%), *K. pneumoniae* (5.7%), *Acinetobacter* spp. (4.9%), *Micrococcus* spp (4.7%), *C. bovis* (4%), *C. ulcerans* (4%), *B. cereus* (3.5%), *Streptococcus* spp. (3.5%), *A. pyogenes* and *E. aerogenes* each (3.2%), *C. pseudotuberculosis* (3%), *S. marscenes* (2.7%), *P. mirablis* (1.2%), *R. equi* (1.2%), *C. freundii*, *C. diversus*, *Actinobacillus equlli* and *P. hemolytica* (each 0.9%), *Y. enterocolitica* and *Candida albicans* (each 0.5%), *Enterococcus fecalis*, *E. agglomerans*,*Y. pseudotuberculosis*, *P. vulgaris* and *P. multocida* (each 0.3%).

Drug susceptibility test was conducted on frequently isolated bacteria and major pathogenic species against eleven antimicrobials (Oxoid, Hamshire, England). All *S. aureus* spp. (100%), 17 (43.6%) *S. epidermidis*, 14 (100%) *Streptococcus* spp., 13 (100%) *A. pyogenes*, 10 (71.4%) *B. cereus*, 11 (34.4%) *E. coli*, 10 (43.5%) *K. pneumoniae* 8 (61.5%) were tested against polymyxin B, methicillin, cefoxitin, naidixic acid, erythromycin, cloxacillin, sulfonamide, chloramphenicol, gentamycin, penicillin G, streptomycin and oxytetracycline. Penicillin G was tested only for *Staphylococcus* spp.

When considering the over all antimicrobial resistance pattern, 75.7 % of the tested isolates were susceptible to the antimicrobials. The most effective drugs were gentamycin, polymixin B, sulfonamide and erythromycin. Nalidixic acid, cloxacillin, methicillin and streptomycin were less effective against the tested pathogens.

The most resistant bacteria were *K. pneumoniae* and *E. aerogenus*. However, they were100% susceptible to polymyxin B, nalidixic acid and gentamycin. *Escherichia coli* were resistant to methicillin, erythromycin and cefoxitin similar to *K .pneumoniae* and *E. aerogens*. The most effective drug against *E. coli* were polymyxin B, nalidixic acid and gentamycin. *Corynebacterium* species and *Arcanobacter pyogenes* were generally susceptible to all drugs except nalidixic acid. *Staphylococcus aureus* was resistant to penicillin and nalidixic acid. All isolates of *S. aureus* were susceptible to sulfonamide and gentamycin. About 82.7% of the *S. aureus* spp. were susceptible to methicillin whereas 17.3% were resistant.

Host risk factors such as udder characteristics (pendulous or non pendulous) and parity has got relation ($p<0.05$) with prevalence of mastitis. High producing goats with pendulous udder and multiparious goats were more susceptible to intrammary infection.

Key words: Lactating goats, mastitis, prevalence, CMT, drug susceptibility test, host risk factor, Adami-Tulu District, Oromia, Ethiopia.

1. INTRODUCTION

The goat (*Capra hircus*) is thought to have been the first animal domesticated for economic purposes and the first animal milked by man (Peacock, 1996). Livestock estimates of FAO (2002) states that a world goat population is 740,431,000 of which the continent Africa accounts for 222,275,000 representing 30.2 percent. Ethiopia possessing 17,000,000 goats stands second in Africa and fourth in the world (FAO, 2002).

Goats are exploited for diverse purposes including meat production, milk and cheese production, cashmere and mohair production, skin for clothes and leather making, cash income and security (Devendra and Burns, 1983).

The use of goat milk for human consumption is as old as the domestication of animals of economic value (Workneh and Peacock, 1991). In temperate countries where goats are used primarily or exclusively for milk production, claims have been made that goat milk has important advantage over cow milk for human consumption and there is great interest to improve the milk yield of goats to satisfy milk requirements and to raise the living standards of inhabitants (Mackenzie, 1980; Devendera and Burns, 1983).

In Ethiopia goats represent 5.3% of the total tropical livestock unit of the country and contribute an estimated 13.9% of meat and 10.5% of the milk production (FAO, 1999). Goats are used as source of food, such as meat, milk and blood, generate cash income, accumulate capital and fulfill cultural obligations in pastoral, agro-pastoral and agricultural production systems (Workneh and Peacock, 1991).

The relative importance of the goats has increased because of declining land holdings and shrinkage of grazing land. The small body size, broad feeding habits, adaptation to un favorable environmental conditions and their short reproductive cycle provide for goats comparative advantage over cattle and sheep to suit the circumstances of the poorer mixed crop–livestock environment These attributes make it easier to adjust goat flock size to match the available resources, facilitate the integration of live stock production into small scale production system (low capital, low risk) and enable flexible production (Peters,1991;Devendera, 1992)

In Ethiopia, all the known indigenous goat types are milked. Milk is widely utilized, Somalis and Boran pastoralists, Tigray, Hararaghe highlands, Arsi and Bale, South Omo and Sidamo (agropastoral), Afar, Surma use goat milk (Farm-Africa and ILRI, 1996). A study conducted by Workneh and Peacock (1991) indicated that 78.5% of the goat keepers in South of the country milk their goats. Goat milk is widely consumed in the study area (Adami-Tulu-Jiddo Kombolcha district).

The integration and full utilization of small ruminant particularly goats is constrained by various production factors of which high prevalence of disease, poor genetic potential, low plane of nutrition, poor management and extensive production systems are the most. Of these factors diseases are rampant with a significant impact on performance of goats (Kassahun, 1985; Ademsoun, 1994).

Despite many years of research, mastitis remains the most economically damaging disease for dairy industry worldwide irrespective of species of animal (Owens *et al.*, 1997). It is a disease that can cause devastating effects to a farmer because of serious economic losses (Ameh and Tarri, 2000). In addition, there is a danger that the bacterial contamination of milk from affected goats may render it unsuitable for human consumption by causing food poisoning or in rare cases provide a mechanism of spread of disease to humans.

Pervious studies conducted in different countries indicate the distribution and economic importance of the disease. Contreras *et al.* (1997) from Spain; Moshi *et al.* (1998) from Tanzania; Ameh and Tarri (2000) from Nigeria; Ndegwa *et al.* (2000) from Kenya and Kozascinki *et al.* (2002) from Croatia reported different prevalence rates of mastitis in goats.

In Ethiopia there is no published information on mastitis of dairy goats except clinical cases of mastitis in goats reported by Birhanu (2004). Therefore, the objectives of this study were:

1. To determine the status of clinical and subclinical mastitis in indigenous dairy goats,

2. To find out bacterial causes of goat mastitis,

3. To determine the diagnostic value of CMT for the detection of subclinical mastitis in goats,

4. To assess antmicrobial susceptibility pattern of the frequently isolated pathogens to the commonly used antimicrobials and

5. To determine the relationship of host risk factors such as udder characteristics and parity number to the prevalence of intramammary infection.

2. LITERATURE REVIEW

2.1. General

Mastitis is an inflammation of the parenchyma of the mammary gland regardless of the cause. It is characterized by physical, chemical and usually bacteriological changes in the milk and by pathological changes in the glandular tissue. It is one of the most complex and costly diseases of the dairy industry (Radostits *et al.*, 1994).

Mastitis may be classified as clinical or subclinical depending on the degree of inflammation (Rowe, 1999; Quinn *et al.*, 1999).

Subclinical mastitis

The infection in the mammary gland is detectable only by bacterial culture or by test to demonstrate a high leukocyte count in the milk. This form of the disease is important because it is 15 to 40 times more prevalent than the clinical form, it is of long duration, it reduces milk production, it adversely affects milk quality, and it constitutes a reservoir of microorganisms that can lead to infection of other animals within the herd.

Clinical mastitis

Clinical mastitis can be focal inflammation having very mild clinical signs or extensive and severe resulting in marked swelling and complete disruption of milk secretion. Clinical mastitis can be peracute, acute, or chronic.

Peracute mastitis: Swelling, heat, pain and abnormal secretion in the mammary gland is followed by signs of systemic disturbance. The clinical picture is usually dominated by depression, fever; dehydration, anorexia and a swollen, discolored gland. *Staphylococcus aureus*, *Pasteurella* spp., colifoms and *Pseudomonas aeuroginosa* are commonly cultured from these cases.

Acute: changes in the mammary gland are similar to those of peracute mastitis but the systemic signs are less severe.

Chronic: There are no systemic signs and very few external signs of changes in the udder, but abnormal secretion in the gland occurs intermittently.

In clinical mastitis of goats with marked changes to the udder, *S. aureus* is still the most commonly isolated organism (80%) of the cases. Some species of CNS such as *S. epidermidis*, coliforms, *Pseudomonas* spp., *Arcanobacterium pyogenes* and *Pasteurella* spp are also seldom isolated (Menzies and Ramanoon, 2001).

2.2. Significance of mastitis

The impact of mastitis can be categorized as economic loss and zoonotic potential. Economic loss caused by mastitis includes reduction in milk production, kid mortality, culling of goats, value of milk that has to be withheld from sale, reduction in quality of milk and cost of treatment (Radostits *et al.*, 1994). Bacterial contamination of milk from affected goats may render unsuitable for human consumption by causing food poisoning, or interference with manufacturing process, or in rare cases, provides mechanism of spread of disease to humans. Zoonotic diseases potentially transmitted by raw goat milk includes, brucellosis, caseous lymphadenitis, leptospirosis, listeriosis, melioidosis, Q-fever, Staphylococcal food poisoning, toxoplasmosis and tuberculosis (Smith and Sherman, 1994; Radostits *et al.*, 1994)

2.3. Etiology

Although variation exists on the type and isolation rate of mastitis pathogens from country to country, the most commonly incriminated and reported causes of infectious mastitis in goats include: *Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus hyicus, Streptococcus agalactiae, Streptococcus dyslgalaltiae, Streptococcus pyogenes, Streptococcus intermidus, Escherichia coli, Actinomyces pyogenes, Pseudomonas aeruginosa, Mycoplasma mycoides* subsp *mycoides* (large cocony), *Mycoplasma agalactiae, Mycoplasma puterificienes, Mycoplasma capricolum, Corynebacterium pseudotuberculosis, Bacillus coagulans, Nocardia asteroids, Listeria monocytogens and Bacillus cereus* (Smith and Sherman, 1994; Radostits *et al.*, 1994; Quinn *et al.*, 1999;

Ameh and Tarri, 2000; Ndegwa *et al.*, 2000; Kozacinsk *et al.*, 2002; Dewani *et al.*, 2002; McDougall *et al.*, 2002).

Coagulase negative staphylococci are the most frequent isolates from the caprine mammary gland. Ndegwa *et al.* (2001) isolated bacterial organisms on 630 clinically normal samples. Bacteria were isolated in 28.7% of the milk samples (181/630). The most prevalent bacterial organisms were *Staphylococcus* spp. (60.3%), followed by *Micrococcus* spp (17.7%), *Acinetobacter* spp. (5%), *Actinomyces* spp. (5%) and *Streptococcus* spp. (1.1%). The *Staphylococcus* spp. were mainly coagulase negative (64.3%). In another study conducted by McDougall *et al.* (2002) to determine the prevalence of bacterial isolation in 110 goats at parturition, 27.3% were found to be infected and coagulase negative staphylococci were the most common isolates.

For the ease of control, mastitis causing pathogens are categorized as contagious pathogens and environmental pathogens (Radostits *et al.*, 1994; Rowe, 1999).

2.3.1. Contagious pathogens

Contagious pathogens are those pathogens capable of being transmitted during milking procedures. Coagulase negative *Staphylococcus*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma* species are the contagious pathogens of greater consequence (Smith and Sherman, 1994; Rowe, 1999).

Staphylococcus aureus is often transmitted from doe to doe during milking procedures and results in elevated SCC, decreased milk production, palpable changes in mammary gland texture from mild to gangrenous (Radostits *et al.*, 1994). Gangrenous mastitis is caused by the alpha toxin that damage vessels, resulting in ischaemic coagulative necrosis of adjacent tissue. Both chronic and subclinical mastitis lead to gradual replacement of secretory tissue with fibrous tissue and a subsequent loss of milk production by the affected halves.

Streptococcus agalactiae resides in the milk and on the surface of milk channels but does not invade the tissue. There is rapid multiplication of bacterium with a great outpouring of neutrophils into the ducts with a damage into the ductual and acinar epithelium Fibrosis or

interalveolar tissue occurs, which also leads to loss of secretory function (Quinn *et al.*, 1999).

Several species of *Mycloplasma* are important causes of mastitis, polyarthritis, pneumonia and conjunctivitis. *Mycoplasma mycoides* subspp. *mycoides* (large colony type), *Mycoplasma puterificens*, *Mycoplasma agalactiae* and *Mycoplasma capricolum* all have been associated with herd outbreaks of mastitis and other diseases in goats. Many kids are infected at birth by ingesting colostroum and milk from infected does. Mycoplasmas are also spread from doe to doe during milking (Rowe, 1999).

Egwu *et al.* (2001) investigated the status of intrammary mycoplasmosis in caprine udder in Nigeria. Out of a total of 57 and 24 milk samples collected from udders of goat affected by mastitis and from apparently normal goat udder respectively, *Mycoplasma agalactiae* and *Mycoplasma capricolum* occurred at significant higher rate in udders affected by mastitis than normal healthy udders. *Mycoplasma bovis* and *Mycoplasma mycoides* sub spp. *mycoides* (large colony type) occurred at a low prevalence.

2.3.2 Environmental Pathogens

Escherichia coli, *Streptococcus dysgalactiae*, *Streptococcus uberis* and other environmental organisms are occasional goat mastitis pathogens. Maintenance of clean dry bedding areas, pre-milking teat dipping and milking clean dry teat of does are elements of environmental control (Radostits *et al.*, 1994; Rowe, 1999).

Pseudomonas aeurginosa is an important pathogen of infected herds. *Pseudomonas* spp often originates from a contaminated water sources in the milking process and may present as a severe and persistent herd infection, being transmitted between does once the infection is established in the herd. *Bacillus cereus* and other *Bacillus* spp. have been associated with mild to gangrenous inflammatory infection is the doe. *Bacillus* spp. may arise from soil or water sources (Rowe, 1999).

2.4. Epidemiology

2.4.1. Host-agent-environmental factors

Infection of the mammary gland is almost always via the teat canal except in the case of tuberculosis where the method of spread may be hematogenous. Interaction of the bacteria- host and environmental factors lead to mastitis (Radostits *et al.*, 1994).

Host risk factors: The host risk factors include breed, physiological state of the mammary gland, stage and number of lactation, teat shape, sphincter tone and immunological factors such as the level of IgA and IgG (Radostits *et al.*, 1994; Quinn *et al.*, 1999).

Agent factors: This includes the agent's ability to survive in the immediate environment of the animal, ability to colonize the teat duct, ability to adhere to the mammary epithelium, ability to resist phagocytosis and antibacterial substance in the udder including resistance to antibiotics (Quinn *et al.*, 1999).

Environmental factors: Environmental factors include milking practice, housing system, budding and management elements related to nutrition, early detection and treatment of clinical cases and removal of chronically infected goats (Schalm *et al.*, 1971).

2.4.2. Distribution of goat mastitis

The distribution of caprine mastitis is worldwide and different researchers from various countries reported different prevalence rates and pathogens at different isolation rates.

Contreras *et al.* (1995) from Spain conducted study on 369 milk samples from 188 goats. Intrammary infection was present in 18% of glands and 30% of goats. The isolated bacteria include staphylococci (71%), corynebacteria (12%), maycoplasmas (9%), enterobacteria (3%), pasteurellas (3%), streptococci (1%) and yeast (1%).

Mishira *et al.* (1996) from India bacteriologically analyzed 44 CMT positive milk samples and isolated *S. aureus* (34.1%), *E.coli* (18.2%) *P.aeuroginosa* (11.4%), *Bacillus* spp.

(9.1%), *Streptococcus* spp and *K. pneumoniae* (6.8%), *Aspergillus niger* (4.5%), *Candida albicans* (2.3%).

Contreras *et al.* (1997) from Spain conducted study on 131 goats and isolated 168 pathogens in the frequency of 82.5% *Micrococcus*, 9.5% Gram-negative bacilli and 8% *Corynebacterium* spp.

Topolko *et al.* (1997) tested 5605 goats and 11310 halves for sub clinical mastitis in Croatia between 1994 and 1996. They found 1435(12.86%) halves mastitis positive. The frequency of isolation were *S. aureus* (3.6%), *Micrococcus* spp. (2.3%), Gram- negative (*E. coli, Kelebsiella* and *P. aeuroginosa* 1.6%), *Streptococcus* spp (0.9%) and other bacteria (*P.hemolytica, Bacillus* spp., and *Corynebacterium* spp. 1.6%). *Streptococcus. agalactiae* was detected in only 0.1 % of the tested milk samples.

Moshi *et al.* (1998) examined 206 halves of 103 goats. The prevalence of mastitis at animal level was 80.5% and 72.8% at halve level. The isolates were *Staphylococcus* spp. (49.2%), *Streptococcus* spp. (14.2%), *Bacillus* spp. (4.8%), *E.coli* (26.9%), *Kelebsiella* spp. (1.6%) and 3.2% *Candida albicans.*

Bhujbal *et al.* (1999) from India analyzed 118 milk samples among which 66 (55.9%) were positive. The major organisms isolated were staphylococci, streptococci and *E. coli.*

Ameh and Tari (2000) from Nigeria conducted study on 300 goats. Fifty-one (17%) goats had mastitis. *Staphylococcus aureus,* CNS, *Bacillus* spp. *Corynebacterium pseudotuberculosis. P. aeuroginosa, E.coli, K. pneumoniae* and *P. mirablis* were isolated.

Dewani *et al.* (2002) from Pakistan conducted study on 50 milk samples and 46 (92%) were positive and the species of bacteria isolated were *A. pyogenes* (10.9%), *B. cereus* (6.5%), *E. coli* (10.9%), *N. aestroides* (6.5%), *P. vulgaris* (2.2%), *S. aureus* (19.6%) and *Str. uberis* (6.5%).

Kozacinski *et al.* (2002) form Croatia analyzed 912 milk samples and 247 (27.1%) were culture positive. Frequency of occurrence among 152 selected bacteria were 39.7%

Micrococcus, 29.8% *Staphylococcus* spp., 8% *Streptococcus* type D; 2.4% *B. cereus*, 0.4% *P. multocida*, and 0.4% *Kelebisella* spp.

McDougall *et al.* (2002) from USA conducted study on 110 goats and 30 (27.3%) milk samples were culture positive. Gomes *et al.* (2003) from Brazil reported a prevalence of 25.4% on a study conducted on 82 goats.

2.5. Diagnosis

2.5.1. Physical examination

The normal mammary gland of goats is bilaterally symmetrical and soft in texture. The superamammary lymph node in goats is normally barley palpable high in the rear udder. Heat, pain and swelling, firmness of the udder half, supramammary lymph node enlargement, irregularities of udder texture are indicators of clinical mastitis. Milk should be examined for flakes, clots, other changes in milk composition or color change (Rowe. 1999). Particular care has to be given in clinical examination of goat milk because of its apparent normality when there are severe inflammatory changes in the udder (Radostits *et al.*, 1994; Quinn *et al.*, 1999). Other indications of clinical mastitis in goats include. lameness on the affected half as the goat attempts to avoid contact of the hind limb with the tender half of the udder, and increased kid mortality (Smith and Sherman, 1994).

2.5.2. California mastitis test (CMT)

The CMT is a simple, semi quantitative test for determining the number of nucleated cells (both neutrophils and epithelial cells) in the milk. Equal quantities of milk and commercial reagent, which contains 3% alkyl aryl sulfonate and bromocresol purple as a pH level indicator, are mixed in the cup on a white paddle and swired. Trace reaction consists of a slight thickening of the milk. Scores of 1, 2 and 3 are given with increasing gel formation (Smith and Sherman, 1994).

Fresh unrefrigerated milk can be tested using the CMT for up to 12 hours. Reliable readings can be obtained from refrigerated milk for up to 36 hours. If stored milk is used. the milk must be thoroughly mixed prior to testing because somatic cells tend to segregate

with milk fat. The CMT reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time. The degree of reaction between the detergent and the DNA of nuclei is a measure of the numbers of somatic cells in milk. The threshold for CMT scores depends on the objective of the study. If it is used to minimize the rate of false negatives, the test should be read as negative versus positive with trace scores regarded as/ recorded as positive. If the CMT is to be used in culling decisions, a threshold with a lower rate of false positives may be desirable (Larsen, 2000).

Goat milk has naturally a higher concentration of epithelial cells than cow milk. It is generally believed that scores of trace or 1 (up to 1 million cells per ml of milk) are no reason for concern (Guss, 1992; Rowe, 1999), Table 1. The negative, trace, 1, 2 or 3 categories of result are less sensitive than quantitative SCC but are helpful to screen animals during physical examination, while plating milk samples for culture or at other times when quantitative SCC is not available. Comparison of CMT results between milk from different halves is useful for detecting infected halves (Rowe, 1999).

Table 1: The relation between CMT scorers and somatic cell count of goat milk

Score	Scand. system	Number of samples examined.	Neutrophil leukocyte /ml	
			Range	Median
0	1	46	0-480,000	60,000
Trace	2	43	0-640,000	270,000
1	3	29	240,000-1,440,000	660,000
2	4	16	2,080,000-5,850,000	2,4000,000
3	5	6	Over 10,000,000	-

Source: Smith and Sherman (1994)

2.5.3. Somatic cell counts (SCC)

Epithelial cells, neutrophils and macrophages are normally present in low numbers in the secretion of an uninfected mammary gland. In infected goat mammary gland, somatic cell counts greatly increase in a manner similar to the somatic cells in cow milk due to a dramatic increase in neutrophils. However, many additional factors are associated with increased SCC in goats, consequently high somatic all counts in the goats are less predictive of intramammary infection than are SCC in cow milk (Rowe, 1999).

The relation of somatic cell count to caprine mastitis is limited, unless test appropriate to caprine milk are used. This is partly because goat milk differs from cow milk due to the presence of cytoplasmic particles and epithelial cells. Milk secretion from the caprine mammary gland is apocrine secretion in contrast to the merocrine secretion of bovine milk. Portions of the cytoplasm of the epithelial cells are pinched off and appear in the milk as DNA free particles similar in size to leukocyte. Also present in variable number in goats are intact epithelial cells sloughed from acini and ducts (Smith and Sherman, 1994). Methods of SCC determination using DNA detection such as fossomatic or direct microscopic examination techniques are appropriate for caprine SCC (Rowe, 1999). In spite of the additional factors with increased SCC in uninfected does, surveillance of SCC is still an extremely useful indicator of mammary gland health. Herds on diary herd improvement association testing programs receive monthly report of individual doe SCC. Sudden increase in SCC or chronic elevation in SCC are indicators of intramammary infection and can be used to identify suspicious does by milk culture. Similarly a decrease in SCC following intramammary therapy is a useful adjunct to culture following in assessing response to treatment (Rowe, 1999).

Inadequacy of much of the literature is in the realm of determining mean SCC. Healthy dairy goats can be expected to produce milk with a somatic cell count under 500,000 cells per milliliter. The presence of mastitis infection in dairy goat herds is reflected in bulk tank milk samples with a CMT of 1 or higher and somatic cell exceeding 10^6 cells/ml of milk (Guss, 1992). Among many cell counting methods developed the common ones are electronic SCC using equipments such as coulter counter, Fossomatic method and Direct microscopic somatic cell count (Quinn *et al.*, 1999).

Coulter counter: Coulter counter enumerates particles as milk follows past electronic eye. Because cytoplasmic particles are similar in size, they too are counted in goat milk. Certain counters with channels that permit categorizing cells by cell diameter may improve differentiating mastitis from non-mastitis samples (Smith and Sherman, 1994).

Fossomatic Counter: It is used for determining somatic cell counts by an automated fluorescent technique that uses a dye that specifically bind to the DNA of the cell nuclei. Both epithelial cells and leukocytes are counted, but counts are not confounded by cytoplasmic particles (Eriskine, 2001).

Direct microscopic somatic cell count (DMSCC): The DMSCC, using a stain appropriate for goat milk is the standard against which other counting methods have to be judged. In this case leukocytes can be counted directly (Smith and Sherman, 1994). A known volume of milk (0.01ml) is spread over cm^2 on a microscopic slide, defatted and stained by a methyl blue-based stain the microscope is calibrated and from an average number of leukocytes per field (counting 50 fields) the number of leukocytes/ml of milk can be calculated. If comparatively large numbers of pathogenic bacteria are present in the milk sample, these may also be seen in the stained smear (Quinn *et al*., 1999).

The Levovitz - Weber modification of the Newman-Lamvert stain is commonly used to stain somatic cell for counting. This stain is inappropriate for goat milk because staining is similar for cytoplasmic particles and cells. Currently the stain preferred for determining SCC in goat milk is the pyronin Y-methyl green stain, often refereed to as green stain. Methyl green is specific for DNA and pyronin Y is specific for RNA. Chromosomes, then, stain blue-Lavender while cytoplasm particles and the cytoplasm of epithelial cells stain red. Leukocytes and epithelial cells in goat milk have also been differentiated by a modified Wright's stain technique (Smith and Sherman, 1994). Leukocytes in milk samples disintegrate quite rapidly on storage so the cell counts should be conducted within 2 hrs of milk collection. Alternatively formalin could be added to the milk samples as a preservative (Quinn *et al*., 1999).

2.5.4. Other tests correlated with mastitis

Some tests are based on the increase in sodium and chloride ions in mastitic milk and consequently an increase in electrical conductivity of the milk (Eriskine, 2001). The chloride content of goat milk is greater than in cow milk, with means ranging from 121 to 204 mg/ml bacterial infectious in the udder alter the cell wall permeability and permit an increased flow of sodium and chloride into the milk (Smith and Sherman, 1994). N-acetyl-B-D-glucosaminidase is a cell associated enzyme in the milk and high level of the enzyme indicate high cell count. NAGase test is easily automated (Quinn *et al.*, 1999; Eriskine, 2001).

2.5.5. Microbiological investigation of mastitis

2.5.5.1. Culture

The microbiological examination of both individual goat and bulk tank milk culture are elements of mastitis control. Most mastitis control programs include the use of individual goat cultures to determine which mastitis pathogens are present on the farm. Culturing can be used in a targeted fashion for specific control programs such as segregation plans for contagious mastitis or for surveillance to detect the presence of new or emerging pathogen. Culturing is also used to evaluate treatment efficiency and to establish susceptibility patterns to aid in the development of rational treatment strategies (Larsen, 2000).

Sensitivity of bacterial culture could be increased by including second and third consecutive samples as this overcomes the problem of cyclic shedders (Sears *et al.*, 1993). The most widely accepted criterion for the diagnosis of intramammary infection is that when the same organism is isolated from two samples or two of the three consecutive samples taken every other day.

Most of the bacterial pathogens causing mastitis grow on ox or sheep blood agar. A MacConkey agar plate is streaked in parallel to detect *Enterococcus faecalis* and any other Gram-negative bacteria that are able to grow on the medium. Edwards's medium is highly selective for streptococci and also acts as an indicator medium for haemolysis and for

hydrolysis of aesculin. A Sabourauds dextrose agar plate can be inoculated if a fungal pathogen is suspected. If large numbers of milk samples are to be cultured on a herd basis, half-plating the samples on aesculin blood agar, alone, is satisfactory. The inoculated plates are incubated aerobically at 37^0C for 5 days to accommodate the slow growing fungi and bacteria such as *Nocardia asteroides* (Quinn *et al.*, 1999).

2.5.5.1.2. Identification

Subculture of the bacterial colony type(s) considered most significant should be made to obtain pure culturing for use in identification tests (Carter, 1984).

Primary identification of bacteria: Once a pure culture is obtained, the results from a few comparatively simple tests can often identify the bacterium to genus level (Quinn *et al.*, 1999). A Gram-stained smear from the culture will establish the Gram reaction (Gram-positive or Gram-negative), and the cellular morphology (coccus or rod). The other tests include growth or absence of growth on MacConkey agar, catalase and oxidase tests, motility tests and an oxidation-fermentation (O-F) tests.

Secondary biochemical tests for the identification of bacteria: Once the bacterium has been identified to a generic level, further tests can be carried out to identify the species. Secondary biochemical tests can be grouped in the following categories (Carter, 1984; Quinn *et al.*, 1999). Some commonly used biochemical tests used for the identification of bacteria include aesculin hydrolysis, carbohydrate fermentations, citrate utilization, decarboxylase (lysine and ornithine), hydrogen sulphide, indole, methyl red (MR), nitrate reduction, phosphatase, urease and Voges-Proskauer (VP) tests.

2.6. Antimicrobial susceptibility testing

The major obstacle in treating mastitis is antimicrobial resistance. Antimicrobial susceptibility testing is useful to carry out effective treatment because certain microorganisms are resistant to certain antimicrobials. For example *Staphylococcus* spp. are resistant to penicillin (Smith and Sherman, 1994).

Although there are several laboratory methods for measuring the invitro susceptibility of bacteria to antimicrobial drugs, the agar disc diffusion technique is used commonly using Kirby-Bauer disc diffusion method. (Quinn *et al.*, 1999).

A standard method has been proposed by the National Committee for Clinical Laboratory Standards (NCCLS, 1990). The disc diffusion method entails preparing a uniform lawn of the test bacterium on an agar plate and placing paper discs, each impregnated with an antimicrobial of known concentration on the agar surface before incubation. Strict standardization of the test procedures is important because many factors influence the zone of inhibition (Baron *et al.*, 1994; Quinn *et al.*, 1999). The size of inoculum, the turbidity of the inoculum is adjusted to a 0.5 McFarland opacity standard to have dense lawn of bacterial growth with the individual colonies just touching each other. Mueller-Hinton or modification of this medium is usually chosen for routine susceptibility test. For bacteria that are unable to grow on Mueller-Hinton agar such as *Streptococcus* and *Actinomyces pyogenes*, blood agar with 5-10 percent defibrinated sheep blood can be used.

The ability of the antimicrobial agent to diffuse through the agar varies and the zones inclination for some drugs, such as streptomycin, is always imperatively small. The concentrations of the antimicrobial agents in the discs have been chosen to give zone sizes that correlate with achievable serum level in the patient. Incubation conditions have been standardized for routine susceptibility tests under aerobic incubation at 35^0C for 16 - 18 hours and 24 hours for staphylococci. The diameter of zone of inhibition are measured to the nearest mm using a rule or calipers. The bacterium is reported susceptible, moderately susceptible, intermediate or resistant to each antimicrobial agent used in the test (Quinn *et al.*, 1999; Carter, 1984).

2.7. Treatment, control and prevention

Treatment and control of mastitis techniques to be used in goat does can be adapted from those used for cattle supported by a laboratory culture and antibiotic sensitivity examination. Both udder infusion and systemic antibiotic administration in case of acute severe mastitis is recommended (Guss, 1992)

The objectives and procedures for mastitis control and prevention are (Radostits *et al.*, 1994):

- Reducing the duration of infection: involves the treatment of lactating does, dry period treatment and culling of chronic clinical cases,
- Reducing the new infection rate: includes milking hygiene especially disinfecting, servicing and maintaining milking machinery, and other relevant management procedures and
- Monitoring the infection rate: test mastitis using simple test or somatic cell counts. This is done at the herd level, at the doe and halves level at regular intervals.

3. MATERIALS AND METHODS

3.1. Study Area

Adami-Tulu Jiddo Kombolcha district is situated in the mid-rift valley east showa zone of oromiya regional state (Figure 1), 167 kms south of Addis Ababa. It has an altitude of 1650 meters above sea level with a bimodal unevenly distributed rainfall pattern. The average annual rainfall for the last 10 years was 760.9 mm and consisted of short rainy season (February to April) and long rainy season (July to September). Adami-Tulu has a minimum mean temperature of 12.7^{0}C with a relative humidity of 760.Vertisol is the predominant soil type with sand-silt-clay in the proportion of 33:48:18, respectively and has a pH of 7.9.The predominant land around the area is suitable for livestock production, goats being the most selected for breeding purpose next to cattle. Goat milk is widely consumed in the area. According to district's agricultural office, there are about 189,870 cattle, 85,365 goats, 11,330 sheep, 17,356 donkey, 256 mule, 1442 horses and 80,270 poultry in the district.

Figure 1: Map showing the study area

3.2. Study Population

The study animals were lactating does in Adami-Tulu district in selected peasant associations and households. According to district's agricultural office, there are 85,365 goats out of which 14,426 are lactating. The predominant goat breeds in the study area are Arsi-Bale breeds, which are managed under extensive system. Traditional housing, feeding and milking procedures are generally practiced. Farmers who own more than five goats keep them in barns while those who own less tan five goats keep them in their own houses. The source of feed is traditional grazing and browsing. Most of the farmers milk their goats in the morning once a day while few of them twice a day and they do not follow pre milking and post milking procedures.

3.3. Study design

A cross-sectional study was conducted from September 2004 to March 2005 by collecting milk samples from lactating goats in Adami-Tulu district. Characteristics of udder attachment (attached or pendulous), parity, any abnormalities of udder and milk was recorded. Milk sample was collected aseptically and transported in an icebox to the laboratory for microbiological analysis. CMT was conducted on collected milk samples and the result recorded. Microbiological culturing was conducted irrespective of CMT results for isolation and identification of bacteria. Antimicrobial susceptibility test was conducted for frequently isolated and major species. The number of isolates from each species to be tested was based on their frequency of isolation. For laboratory techniques and data collection standard procedures was followed (Quinn *et al.*, 1999).

3.3.1. Prevalence study

The prevalence of mastitis was determined cross sectionally from September 2004 to March 2005 at goat level and at halve level based on clinical manifestations for clinical mastitis and indirect tests (CMT and culture) for subclinical prevalence. Prevalence was calculated according to the formula recommended by Thrustfield (1995).

$$P = \frac{\text{No. f animals with disease}}{\text{No. of animals at risk}}$$

3.3.1.2. Definitions

This definition was according to International Dairy Federation recommendation, IDF (1987).

Clinical mastitis: A goat or halve was considered clinically sick for mastitis when abnormality was observed in milk (the presence of flakes, clots, bloody or watery appearance), in the udder (like swelling, pain, hottness) or in the goat (systemic signs together with the above manifestations).

Subclinical mastitis: A goat or halve was considered to have subclinical mastitis if CMT score was 1, 2, 3 or when a mastitis pathogen was isolated.

3.3.2. Sample size and sampling method

Since there was no any previous study conducted on goat mastitis, sample size was determined according to Thrusfield (1995) recommendation by taking 20%, for a disease of unknown prevalence. The sampling method used was a two-stage cluster sampling by fixing the total sample size. One household was considered as one cluster. The study was planned to include 340 lactating goats and the number of clusters (g) to be included in the study was calculated using the formula for two-stage cluster sampling as follows:

$$g = \frac{1.96^2 \, Ts \, Vc}{d^2 \, Ts - 1.96^2 \, pex \, (1-pex)}$$

Where, g = no. of clusters(house holds)

Ts = Total sample size

Vc = cluster variance,

$Vc = s.d^2$, $s.d = pexp^2$

s.d = standard deviation

Pexp = expected prevalence

d = desired absolute precision

If Pexp = 20% d = 0.05, then , g = 9.

When we divide 340 by 9 to get the number of animals in each cluster it was 38 goats. In this particular case since we cannot get 38 lactating goats in 9 households, 21 households were added making the total households included in the study 30. Ten PA's (Peasant Associations) were randomly selected and three households were selected randomly from each PA.

3.3.3. Study methodology

3.3.3.1. Physical examination

Udder attachment, parity number, any physical abnormalities such as swelling of the udder, presence of lesions, anatomical malformations and tick infestation were recorded. The milk was examined for its color, odor, consistency and other abnormalities.

3.3.3.2. Milk sample collection

Aseptic procedures for collecting milk samples recommended by Schalm *et al.* (1971). Sears *et al.* (1993) and Quinn *et al.* (1999) were followed. The time chosen for milk sample collection was before milking. Udders and especially teats were cleaned and dried before sample collection. Each teat end is scrubbed vigorously with a pledged of cotton moistened with 70% ethyl alcohol. A separate pledged of cotton was used for each teat. The first few streams of milk was discarded and 10 ml of milk was collected into horizontally held vial. After collection, the sample was placed in an icebox and transported to the laboratory for analysis.

3.3.2.3. California mastitis test (CMT)

The California Mastitis was carried out using the method described by Schalm *et al.* (1971) and Quinn *et al.* (1999). A squirt of milk, about 2ml from each half was placed in each of two shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 15 seconds. The result was interpreted and scored from 0-3 (Annex 1).

3.3.2.4. Bacterial isolation

Bacteriological examination of the milk was carried out irrespective of CMT results following standard procedures (Carter, 1984; Sears *et al.*, 1991; Quinn *et al.*, 1999). Milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature (25^0C) for about an hour and then mixed by shaking. The samples were allowed to stand for a while for the foam to disperse and just before inoculation the tube was inverted gently (NMC, 1990).

One standard loop (0.01ml) of milk sample was streaked on 7% sheep blood agar (BBL®, Becton Dickinson, USA). The inoculated plates were incubated aerobically at 37 ^{0}C. The plates were checked for growth after 24, 48, and 72 hours to rule out slow growing bacteria. The plates were examined for growth, morphologic features such as colony size, shape, color and hemolytic characteristics. Suspected colonies were sub-cultured onto blood agar and MacConkey agar (Oxoid, Hampshire, England) plates for further investigation. For primary identification of bacteria, once a pure culture is obtained, the results from a few comparatively simple tests can often identify the bacterium to a genus level (Quinn *et al.*, 1999). A Gram-stained smear from the culture will establish the Gram reaction (Gram-positive or Gram-negative), and the cellular morphology (coccus or rod). The other primary tests include growth or absence of growth on MacConkey agar, catalase and oxidase tests, motility tests and oxidation-fermentation (O-F) tests (Annex 2, 3, 4 and 5).

For secondary identification of bacteria to species level examination of other biochemical or metabolic properties of the organism in question are required as indicated in Annex 6, 7, 8, 9, 10 and 11 (Carter, 1984; Quinn *et al.*, 1999).

3.3.2.5. Antimicrobial susceptibility test

Susceptibility of bacteria to the commonly used antimicrobials was conducted using Kirby-Bauer method. The selections of antimicrobial agents for use in the disc diffusion test depend on clinical considerations, drugs that are available and in general use, mode of

action of antimicrobials. Based on this consideration the following antimicrobials were used (Table 2). Streptomycin, tetracycline, erythromycin, penicillin G, gentamycin, chloramphinicol, polymyxin B, cloxacillin, sulfonamide, methicillin, cefoxitin, nalidixic acid were selected (Oxoid, Hampshire, England). The procedure was as recommended by Carter, 1984 and Quinn *et al.*, 1999.

A distinct colony was inoculated into 5ml of nutrient broth and incubated at 35-37^{0}C for about 5 hours. Then the turbidity is compared with 0.5 McFarland standard. This standard is prepared by adding 0.5 ml of Solution A (0.048M $BaCl_2 2H_20$) to 99.5ml of solution B (0.18 M H_2SO4).

Mueller-Hinton Agar was cooled to 50^{0}C and poured into a sterile Petri dish on a level surface to a depth of 4 mm.. For slow growing bacteria such as *Streptococcus* spp. and *Arcanobacter pyogenes*, 5% defibrinated whole blood was added. Then a sterile cotton swab on a wooden applicator stick is used to transfer the diluted bacterial suspension to a plate; excess fluid was squeezed out by rotating the swab against the sides of the tube. The plate was seeded uniformly by rubbing the swab against the entire agar surface in three different planes roughly 60 degrees to each other.

After the plates were inoculated within 15 minutes (time used to dry the inoculum), antimicrobial impregnated discs were applied to the surface of the inoculated plates by using a sterile forceps. All discs were gently pressed down on the agar surface with forceps to ensure complete contact with the agar surface. The discs were placed 1.5 cm from the edge of the plate and 3 cm apart from each other. The invented plates were incubated aerobically for 16 to 18 hours and 24 hours for *Staphylococcus* at 35^{0}C. Inhibition zone was measured in millimeters using a caliper on the under surface of the petri dish. For measuring purpose the end point was taken as complete inhibition of growth as determined by naked eye. The result was interpreted according to Table 2.

Table 2: Zone size interpretive chart for antimicrobials (inhibition zone diameter in mm)

Antimicrobial agent	Disc potency	Resistance(≤)	Intermediate	Susceptible (≥)
Streptomycin	10µg	11	12-14	15
Oxyetracycline	30µg	14	15-18	19
Erythromycin	15µg	13	14-22	23
Penicillin G for staph.	10U	28	-	29
Methicillin	5 µg	10	11-12	13
Gentamycin	10µg	12	13-14	15
Chloamphinicol	30µg	12	13-17	18
Polymyxin B	300U	8	9-11	12
Cefoxitin	30 µg	14	15-17	18
Nalidixic acid	30 µg	13	14-18	19
Cloxacillin	5 µg	10	11-12	13
Sulfonamide	300 µg	12	13-16	17

Source: Carter, 1984; Quinn *et al.*, 1999

3.4. Data entry and analysis

For data entry, culture positive was denoted by 1 and culture negative by 0. Udder characteristic pendulous was denoted by1 and attached by 0. Goats with parity number four and greater was recorded as four.

Percentage with confidence interval was used for determination of prevalence rate and chi-square for significance of difference. Association of host risk factors with prevalence of intramammary infection was calculated using Stata 7 Software. Sensitivity, specificity and Kappa statistics were calculated using Win Episcope 2 Software.

4. RESULTS

4.1. Physical examination

Physical examination and visual inspection was conducted on udder, teats and milk of 340 lactating goats and abnormalities such as clinical mastitis, udder injuries, tick infestation, supernumery teats and change in milk were observed (Table 3).

Table 3: Prevalence of physical abnormalities of udder and milk of goats

Physical condition	No.	Percentage
Clinical mastitis	8	2.4
Udder injuries	4	1.17
Tick lesion	3	0.88
Supernumerary teats	15	4.41
Change in milk	6	1.76

4.2.Sensitivity, specificity and test agreement of California mastitis test

California mastitis test was conducted on 680 milk samples collected from 340 lactating goats for the presence of subclinical mastitis. Considering CMT scores of 0 and T as negative and 1, 2, 3 as positive, 278 milk samples were CMT positive, while 402 samples were CMT negative. To assess the diagnostic value of CMT as a diagnostic tool for indigenous goat percentage agreement at each CMT level was calculated (Table 4).

Table 4: Status of culture at different CMT level

Agent	0	T	1	2	3	Total
Negative	248	30	21	6	1	306
Positive	80	44	143	74	33	374
Total	328	74	164	80	34	680
% Agreement	75.6	40.54	87.2	92.5	97.05	

Considering CMT scores of 0and trace as negative and 1, 2, and 3 as positive, the sensitivity, specificity of CMT was calculated by Win Episcope 2 using culture as gold standard (Table 5).

Table 5: Comparison of culture and CMT status

Culture	CMT Status		Total
	Negative	Positive	
Negative	278	28	306
Positive	124	250	374
Total	402	278	680

The sensitivity and specificity of CMT was 66.9% and 90.9% respectively. Test agreement between CMT and culture using kappa statistics was 0.56, which is a moderate agreement.

4.3. Bacterial isolation

Milk sample was collected from 680 halves of 340 lactating goats. A total of 374 halves, 177 right halve and 197 left halves from 236 goats were culture positive. The prevalence at halve level was 55% and 69.4% at goat level. One hundred thirty eight animals had bilateral (40.6%) infection and 31(4.6%) milk samples yielded more than one bacteria. The prevalence of clinical mastitis at animal level and halve level was 2.4% and 2.1% respectively. The prevalence of subclinical mastitis at animal level and halve level was 67.1% and 53% respectively (Table 6 and 7).

Table 6: Prevalence of clinical and subclinical mastitis at goat and halve levels.

Flock size	No. of farms	No. of goats	Clinical mastitis	Subclinical mastitis	Clinical mastitis	Subclinical mastitis
					Halve	Halve
Small	15	113	3	73	5	116
Intermediate	15	227	5	155	9	244
Total	30	340	8	228	14	360

By categorizing households, which have less than ten goats as small and those, which has greater than ten as intermediate, the relation of intramammary infection to flock size was assessed. The result showed insignificant difference ($p>0.05$) in the categories of flock size in relation to prevalence of mastitis (Table 7).

Table 7: Prevalence of mastitis related to flock size

Flock size	Number of flocks	Number of goats		Prevalence (95%CI)
		Examined	Positive	
Small	15	113	76	67.3%
Intermediate	15	227	160	70.5%
Total	30	340	236	69.4%

$\chi 2$ =2 (1), p= 0.157 insignificant at p >0.05

All flocks had intramammary infection and the flock prevalence ranged from 17.6% to 100%. The number of infected animals ranged from 3-18 and all animals in five flocks were infected. The flock prevalence at halve level ranged from 11.8 %-88.9 (Table 8).

Table 8: Prevalence of mastitis at animal level and halve level by flock

Flock	No.of goats		Goat level (%)	No. of halves infected	Halve level (%)
	Examined	Infected			
1	10	7	70	12	60
2	8	5	62.5	5	31.3
3	6	4	66.7	6	50
4	8	4	50	6	37.5
5	11	7	63.6	8	36.4
6	13	9	69.2	14	53.9
7	5	2	40	3	30
8	8	2	25	3	18.8
9	11	9	81.8	16	72.7
10	9	6	66.7	12	66.7
11	6	9	100	10	83.3
12	11	10	90.9	17	77.3
13	10	7	70	9	45
14	5	3	60	4	40
15	15	7	46.7	13	43.3
16	7	6	85.7	12	85.7
17	8	5	62.5	8	50
18	5	5	100	7	70
19	16	7	43.8	8	25
20	8	4	50	5	31.3
21	17	3	17.6	4	11.8
22	14	6	43	9	32.1
23	17	17	100	24	70.6
24	14	1	78.6	19	67.6
25	18	14	77.8	25	69.4
26	10	10	100	19	95
27	18	13	72.2	21	58.3
28	14	11	78.6	14	50
29	20	18	90	29	72.5
30	18	18	100	32	88.9
Total	340	236		374	

Out of the total 680 milk samples examined bacteriologically 405 isolates were obtained from 374 halves of 236 animals. The bacteria were diverse in species. The most predominant species were *Staphylococcus* species accounting for 22.5% of the total isolates. Among the *Staphylococcus* species *S. aureus* was the most dominant and accounts for 57.1% of the *Staphylococcus* spp. and 12.8% of the total isolates. *Staphylococcus epidermidis* accounts for 9.6% of the total isolates and 42.9% of the *Staphylococcus* spp. *Bacillus* spp. were another frequently isolated bacterial spp. (10.4%). Other bacteria isolated were *E. coli* (7.9%), *P. aeuroginosa* (7.9%), *Acinetobacter* spp. (5.4%), *Micrococcus* spp (4.7%), *C. bovis* (4%), *C. ulcerans* (4%), *C. pseudotuberculosis* (3%), *B. cereus* (3.5%), *Streptococcus spp.* (3.5%) and *A. pyogenes* (3.2%). Fourteen halves of eight animals had clinical mastitis. *Staphylococcus aureus* (8), *E. coli* (2), *K. pneumoniae* (1) and *P. aeuroginosa (1)* were isolated from these clinical cases of mastitis (Table 9).

Table 9: Frequency distribution of bacteria isolated from clinical and subclinical mastitis

Microorganisms	Clinical mastitis	Subclinical mastitis	Total	%	% of animal	% of flock
Staphylococcus epidermidis	-	39	39	9.6	9.7	76.7
Staphylococcus aureus	8	44	52	12.8	13.8	70
Streptococcus agalactiae	-	7	7	1.7	1.8	16.7
Streptococcus dysgalactiae	-	2	2	0.5	0.6	6.7
Streptococcus pyogenes	-	3	3	0.7	0.9	10
Streptococcus uberis	-	2	2	0.5	0.3	3.4
Entrococcus fecalis	-	1	1	0.2	0.3	3.4
Micrococcus spp.	-	19	19	4.7	4.7	36.7
Rhodococcus equi	-	5	5	1.2	1.5	13.3
Arcanobacter pyogenes	-	13	13	3.2	3.6	23.4
Corynebacterium bovis	-	16	16	4	4.5	40
Corynebacterium ulcerans	-	16	16	4	4.2	36.7
Cor. pseudotuberculosis	-	12	12	3	3.3	30
Bacillus cereus	-	14	14	3. 5	3.3	23.3
Other *Bacillus* spp.	-	42	42	10.4	10.6	60
Escherichia coli	2	30	32	7.9	7.4	43.3
Klebsiella pnemoniae	1	22	23	5.7	6.2	23.3
Enterobacter aerogens	-	13	13	3.2	3.8	20
Enterobacter agglomerans	-	1	1	0.2	0.3	3.3
Citrobacter freundii	-	4	4	1	1.2	6.7
Citrobacter diversus	-	4	4	1	1.2	10
Serratia marcescens	-	11	11	2.7	2.6	20
Proteus vulgaris	-	1	1	0.2	0.3	3.3
Proteus mirablis	-	5	5	1.2	1.5	13.3
Yersina pseudotuberculosis	-	1	1	0.2	0.3	3.3
Yersina enterocolitica	-	2	2	0.5	0.7	6.7
Pseudomonas aeuroginosa	1	31	32	7.9	7.4	33.3
Acinetobacter spp.	-	22	22	5.4	5	23.3
Actinobacillus equli	-	4	4	1	0.9	3.3
Pasteurella hemolytica	-	4	4	1	0.9	10
Pasteurella multocida	-	1	1	0.2	0.3	3.3
Candida albicans	-	2	2	0.5	0.3	3.3
Total	12	393	405			

4. Drug susceptibility test

Drug susceptibility test was conducted for frequently isolated and major pathogenic 150 bacterial species against eleven antimicrobials selected from main class of antimicrobials (Oxoid,Hamshire, England).

Over all gentamycin was effective drug and 90.6% of isolates were susceptible. *Str. dysgalactiae* and *B. cereus* were resistant to gentamycin. The second effective drug was Polymyxin B where 89.6% of the tested isolates were susceptible. *Streptococcus* species were resistant to polymyxin B. Sulfonamide and chloramphenicol was the third effective drugs where 81.3% of isolates were susceptible. *K. pneumoniae* and *E. aerogenes* were resistant to sulfonamide and chloramphenicol. Erythromycin was also effective drug where 80.7% of tested isolates were susceptible. *E. coli, K pneumonia* and *E. aerogenes* were resistant to erythromycin.

Nalidixic acid was the weakest of all drugs used in the animicrobial susceptibility test where only 43.3% of the isolates were susceptible. However all isolates of *B. cereus, E. coli, K. pneuomoniae, E. aerogenes* were susceptible. Cloxacillin was the second tolerated drug where only 63.3% of the isolate were susceptible. However it was effective drug for *Streptococcus agalactiae, A. pyogenes* and *S. aureus*. Methicillin was another less effective drug drug where only 74% of the bacteria tested were susceptible. All tested coliforms were resistant to methicillin. It was an effective against *Str. agalactiae, Str. uberis and A. pyogenes*. Streptomycin was also relatively less effective where only 74.7% of tested bacteria were susceptible. *Streptococcus* spp., *K. pneumoniae* and *E. aerogenes* were resistant to streptomycin. However, *B. cereus, A. pyogenes* and *S. aureus* were susceptible. Only 14.46% of *Staphylococcus* spp. were susceptible to penicillin G (Table 10).

Table 10: Percentage of susceptible isolates to different antimicrobials

Isolate	PB*	MET	FOX	NA	E	OB	S3	C	G	P	S	OXT
S. aureus (52)**	98.1	82.7	96.2	11.5	98.1	94.2	100	82.7	100	7.7	96.1	86.1
S.epidermidis (17)	100	88.2	82.4	47.1	94.1	70.6	70.6	94.1	100	35.3	88.2	100
Str. agalactiae (7)	42.9	100	100	0	100	100	100	100	14.3	-	0	100
Str. dysgalactiae (2)	0	50	100	0	100	50	50	100	100	-	0	100
Str. pyogenes (3)	0	66.7	100	0	100	33.3	33.3	100	100	-	0	100
Str. uberis (2)	50	100	100	0	100	0	100	100	100	-	50	100
Coryne. spp. (15)	100	93..3	86.7	53.3	100	80	86.7	93.3	100	-	86.7	93.3
A. pyogenes (13)	76.9	100	92.3	7.7	100	100	100	100	100	-	100	100
B. cereus (10)	80	80	0	100	100	0	100	100	20	-	100	60
E. coli (11)	100	0	90.9	100	0	0	90.9	90.9	100	-	81.8	81.8
K.pneumoniae (10)	100	0	10	100	20	0	0	10	100	-	0	0
E.aerogenes (8)	100	0	0	100	0	0	12.5	12.5	100	-	12.5	12.5
Over all 75.6	89.6	74	76	41.3	80.7	63.3	81.3	81.3	90.7	14.5	74.7	79.3

*PB = Polymyxin B NA = Nalidixic acid P = Penicillin

MET = Methicillin OB = Cloxacillin C= Chloramphenicol

S = Streptomycin S3 = Sulfonamide E = Erythromycin

FOX = Cefoxitin G = Gentamycin OXT = Oxytetracycline

**The numbers in bracket indicate number of isolate tested

4.5. Prevalence of mastitis in relation to host risk factors

4.5.1. Prevalence of mastitis in relation to udder attachment

The prevalence of mastitis in relation to type of udder characteristics (attached or pendulous) is assessed. Out of 340 lactating goats examined 133 (39.1%) had pendulous udder while 207 (60.9%)of the goats had attached udder. Goats with pendulous udder were more affected with mastitis than others (Table 11) and the difference was statistically significant ($p<0.05$). .

Table 11: Prevalence of mastitis in relation to udder characteristics

Goat level culture	Udder attachment		Total
	Attached	pendulous	
Negative	77	27	104
Positive	130	106	236
Total	207	133	340

$\chi 2$ 2(1) =10.8891, p = 0.001 significant at p<0.05

4.5.2. Prevalence of mastitis in relation to parity number

Prevalence of mastitis in relation to parity number was assessed, goats with parity greater than 3 were categorized as parity 4. Multiparious goats were more affected with mastitis than others (Table 12) and there was a statistically significant difference (p<0.05).

Table 12: Prevalence of mastitis in relation to parity number

Goat level	Parity number				Total
	1	2	3	4	
Negative	29	54	17	4	104
Positive	42	96	73	25	236
Total	71	150	90	29	340

$\chi 2$ (3)=15.2419, p = 0.002 significant at p<0. 05.

5. DISCUSSION

5.1. Physical examination

Prevalence of clinical mastitis 2.4% was low only 8 goats, 14 halves had clinical mastitis and 6 goats had bilateral mastitis, while 2 goats had unilateral mastitis. This low prevalence coincides with findings of Ryan and Green (1990) who found only one doe with clinical mastitis in a study conducted in south Wales and Contreras *et al.* (1998) in Spain who reported occurrence of 0-2 % prevalence in ten-year time. This low prevalence might be due to goats with such problems are sold or culled. The most dominant bacteria isolated from clinical mastitis *was S. aureus* isolated from 8 halves of 4 goats. This finding agrees with findings of Anyam and Adekeye (1995) in Nigeria. *E. coli* was isolated from two halves of one animal. *K. pneumoniae* was isolated from one halve and *P. aeuroginosa* was isolated from one halve. One animal with clinical mastitis yielded no bacteria. This could be due to the result of earlier infection which had been over come by body defence or udder may have been infected with organisms which need special media and missed in ordinary course of bacterial isolation.

Four goats had udder injuries and all of them had intramammary infection, which agrees with findings of Ameh and Tari (2000) who found significant association between udder injuries and mastitis. Fifteen goats had supernumery teat, twelve of them had intramammary infection. Only three goats were infested with tick and two of them had intramammary infection. Among 16 clinically affected halves only six milk samples (0.9%) showed change in consistency and color. This is because goat milk is apparently normal when there are severe inflammatory changes in the udder (Radostitis *et al.*,1994; Quinn *et al.* 1999). In this study no blind teat was observed. Goats with blind teats might have been sold or culled.

5.2. California mastitis test

The 66.9% sensitivity and 90.9% specificity agrees with findings of Menzies and Ramanoon (2001) who stated 20%-30% false negative and 20%-40% false positive results. This present finding nearly agrees with findings of Hueston *et al.* (1986) who stated 69.3%

sensitivity and 76.5 % specificity. This study indicated that CMT may be used as diagnostic tool in detection of subclinical mastitis in indigenous goats at field level where laboratory facilities are not equipped. The percentage agreement at each CMT level ranged from 75.7%-97.1% .The percentage agreement increased as CMT sore level increased except for CMT level T that is 40.5%. This is because CMT sore is subjective and sore T is considered as negative while 59.5% of them were bacteriologically positive.

Twenty-eight milk samples with CMT result positive yielded no bacteria. This may be due to the fact that goat milk naturally has higher number of somatic cell count or the organism which requires special media and can not be identified in ordinary course of bacterial isolation are missed (Rowe, 1999).

One hundred twenty four CMT negative milk samples yielded bacteria. This may be due to less pathogenic bacteria that don't induce release of somatic cell count. This agrees with findings of Boscos *et al.* (1996) who find out that CMT is good to detect major pathogens such as *S. aureus* . This also agrees with the findings of Nedgewa *et al.* (2000) who isolated bacteria from 22.5% of 568 CMT negative milk samples. These bacteria cause latent infection or they don't stimulate detectable increase in somatic cell count.

The over all agreement between CMT and culture calculated using kappa statistics was 0.56, which is a moderate agreement (Thrusfield, 1995). This finding disagrees with Moshi *et al.* (1998) who reported 0.35 agreement. The difference might be due to breed differences.

5.3. Bacterial isolation

The prevalence of intramammary infection was 55% at halve level and 69.4% at goat level. 374 halves of 236 goats had intramammary infection. This high prevalence is similar to findings of Anyam and Adekeye (1995) from Nigeria who found 56% prevalence and Moshi *et al* (1998) from Tanzania who found 72.8% prevalence. However, Topolko and Benic (1997) from Croatia found prevalence rate of 12.9% and Gomes *et al.* (2003) from Brazil reported prevalence rate of 25.4%. The variation in prevalence rate is influenced by factors such as breed differences, different hygienic and management practices. The high

prevalence of intramammary infection in this study might be due to poor management practices, poor sanitation of housing, absence of pre milking and post milking hygienic procedures.

A total of 405 isolates were obtained from 374 halves of 236 animals. One hundered fifty eight (58.5%) had bilateral infection. left halves (197) were more affected than right halves (177. This is similar to observation by Ndegwa *et al.* (2000) who suggested that this was possibly related to the milking process. They observed that most milkers were right-handed and consequently milked the right mammary gland halves more efficiently that the left halves, hence predisposing the latter to a higher rate of bacterial infection.

Three hundred forty three milk samples that is 91.7% of 374 halves grew only one type of organism while 31(8.2%) of them grew more than one type of bacterium. The isolated microorganisms were diversified in species. The most prevalent bacteria were *Saphylococcus* species accounting for 22.5% of the total isolate. This was in agreement with findings of Contreras *et al.* (1995) and Nedegewa *et al.* (2000). Among the *Staphylococcus* species, *S. aureus* was the dominant bacterial isolate accounting for 57.1% of *Staphylococcus* species and 12.8% of the total isolate, while *S. epidermidis* accounts for 42.9% of *Staphylococcus* species and 9.6% of the total isolate. Tthis finding contradicts with findings of Contreras *et al.* (1995) and Nedgewa *et al.* (2000) who found dominant number of CNS over *S. aureus*. For instance Nedgewa *et al.* (2000) isolated CNS, which is 64.3% of *Staphylococcus spp.* The present finding was inagreement with Ameh and Tari (2000) in Nigeria and Topolko and Benic (1997) in Croatia who found dominant number of *S. aureus* over CNS in goat mastitis.

Staphylococcus aureus: *Staphylococcus spp.* were widely distributed in 13.8% of the animals and 70% of the herds. This is because *S. aureus* is contagious pathogen transmitted from doe to doe during unhygienic milking procedures (Rowe, 1999). Most of the clinical cases of mastitis were caused by *S. aureus* affecting 50% of the gland and 50% of the animals, which was in agreement with findings of Anyam and Adekeye (1995). The higher prevalence of *S. aureus* intramammary infection can be a public health concern.

CNS *(S. epidermidis)*: It was the second bacterial spp. isolated accounting for 42.9% of the *Staphylococcus* spp. and 9.6% of the total isolate. It was isolated from 9.7% of animals and 76.7% of herds. It is a contagious pathogen found on the skin of human hand and skin of goats. It is transmitted during unhygienic milking procedures (Guss, 1992)

Bacillus spp.: *Bacillus* spp. were the second most bacterial *spp.* next to *Staphylococcus* spp. They accounted for 10.4% of total isolate. This high prevalence agrees with findings of Anyam and Adekeye (1995) in Nigeria who found 14.7% prevalence next to *S. aureus.* Bacillus spp. is environmental pathogen and high prevalence of Bacillus spp. might be due to unclean bedding and dusty environment of goats (Rowe, 1999).

Streptococcus agalactiae: *Str. agalactiae* accounted only for 1.7% of the total isolate. This low prevalence agrees with findings of other researchers including Nedegawa *et al.* (2000) in Kenya and Contreras *et al.* (1995) from Spain. *Streptococcus agalactiae* and other *Streptococcus* spp. are not nearly as prevalent or economically important as they are in dairy cows (Guss, 1992)

Arcanobacteium pyogenes: *A. pyogenes* accounted for 3.2% of the total isolate and it occurred mostly mixed with other bacterias (Radostitis *et al.*,1994) This low prevalence agrees with findings of Nedgewa *et al.* (2000).

Coliforms: Coliforms (*E.coli, K.pneumoniae, E. aerogenes, Citrobacter* spp.) and *P. aeuroginosa* together make up 26.6% of the total isolates. This high prevalence was in agreement with findings of Moshi *et al.* (1998) in Tanzania who found 28.5% from 206 quarters of 103 goats and Ameh and Tari (2000) who found 30% of coliforms. This high prevalence might be due to un clean environment of goats and bedding. This finding disagrees with findings of Rayan and Green (1990) who found coliforms in 2% of of halves.

Pasteurella hemolytica: *P. hemolytica* was isolated from three goats. This bacterium resides in the nasopharnex of goats, and udder infection can originate during nursing (Rayan and Green, 1990).

5.4. Prevalence of mastitis in flocks

In all flocks, at least one animal was found infected and the flock prevalence ranged from 17.5%-100% at animal level. All animals in five flocks were infected. Only 10% of the herds had less than 50% prevalence and 90% of the herds had more than 50% prevalence at goat level. The prevalence of intramammary infection at halve level in flocks ranged from 11.8%-88.9%. Forty percent of the herds had less than 50% prevalence and 60% of the herds had greater than 50% prevalence at halve level. This high prevalence of intramammary infection in flocks indicate general poor management of animal prevailing in the extensive system that is poor housing, poor hygiene of animals environment. Absence of milking procedure and absence of premilking and post milking udder washing or disinfections are the most contributing factors.

When considering flocks size in relation to the prevalence of mastitis the difference was not statistically significant ($P>0.05$). The conventional outlook is that as herd size increases contagious mastitis prevalence and incidence increases. Herd size in particular is said to be associated with the incidence of clinical mastitis. With increasing herd size, manure disposal and sanitation problems may also increase exposure to coliforms and environmental streptococci (Bartlett *et al.*, 1992) but in this particular study the flock size showed no difference on the occurrence of mastitis because the number of animals in households do not vary much and the sanitation problem is the same in almost all flocks.

5.5. Prevalence of mastitis in relation to host risk factors

The prevalence of mastitis in relation to host risk factors such as udder characteristics (pendulous or attached) and parity number was assessed. Both host risk factors have got relation with prevalence of mastitis. Although the number of goats with pendulous udder is only 133, it has got relation with prevalence of mastitis ($p<0.05$). This finding was in agreement with that of Schulz *et al.* (1999). They found association between loose tightening of udder and SCC. Genetic variation in conformation of the udder, teats, sphincter tone and anatomy of the teat canal is determined in part by heredity and may be considered one component of genetic resistance (Shook, 1989). Pendulous udder are also

susceptible to weakening of suspensory ligament and mastitis (Young and Legates, 1960; Quinn *et al.*, 1999).

In this study parity number had relation with occurrence of mastitis ($p<0.05$). This is in line with Boscos *et al.* (1996) who found higher proportion of bacteriologically positive samples in multiparious than in primiparious goats. Sanchez *et al.* (1999) from Spain also found a positive relation between subclinical intrammary infection and parity number.

5.6. Drug susceptibility patterns

The purpose of antimicrobial test is to generate information about which drug is effective against most frequent and pathogenic isolates of goat mastitis in the study area. No work is done on antimicrobial resistance of pathogens of goat mastitis in Ethiopia and literature is not available on the subject. Since the causative agent of mastitis is diverse antimicrobial susceptibility test is important before instituting treatment.

When considering the over all antimicrobial resistance pattern, 24.4% of the tested isolates were resistant. This might be due to pre-existent resistance in which the cellular mechanisms required for antimicrobial susceptibility are absent from the bacterial cell or acquired genetically because of chromosomal mutation and accusation of transferable genetic material. Antimicrobial resistance can be present in a bacterial population before exposure to a particular antibiotic. Treatment with a specific antimicrobial agent selects for those microorganisms that have pre-existent or acquired resistance (Pyorala and Myllys, 1991;Quinn *et al.*, 1999). This finding indicates that care has to be taken by the Veterinarian and farmers in selection of antimicrobials for treatment of mastitis. In this study over all the most effective drugs were gentamycin, polymyxin B. Sulfonamide, chloramphenicol and erythromycin. This is in line with Almaw (2004) who reported sulfisoxazole, erythromycin, chloramphenicol effective drugs on bovine isolates. The most resistant bacteria were *K. pneumoniae* and *E. aerogenes*. They were resistant to eight antimicrobials, namely, methicillin, cefoxitin, erythromycin, cloxacillin, sulfonamide, chloramphenicol, streptomycin and oxytetracycline . This finding was in line with Hussein (1999) who found that *K. pneumoniae* and *E. aerogenes* resistant to four antimicrobials, namely ampicillin, penicillin, streptomycin and tetracycline in bovine. Similar result was

found by Abdel-Gadir (2001) in camel that *K. pneumoniae* and *E. aerogenes* were resistant to oxytetracycline, erythromycin and penicillin G. However, *K. pneumoniae* and *E. aerogenes* were 100% susceptible to polymyxin B, nalidixic acid and gentamycin similar to findings of Hussein (1999) in bovine and Abdel-Gadir (2001) in camel.

Eschercia coli were resistant to methicillin, erythromycin and cefoxitin similar to *K. pneumoniae and E. aerogenes*. The most effective drug for *E.coli* were polymyxin B, nalidixic acid and gentamycin. Similarly Abdel-Gadir (2001) in camel reported that *E.coli* was resistant to erythromycin but susceptible to gentamycin.

Streptococcus spp. showed resistance to polymyxin B, nalidixic acid and streptomycin. The most effective drug for *Streptococcus* spp. were Cefoxitin, erythromycin, chloramphenicol, gentamycin and oxytetracycline. Similarly Hussein (1999) in bovine found that *Streptococcus* spp were susceptible to chloramphenicol, oxytetracycline, erythromycin and gentamycin.

Corynebacterium species were generally susceptible to all drugs except nalidixic acid. The most effective drug for *Corynebacterium* species were polymyxin B, erythromycin and gentamycin. *Arcanobacterium pyogenes like Corynebacterium species* were susceptible to all drugs except nalidixic acid. All isolates of *Arcanobacterium pyogenes* were susceptible to eight drugs, namely, methicillin, erythromycin, cloxacillin, sulfonamide, chloramphenicol, gentamycin, streptomycin and oxytetracycline. This finding was in agreement with Hussein (1999) and Almaw (2004) who found similar result in bovines.

Bacillus cereus was resistant to Cefoxitin, Cloxacillin and Gentamycin. The most effective drug for *B. cereus* was nalidixic acid, erythromycin, sulfonamide, chloramphenicol and streptomycin. This finding is in line with Almaw (2004) in bovine who found that *B. cereus* susceptible to erythromycin (100%), Sulfisoxazole (100%), chloramphenicol (100%).

Staphylococcus aureus was resistant to penicillin and nalidixic acid. However, Abdel-Gadir (2001) in camel reported that *S. aureus* were susceptible (91.4%) to penicillin G. Although *S. epidermidis* similar to S. aureus were resistant to penicillin and nalidixic, it is less resistant to these drugs. *Staphylococcus epidermidis* was more susceptible to

methicillin than *S. aureus*. The variation might be due to the fact that *S. aureus* is mostly involved in clinical mastitis. All isolates of *S. aureus* were susceptible to sulfonamide and gentamycin. Also 82.7% of *S. aureus* spp. were susceptible to Methicillin. This is in agreement with Almaw (2004) who found that *S. aureus* susceptible to sulfisoxazole (100%) in bovine. Hussein (1999) in bovine also found that *S. aureus* was susceptible to gentamycin.

6. CONCLUSIONS AND RECOMMENDATIONS

In developing countries like Ethiopia there is high deficiency of protein. The goat as a dairy animal can play an important role in reducing the gap between the high requirements of protein of animal origin and the low availability. Dairy goat milk, though small in quantity, provides an all year round source of animal protein. Nevertheless, mastitis could be an important factor limiting milk production in goats. It also causes kid mortality, culling of does and mastitis pathogens have zoonotic potential.

I n the present study, the prevalence of mastitis at goat level, halve level and flock level was high. This indicates that mastitis is one of the major problems of indigenous goats in milk production. The large number of bacteria isolated, the diversity in species and their distribution in flocks, indicate the economic importance of the disease. Isolation of high number of *Staphylococcus aureus* and *B. cereus* and other mastitis pathogens indicate the potential risk of consumption of raw goat milk.

The majority of the tested isolates were susceptible to the various antimicrobial agents. Gentamycin, polymixin B, chloramphenicol and erythromycin were effective drugs whereas nalidixic, cloxacillin acid, methicillin and streptomycin were relatively less effective against the pathogens.

The sensitivity, specificity and agreement of CMT with culture indicate good diagnostic value of the test for the detection of subclinical mastitis in goats.

Goats with loosely attached udder and multiparious ones were more susceptible to intramammary infection.

Based on the results of present study the followings are recommended.

- Awareness creation has to be done for the farmers concerning the economic and public health importance of mastitis in goats.
- Pre milking and post milking udder washing and disinfections need to be practiced.
- Hygienic milking procedures such as milking mastitic goats last have to be practiced.
- Boiling of milk before consumption is required.
- Care has to be taken in selection of drugs before instituting treatment against mastitis.
- CMT can be used at field level to detect subclinical mastitis where laboratory facilities are not available.
- Clinical cases have to be treated and chronic and untreatable cases have to be culled.
- High producing goats with pendulous udder and multiparious goats requires special care and follow up.

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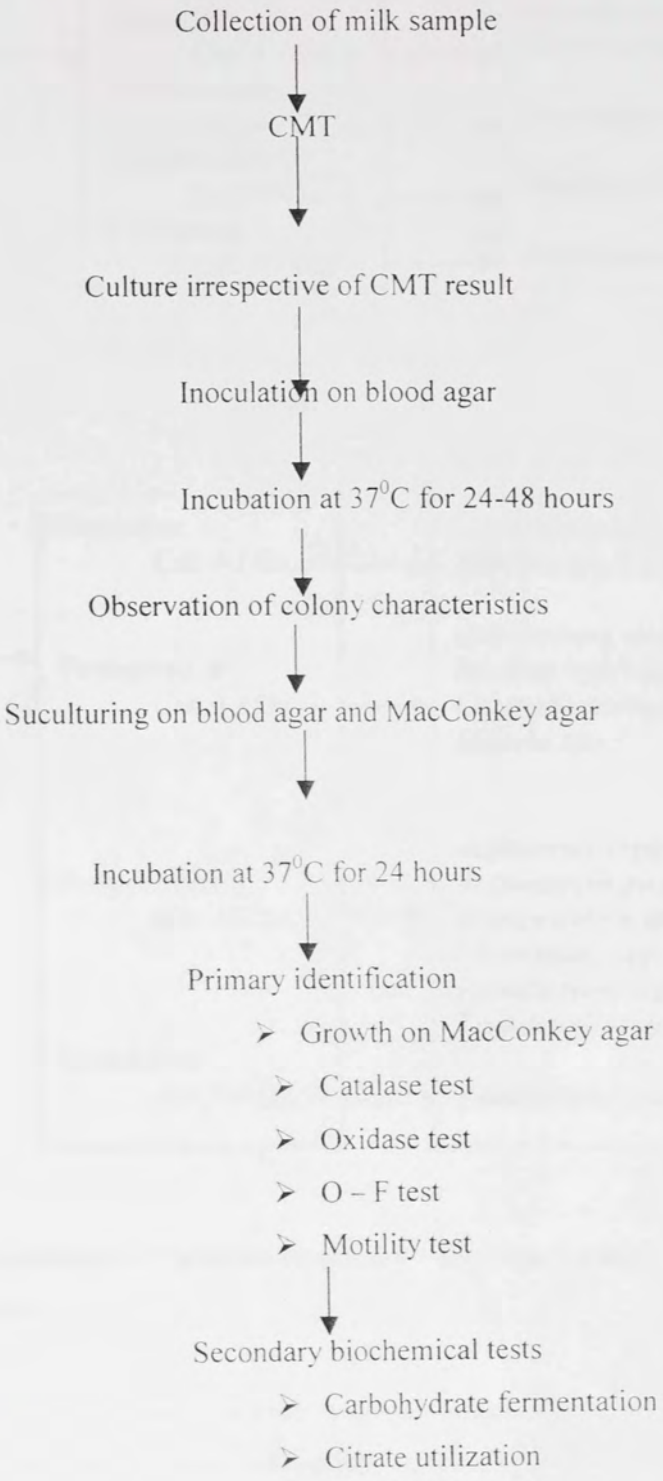
8. ANNEXES

Annex 1: Interpretation of CMT scores on goat milk

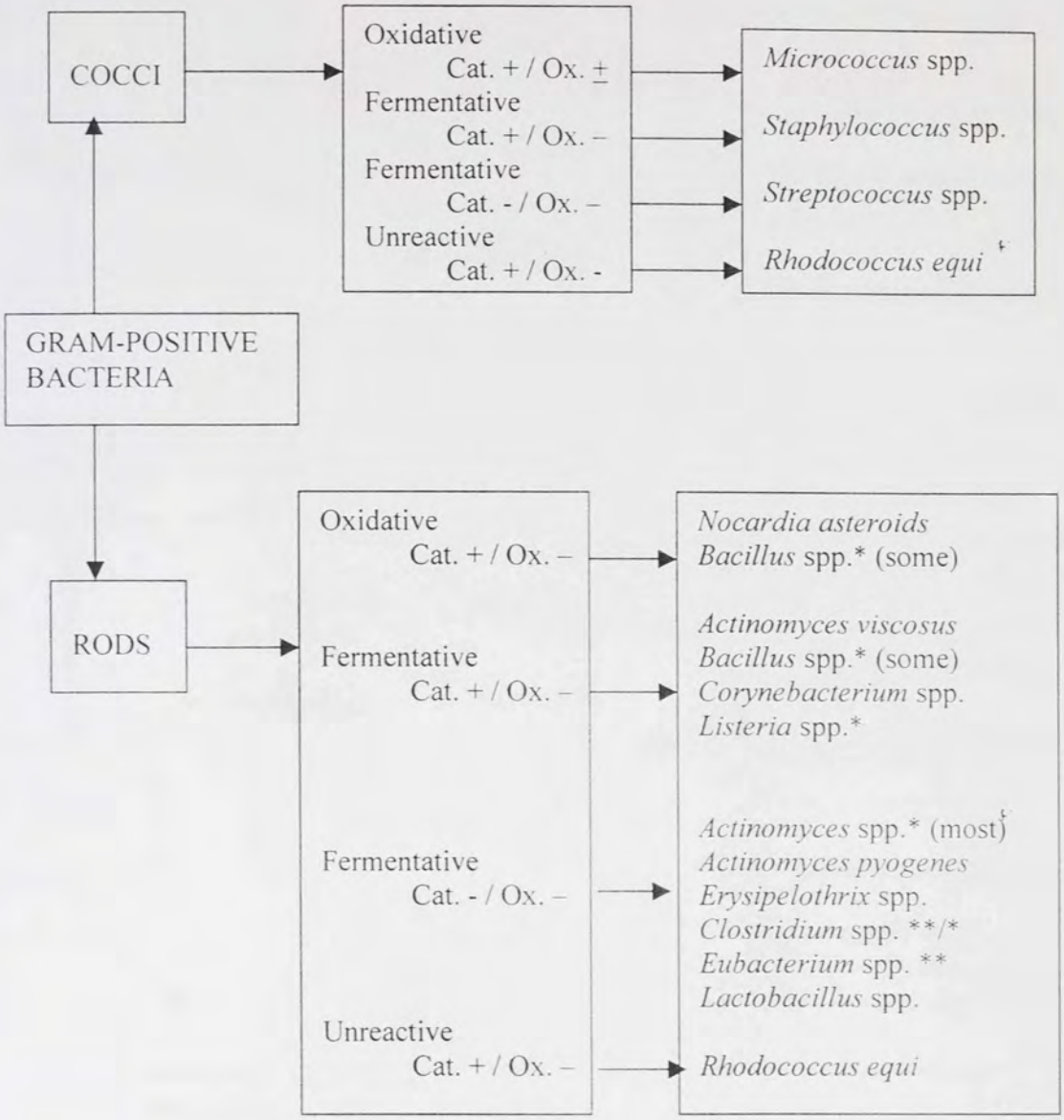
Score	Interpretation	Visible reaction	Mean number of leukocytes per ml
0	Negative	No reaction	68,000
T	Trace	Slight precipitation	268,000
1	Weak positive	Distinct precipitation but no gel formation	800,000
2	Distinct positive	Mixture thickens with gel formation	2,560,000
3	Strong positive	Viscosity greatly increased. strong gel i.e. cohesive with a convex surface	$\geq$ 10,000,000

Source: Schalm *et al.* (1971); Quinn *et al.* (1999)

Annex 2: Flow chart for isolation and identification of bacteria from milk

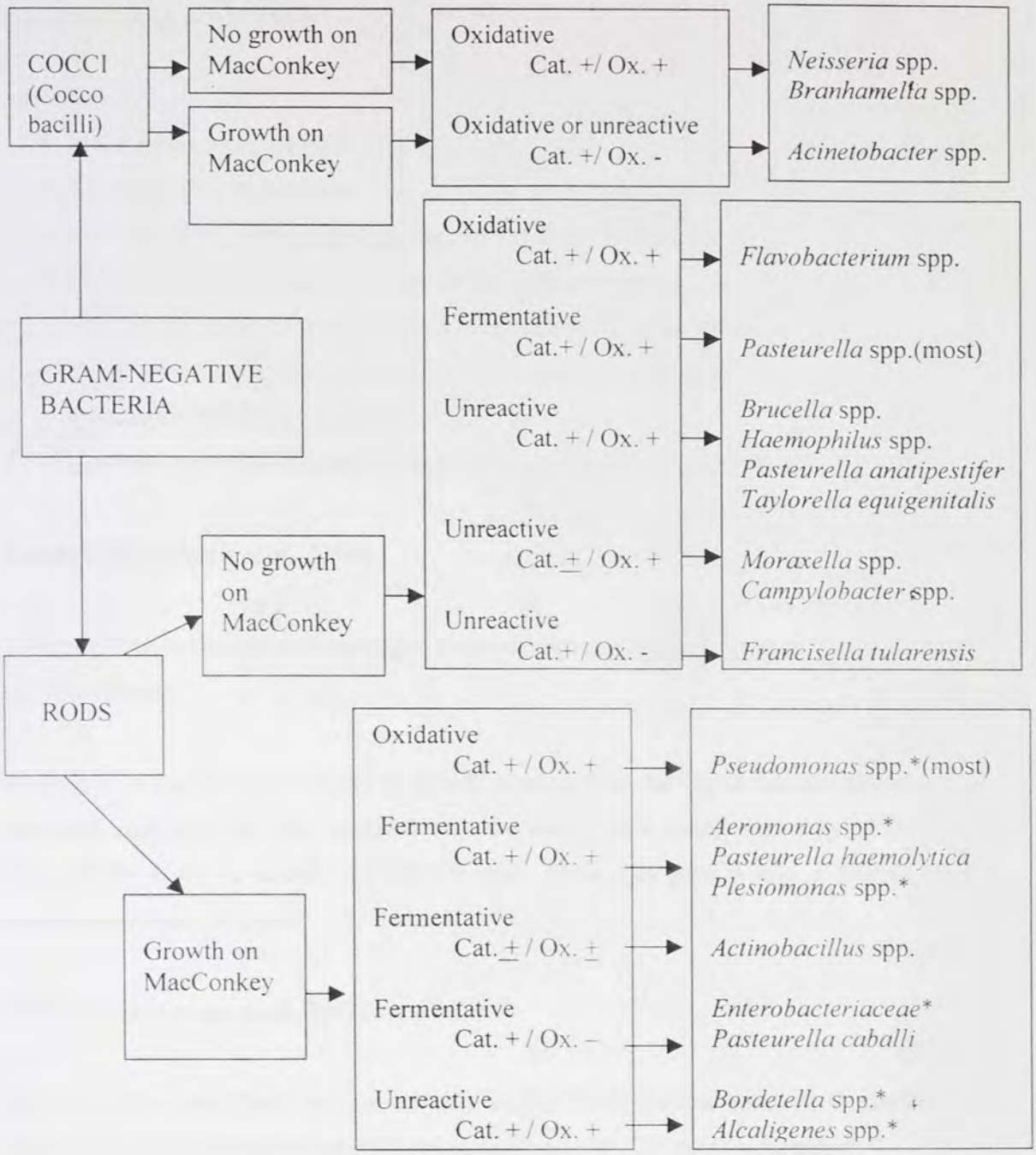


Annex 3: Primary identification of Gram-positive bacteria



(Cat. = Catalase; Ox. = oxidase; + = positive reaction; - = negative reaction; ± = variable; *= motile; ** = anaerobic)

Annex 4: Primary identification of Gram-negative bacteria



(Cat. =Catalase; Ox. =oxidase; += positive reaction; - = negative reaction; ± = variable,*= motile)

Source: (Quinn et al., 1999)

Annex 5: Primary identification tests

Gram's stain (Carter, 1984)

Procedure:

- Make a thin smear or film
- Allow the film to dry in air
- Fix the film by passing through the Bunsen flame several times
- Flood the slide with crystal violet for 30 to 60 seconds
- Pour of the stain and wash the remaining stain with iodine solution
- Wash off the iodine and shake the excess water from the slide
- Decolorize with acetone alcohol
- Counter stain with safranin for 30 to 60 seconds and wash with water

Catalase test (Quinn *et al.*, 1999)

Principle: The break down of hydrogen peroxide into oxygen and water is mediated by the enzyme catalase.

Procedure: A loopful of the bacterial growth is taken from the top of the colonies avoiding the blood agar medium. The bacterial cells are placed on a clean microscope slide and a drop of 3% H_2O_2 is added. An effervescence of oxygen gas, within a few seconds, indicates a positive reaction.

Oxidase test (Quinn *et al.*, 1999)

Principle: The cytochrome oxidase enzyme is able to oxidize the substrate tetramethyl-p-phenylenediamine dihydrochloride,forming a coloured end product, indophenol.

Procedure: Prepare a solution of 1 % tetramethyl-p-phenylenediamine dihydrochloride. then a piece of filter paper is moistened in a Petri dish with fresh reagent and the test bacterium is streaked firmly across the filter paper with a glass rod. A dark purple colour along the streak line with in 10 seconds indicates a positive reaction. *Pseudomonas aeruginosa* can be used as a positive control organism

O-F test (Quinn *et al.*, 1999)

Procedure: Prepare O-F base medium and when the O-F base has cooled to 50 ^{0}C add 20 ml of sterile glucose solution into 200 ml of O-F base, for a final concentration of 1 % glucose and dispense in to tubes. Two tubes of the O-F medium are heated in a beaker of boiling water immediately before use to drive off any dissolved oxygen and the tubes are then cooled rapidly under cold running water. Both tubes are stab-inoculated with the bacterium and a layer of sterile paraffin oil is layered on top of one of the tubes (sealed tube) to a depth of about 1cm and the tubes are incubated at 37^{0}C and examined in 24 hours and then daily for up to 14 days.

Motility test (Quinn *et al.*, 1999)

Procedure: SIM medium (BBL) was used to detect motility and the medium was stab inoculated using a straight wire. Then the tube is examined for motility after 24 and 48 hours. If there is a diffuse growth throughout the medium, the bacterium is motile. The growth of a non-motile bacterium is confined to stab line. To interpret the results, hold the tube against a good light and compare the inoculated tubes with un inoculated one.

CAMP test (Quinn *et al.*, 1999)

Procedure: A culture of the *Staphylococcus aureus*, with a wide zone of partial haemolysis (beta-haemolysin) is streaked across the center of a sheep or ox blood agar plate. A streak of the suspect Group B streptococcus is made at right angles to, and taken to with in 1 to 1.5 mm of staphylococcal streak. The plate is incubated at 37^{0}C for 18 – 24 hours. A positive CAMP test is indicated by an arrow–head of complete haemolysis. The group B streptococci produce a diffusible metabolite that complete the lysis of the red cells, only partially haemolysed by the beta –haemolysin of the staphylococcus.

Annex 6: Secondary identification tests

Indole test (Quinn *et al.*, 1999)

Principle: Indole positive bacteria possess an enzyme tryptophanase which converts tryptophan to indole.

Procedure: Stab inoculate SIM medium with test bacterium and incubate at 37 ^{0}C for 18 to 24 hours. Then add Kovac's reagent (0.2 ml) to tube and stand for 10 minutes.

Interpretation: The formation of dark red ring indicates positive reaction while in negative reaction a yellow ring is formed.

Methyl red (MR) test (Quinn *et al.*, 1999)

Principle: It is a quantitative test for acid production, requiring positive organisms to produce strong acids (lactic, acetic, and formic)

Procedure: Inoculate MR-VP broth with pure culture of test organism and incubate at 37^{0}C for two days, then add 5 drops of MR solution in to the media.

Interpretation: Production of red colour indicates a positive result and yellow colour negative in methyl red test.

Voges-Proskauer (VP) test (Quinn *et al.*, 1999)

Principle: Some organisms produce acetoin as the chief end product of glucose metabolism and form less quantity of mixed acids.

Procedure: Inoculate MR-VP broth with pure culture of the test organism and incubate at 37^{0}C for 2 days. Then aliquot 1 ml of broth to a clean test tube and add 0.6 ml of 5 % α-naphthol followed by 0.2 ml of 40 % KOH. Shake the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15 minutes.

Interpretation: A pink colour indicates a positive reaction.

Urease test (Quinn *et al.*, 1999)

Principle: Urease is an enzyme possessed by many species of microorganism that can hydrolyze urea with the formation of ammonia (alkaline).

Procedure: The surface of the agar slant is streaked with the test organism and incubated at 37^0C for 18 to 24 hours.

Interpretation: Organisms that hydrolyze urea rapidly may produce positive reaction with in 1 or 2 hours. Red (pink) colour through out medium indicates positive reaction.

Annex 7: Differentiation of mastitis causing *Staphylococcus spp and Micrococcus* spp

Test	*S. aureus*	CNS	*Micrococcus*
Catalase	+	+	+
Coagulase	+	–	–
Haemolysis	+	–	–
Manitol (A)	+	–	–
Maltose (A)	+	V	–
Glucose (A	+	+	–

+ =Positive reaction, -= Negative reaction, v =variable reaction, A= acid production

Annex 8: Differentiation of mastitis causing *Streptococcus* spp.

Species	CAMP Test	Growth on MacConkey	Easculin hydrolysis	Other Confirmatory Tests
Str. agalactiae	+	-	-	
Str. uberis	±	-	+	Manitol +(A)
Str. dysgalactiae	-	-	-	Salicin +(A)
Str. faecalis	-	+	+	salicin +,Manitol +
Str. pyogens	-	-	-	Salicin (-)
Str. pnuemoniae	-	-	+	Manitol (-)

+ = Positive reaction, - = Negative reaction, ± =Positive or Negative

Annex 9 : Differential test used for *Bacillus* spp.

Bacillus spp.	Citrate	Arabinose	Manitol	Voges Proskauer
B. steariothermophilus	–	v	–	–
B. cereus	+	–	–	+
B. pumilus	+	+	+	+
B. brevis	d	–	d	–
B. coagulans	d	d	d	d

V=variable reaction, d=11-89% strains are positive

Source: Carter (1984)

Annex 10 : Differential tests used for *Corynebacterium* and *Actinomyces* spp

Corynebacterium and *A. pyogenes*	Catalase test	Hemolysis	Glucose	Lactose	Maltose	Trehalose
C. ulcerans	+	V	+	-	+	+
C. bovis	+	-	-	-	-	-
C.pseudotuberculosis	+	+	+	+	+	+
A. pyogenes	-	+	+	+	+	V

V=Variable reaction,

Source: Carter (1984), Quinn *et al.* (1999)

Annex 11: Differential test used for Gram- negative rods

G-ve bacteria	1	2	3	4	5	6	7	8	9
E.coli	+	+	-	-	(+)	-	Y/Y, Gas+, H_2S^-	-	+
k. pneumoniae	-	-	+	+	+	+	Y/Y, Gas+, H_2S^-	-	+
Cit. freundi	-	+	-	+	-	+	Y/Y, Gas+, H_2S^+	-	+
P. aeuroginosa	-	-	-	+	-	-	R/R, Gas+, H_2S^-	+	+
P. mirablis	-	+	-	±	-	+	Y/Y, Gas^+, H_2S^+	-	+
P. vulgaris	+	+	-	D	-	+	Y/Y, Gas^+, H_2S^+	-	+
E. aerogenes	-	-	±	D	+	-	Y/Y, Gas^+, H_2S^-	-	+
E. agglomerans	+	-	+	+	D	D	Y/Y, Gas^+, H_2S^-	-	+
P. multocida	+	-	-	-	-	-	H_2S^+	±	-

1=indole test, 2=Methyl red test, 3=VogesProskauer test, 4=Citrate utilization, 5=lysine decarboxylase test, 6=urase test, 7=TSI test, 8=oxidase test, 9=Growth on MacConkey agar D = 26-75% of strains positive, (+)=76-89% 0f strains are positive

Annex 12: Media used for isolation and identification of bacteria

1. Blood Agar Base (BBL®, Becton Dickinson, USA)

Composition (g/l): Heart muscle, infusion from (solids) 2.0; pancreatic digest of casein 13.0; Yeast extract 5.0; sodium chloride 5.0; agar 15.0

Preparation: Suspend 40.0g of the powder in 1 liter of distilled water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Cool the base to 45 to 50°C and add 5% sterile defibrinated blood.

2. MacConkey Agar (Oxoid, Hampshire, England)

Composition (g/l): Peptone 20.0; lactose 10.0; bile salts No.3 1.5 ; sodium chloride 5.0; neutral red 0.03; crystal violet 0.001; agar 15.0

Preparation: Suspend 51.5g in 1 liter of distilled water. Bring to boil completely. Sterilize by autoclaving at 121°C for 15 minutes.

3. SIM Medium (BBL®, Becton Dickinson, USA)

Composition (g/l): Pancreatic digest of casein 20.0; peptic digest of animal tissue 6.1; ferrous ammonium sulfate 0.2; agar 3.5

Preparation: Suspend 30g of the powder in 1 liter of distilled water. Mix thoroughly. Heat with frequent agitation and boil for 1minute. Autoclave at 121°C for 15 minutes.

4. O-F basal medium (Merck, Darmstadt, Germany)

Composition (g/l): Peptone from casein 2.0; yeast extract 1.0; sodium chloride 5.0; Di-potassium hydrogen phosphate 0.2; bromothymol blue 0.08; agar-agar 2.5.

Preparation: Suspend 11g in 1 liter of distilled water by heating in a boiling water bath or in a current steam, autoclave (15 minutes at 121°C); at approximately 50°C mix in 100ml/ liter of filter sterilized 10% solution of D (+) glucose, lactose, sucrose, or other carbohydrates; dispense in to tubes to give a depth of approximately 5cm.

5. Phenol-red broth base (Merck, Darmstadt, Germany)

Composition (g/l): Peptone from casein 5.0; peptone from meat 5.0; sodium chloride 5.0; phenol red 0.018.

Preparation: Suspend 15g in 1liter of distilled water; dispense into tubes and insert fermentation tubes; if necessary; autoclave (15 minutes at 121°C). At less than 60°C add the reactants (final concentration 5 to 10g / liter) as sterile solutions.

6. Mannitol Salt Agar (Difco, Detroit, USA)

Composition (g/l): Proteose peptone No.3 10.0; Bacto-beef extract 1.0; D-mannitol10.0; sodium chloride 75.0; Bacto-agar 15.0; phenol red 0.025

Preparation: Suspend 111g in 1 liter distilled water and heat to boiling to dissolve completely. Sterilize in the autoclave for 15 minutes at 15 pounds pressure (121^{0}C). Cool to 45 to 50^{0}C and dispense in to Petri dishes.

7. Nutrient agar (Oxoid, Hampshire, England)

Composition (g/l): "Lab-Lemco" powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0.

Preparation: Suspend 28g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121^{0}C for 15 minutes.

8. Triple sugar iron agar (Merck, Germany)

Composition (g/l): Peptone from casein 15.0; peptone from meat 5.0; meat extract 3.0; sodium chloride 5.0;lactose 10.0; sucrose 10.0; D(+) glucose 1.0; ammonium iron (III) citrate 0.5; sodium thiosulfate0.5; phenol red0.0024; agar –agar 12.0.

Preparation: Suspend 65g in 1 liter of distilled water by heating in a boiling water bath or in a current steam; dispense in to tubes; autoclave (15 minutes at 121^{0}C). Allow to solidify to give agar slants.

9. Simmons Citrate Agar (BBL®, Becton Dickinson, USA)

Composition (g/l): Ammonium dihydrogen phosphate 1.0; dipotassium phosphate 1.0; sodium chloride 5.0; sodium citrate 2.0; magnesium sulfate 0.2; agar 15.0; bromothymol blue 0.08.

Preparation: Suspend 24.2g of the powder in 1 liter of distilled water. Mix thoroughly, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121^{0}C for 15 minutes. Cool tubed medium in a slanted position for slants.

10. Edwards medium, modified (Oxoid, Hampshire, England)

Composition (g/l): "Lab-Lemco" powder 10.0; peptone 10.0; aesculin 1.0; sodium chloride 5.0; crystal violet 0.0013; thallus sulphate 0.33; agar 15.0.

Preparation: Suspend 41g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115^{0}C for 20 minutes. Cool to 50^{0}C; add 5 to 7% of sterile bovine or sheep blood. Mix well and pour plates.

11. Eosin Methylene Blue Agar (modified) Levine (Oxoid, Hampshire, England)

Composition (g/l): Peptone10.0; lactose 10.0; Dipotassium hydrogen phosphate 2.0; eosin Y 0.4; methylene blue 0.065; agar15.0.

Preparation: Suspend 37.5g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{0}C for 15 minutes. Cool to 60^{0}C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue colour and to suspend the precipitate which is an essential parts of the medium.

13. MR-VP medium (Oxoid, Hampshire, England)

Composition (g/l): Peptone 7.0; Glucose 5.0; Phosphate buffer 5.0

Preparation: Suspend 17g in 1 liter of distilled water. Mix well, distribute in to final containers and sterilize by autoclaving at 121 ^{0}C for 15 minutes.

14. Urea Agar Base (BBL®, Becton Dickinson, USA)

Composition (g/l): Pancreatic Digest of Gelatin 1.0; Dextrose 1.0; Sodium Chloride 5.0; Potassium Phosphate 2.0; Urea 20; Phenol red 0.012.

Preparation: Suspend 29g of the powder in 100 ml of distilled water. Mix thoroughly and Sterilize by filtration. Suspend 15g of Agar in 900 ml distilled water. Autoclave at 121^{0}C for 15 minutes. Cool to 50^{0}C and add 100 ml of urea agar base. Mix thoroughly and dispense aseptically in sterile tubes. Cool tubed medium in a slanted position so that deep butts are formed.

15. Muller Hinton Agar (BBL®, Becton Dickinson, USA Composition(per litter purified water): Beef extract 2gm, Acid Hydrolysate of casein 17.5gm, Starch 1.5gm, Agar 17gm.

Preparation: Suspend 38gm of the powder in 1 litter of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 min. to completely dissolve the powder. Autoclave at 121^{0}C for 15 minutes. Do not over heat.

9. CURRICULUM VITAE

A. Biographical Data:

Name	Asefa Wakwoya
Date of birth	September 23,1963 E.C
Place of birth	Horro, east wollega
Marital status	Single
Nationality	Ethiopian
Profession	Veterinarian
Occupation	District team leader

B. Educational background

1975-1982	Elementary School Shambu Elementary School
19 83-1986	Shambu senior secondory school Achievement: Ethiopian School leaving Certificate Examination
1987-1993	University/Under graduate program Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia Achievement: Doctor of Veterinary Medicine, D.V.M Degree
2003/04-2004/05	Postgraduate study Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia Achievement: MSc in Tropical Veterinary Medicine

C. Work Experience

1995-1997	Zonal Veterinary Officer Kambata, Alaba,Tambaro zone
1997-2003	Alem Teferi, Gimbii, Nadjjo Wereda Veterinary Officer

D. Research out put/Technical paper

a. Prevalence of bovine mastitis in Ethiopia (Seminar paper,1991)
b. Serological study of *S. pullourum* and *S. Gallinarium* using RBAT in large and small scale poultry farms in and around Addis Ababa (DVM Thesis, 1992)
c. Bacteriological study of raw milk of dairy goats with special reference to mastitis. (MSc Thesis, 2005)

E. Membership

Member of the Ethiopian Veterinary Association

F. Language

Afaan Oromo	Mother Tongue
Amharic	Writing and speaking
English	Writing and speaking

G. Computer Skill

MS Dose
Microsoft Word
Microsoft Excel
Microsoft Access

10. SIGNED DECLARATION SHEET

"This Thesis is my original work, has not been presented for any degree in any other university and that all sources of material used for the thesis have been duly acknowledged".

Name: _____

Signature: _____

Date of submission: 16/06/2005

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

Dr. BayleyegnMolla (DVM, Msc. PhD) _____

Dr. Kelay Belihu (DVM, PhD) _____

Bcteriological Study Of RawMilk Of Dairy Goats With Special Ref erence To Mastitis In Adami-Tulu Jiddo-Kombolcha District, Oromia Regional State, Ethiopia

Assefa Wakwoya