A cross-sectional study on the prevalence of subclinical mastitis and antimicrobial susceptibility patterns of the bacterial isolates in milk samples of smallholder dairy goats in Kenya

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Abstract

Dairy goat production is an emerging enterprise, which has a lot of potential for poverty alleviation, improved nutrition, and increased income for the poor and can play a role in contribution towards Kenya's development plan. Inadequate information on prevalence of subclinical mastitis and antibiotic sensitivity are some of the challenges facing this industry. This study was carried out in dairy goats under zero grazing system in Mount Kenya region, from January 2012 to December 2012 to determine the prevalence of subclinical mastitis in lactating goats and the antibiotic sensitivity of the isolated bacteria.

A total of 310 lactating goats were randomly selected from populations in Meru Nyeri and Embu counties and screened for bacterial carriage, as evidence of subclinical mastitis. Six hundred and twenty (620) milk samples from the 310 goats (right and left quarters) were aseptically collected; first screened using California Mastitis Test (CMT), then cultured for bacterial isolation and characterization. Antibiotic sensitivity testing was also performed on the isolated bacteria.

Based on culture results, the prevalence of subclinical mastitis was 59% in Meru County, 58% in Embu County and 54 % in Nyeri County. An overall mean prevalence of 57% was estimated in the three counties. There was no significant difference in subclinical mastitis prevalence in the three counties (P=0.75). Based on CMT, the prevalence of subclinical mastitis was estimated to be 61% in Meru County, 61% in Embu and 60% in Nyeri County. The overall mean prevalence was estimated to be 61%.There was no significance difference between prevalence of subclinical mastitis in the three counties (P=0.96).

Among the 620 milk samples collected from the 310 lactating goats, 317 (51%) were California mastitis test positive, and on culturing, 304 (96%) yielded bacterial growth. The following bacteria were isolated from the milk samples; Coagulase Negative *Staphylococcus* was the most prevalent - at 28.3% (176/620), followed by *Staphylococcus aureus* - at 13.5% (84/620), *Streptococcus - at* 8.8% (46/620), *Escherichia coli -* at 3% (19/620), *Micrococcus -* at 4% (24/620), *Corynebacterium -* at 1% (7/620), *Pseudomonas -* at 0.1% (1/620). Of the *Streptococcus* isolates, 1.5% (9/620) were *Streptococcus agalactiae.*

Norfloxacin and gentamycin were antibiotics that the organisms were most sensitive to while kanamycin and amoxycillin were antibiotics that the organisms were least sensitive to.

The study revealed that there is high prevalence of subclinical mastitis in dairy goats in Mount Kenya region. The high prevalence of subclinical mastitis recorded in this study has a negative impact in dairy goat productivity; and there is, therefore, need to create awareness on the problem with a view to instituting appropriate control measures. The study also revealed that CMT is a reliable test for subclinical mastitis in goats. Since it is easy to carry out, rapid and

cheap, it is recommended that goat associations make use of it as part of the control measures; they can train specific personnel to carry out and interpret the test.

Introduction

The dairy goat industry is rapidly gaining importance throughout the world (Boscos *et al.,* 1996). In Kenya dairy goat farming is emerging as a high-return option for Kenyan small-scale farmers, although it has been challenged in most regions by marketing and distribution problems (Ndegwa *et al.,* 2000).

Kenya has an estimated 28 million goats and about 80,000 dairy goats (MoLFD, 2009). The goat population in Kenya is predominantly indigenous Galla and East African goats which are reared in arid and semi arid areas (Kinuthia, 1997). Dairy goats in Kenya were obtained through a cross breeding programme between the indigenous goats and the exotic breeds and about eighty percent of these are reared in Mt Kenya Region (MoLFD, 2009). They provide a quick source of

milk for consumption or sale and are thus of immense value especially to poor households. The fact that they can be reared in small land holdings is especially useful in these highly populated areas (Kinuthia, 1997).

Goats form the most important group of milk producing animals after dairy cattle in both temperate and tropical agriculture (Farnworth, 2002). The demand of dairy goats milk is increasing because of the growing population of people, the increasing awareness of medicinal and nutritional status associated with goat milk and also the special interest in goat milk products, especially cheeses and yoghurt, in many developed countries which has led to increasing levels of disposable incomes (Epitaufik, 2007). Dairy goat has been used as source of income and source of food (meat and milk) especially to the poor (Haenlein, 2004).

Though dairy goat production is playing an important role in the improvement of income of the poor farmers, poverty and hunger alleviation the dairy goat production is still faced by challenges such as diseases (diarrhea and pneumonia), inbreeding, poor feeding, lack of market and poor management practices (Ndegwa *et al*., 2000). Among infectious diseases mastitis is one of the major diseases affecting dairy goat productivity (Gebrewahid *et al.,* 2012). Several causative agents and predisposing factors have been implicated in dairy goat mastitis. Etiological agents include bacteria, viruses and yeasts. Several risk factors including, milking hygiene, management practice, feeding, number of lactation days and geographical locality have influenced the type and the frequency of isolation of organisms causing mastitis (Ndegwa *et al*., 2000).

Milk is one of the most important foods of human beings. It is universally recognized as a complete diet due to its essential components (Javaid *et al*., 2009). Mastitis reduces the quality

and quantity and is one of the most important and expensive disease of dairy industry. It results in severe economic losses from reduced milk production, treatment cost, increased labor, milk withheld following treatment and premature culling (Sharif *et al.,* 2009).

Subclinical mastitis is the most common in goats and is mainly caused by contagious bacteria (Persson *et al*., 2011).Early diagnosis of mastitis with reliable tests facilitates successful treatment and control. The main control principles include: sound husbandry practices and sanitation, post milking teat dip, treatment of mastitis during non-lactating period, and culling of chronically infected animals (Sharif *et al.,* 2009).

Dairy goat milk is routinely consumed in rural and urban areas of Kenya. Sub-clinical mastitis reduces the quality and quantity of milk and yet only a few studies have been done on the prevalence, while no studies have been done on the antibiotic sensitivity and disease situation in the country as compared to the disease in the cow (Ndegwa *et al*., 2000). This study is therefore geared towards establishing the prevalence of sub- clinical mastitis with associated antibiotic sensitivity which can be used to design control programmes that will lead to improved dairy goat milk production in Kenya.

Material and methods

Study site

This cross-sectional study was carried out between January and December 2012 in Mount Kenya region and three counties were included; Meru, Nyeri and Embu counties of Kenya to access the prevalence of subclinical mastitis. The study area straddles the equator and is in Zone II which

is in the highlands of Kenya with an altitude ranging from 1600 to 3000 m with a forest vegetation on the slopes of Mt. Kenya. The area is characterized by fertile volcanic soil, adequate water supply from two rainfalls in a year and has a high population density practicing mixed farming.

These are high potential highland (Altitude 1600-3000). The main livelihood practices include dairy and crop farming. These areas are densely populated and the dairy goat population is also high. The does were mainly German Alpine crosses and Toggenburg crosses.

Study design and strategy

A total of 620 milk samples were collected from 310 randomly selected lactating goats and screened for subclinical mastitis using the California Mastitis Test (CMT) .The screening was done according to the procedure described by Quinn *et al.,* 2000. The milk samples were cultured for isolation of pathogens and characterization of the causative bacteria done.

The sample size was determined using formula by Martin *et al.,* 1987. Sample size $=$**n=Z^2αpq/L^2.**where n=the required sample size, **Zα=1.96=**the normal deviate at 5% level of significance. P=the estimated prevalence (in percentages), **q=1-P** and **L=** the precision of estimate which is considered to be 5%**=0.05**

Since the prevalence of mastitis in dairy goats in Kenya is estimated at 28.7% (Ndegwa *et al*., 2000). **Sample size n=Z^2αpq/L^2**

$1.96^2 \times 0.287 \times 0.713/0.05^2$=314

Sample collection

On the day of sampling the udders were examined visually for physical injury or swellings and by palpation for consistency and warmth. The first streams of fore milk from each mammary - gland half were examined macroscopically for any abnormalities. After discarding the first three streams of fore milk, 20 ml of milk were aseptically collected from each mammary gland half into sterile Bijoux bottles and transported at 40^c to the laboratory for microbial culture.

California Mastitis Test (CMT)

The California mastitis test was conducted to diagnose the presence of subclinical mastitis. This screening test was performed according to the standard procedure described for mastitis by Quinn *et al*., 1994. The results were read within 10 seconds and scored as 0, +1, +2 or +3 depending on the intensity of reaction**.** After each test the plate was washed and rinsed before another set of samples was tested. All milk samples done for CMT were further taken for bacteriological examination.

Isolation and Identification

The samples were streaked on sheep blood agar and McConkey agar plates and incubated aerobically at 370 C for 48 hours. Significant microbial colonies at 24 and 48 hours were selected and sub-cultured for 24 hours after which they were gram stained and biochemically tested and classified according to standard methods. Apart from a few cases most organisms were classified up to genus level. Gram stain procedure was performed according to the method described by Forbes *et al.,* (2002), Bebora *et al.,* (2007). Bacteriological examination was carried out following standard methods (Quinn *et al.,* 1994, Sears *et al.,* 1993). Briefly a loopful of each

milk sample was streaked on 7% sheep Blood Agar (BA). MacConkey Agar plates were used in parallel to detect *Enterococcus* species and any gram-negative bacteria. Inoculated plates were incubated aerobically at 37^0 C for 24 - 48hours. Presumptive identification of bacterial isolates on primary culture were made based on colony morphology, haemolytic characteristics on blood agar, Gram stain reaction, catalase and oxidation – fermentation tests (Quinn *et al.,* 1994, Sears *et al.,* 1993, Forbes *et al.,* 2002). *Staphylococcus* and *Micrococcus* species were identified based on their growth characteristics on Mannitol Salt agar (MSA), coagulase production, catalase, and oxidase tests. *Streptococcus* species were evaluated according to CAMP reaction (*Streptococcus agalactiae* potentiates *Staphylococcus aureus* hemolysin leading to complete or Beta (β) haemolysis of the red blood cells on Bovine blood Agar – a positive CAMP test), growth characteristics on 7% sheep blood agar, catalase production and sugar fermentation tests (Quinn *et al.,* 1994, Sears *et al*., 1993, Forbes *et al.,* 2002). Gram-negative isolates (Enterobacteriaceae) were sub-cultured on MacConkey agar and further tested using Triple sugar Iron (TSI) Agar, the IMViC test (Indole, Methyl red, Voges-Proskauer and Citrate utilization test) and oxidase reaction (Quinn *et al.,* 1994, Sears *et al.,* 1993, Forbes *et al*., 2002).

Antibiotics susceptibility test

Antibiotic susceptibility test was performed using disk diffusion method on nutrients agar (Oxoid) according to the procedure described by National Committee of Clinical Laboratory Standards 2006. All isolated bacteria were tested with different antibiotics: Tetracycline, Gentamicin, Kanamycin, Norflaxacin, Chloramphenical and amoxicillin all of which are widely used in veterinary practice in Kenya. Briefly ten colonies from the Blood agar medium, incubated at 37°C for 24 h, were suspended in 2 ml of sterile saline to a density approximately

equal to McFarland Opacity Standard No. 0.5. A dry sterile cotton wool swab was placed in the suspension and excess liquid was expressed against the inside of the tube. The bacterial suspension was inoculated onto nutrients agar with the swap in such a way that the whole surface of the agar was covered and the antibiotic disks were then placed on the agar. The antibiotics disks contained six different antibiotics Gentamycin, Norfloxacine, Kanamycin, Chloramphenicol, Tetracycline and Amoxicillin. The results were recorded as resistant or susceptible by measurement of inhibition zone diameter according to the interpretive standards of National Committee for Clinical Laboratory Standards (2006).

RESULTS

California Mastitis Test

California mastitis was performed in all 620 samples collected from the 310 goats. Using a CMT score of <+2, Of all the does screened for mastitis using CMT (190/310) 61% tested positive for mastitis. In the specific counties subclinical mastitis was estimated to be 61% (41/67) in Meru County, 62% (54/86) in Embu and 60% (95/186) in Nyeri County. There was no significant difference in prevalence in the three counties (P=0.96).

Of all the 620 milk samples collected from 310 lactating goats for the presence of subclinical mastitis 317 (51%) milk samples were CMT positive, while 303 (49%) samples were CMT negative (Table 1). On the other hand, 13 (4%) of the 317 CMT-positive milk samples yielded no bacterial growth while the remaining 304 (96%) samples were also culture positive in which

diverse bacterial pathogens were identified (Table 2) Of the 303 (49%) CMT negative sample

2(0.6%) yielded bacteria.

Table 1 Results of California Mastitis Test (CMT) in Comparison with the Bacteriological Examinations			
Number of samples (%)			
CMT score	Examined	Culture positive	Culture negative
0	256	0 (0%)	256 (100%)
Trace	30	0 (0%)	30 (100%)
1+	17	2(2%)	15 (88%)
≥2+	317	304(96%)	13(4%)
Total	620	306 (49%)	314(51%)

The prevalence of subclinical mastitis based on culture results was 59% in Meru County, 58% in

Embu County and 54 % in Nyeri County. An overall mean prevalence of 57% was estimated in

the three counties. There was no significant difference in cultured mastitis prevalence in the three

counties (P=0.75) (Table 2)

Table 2: Summary of the laboratory bacterial culture results for the three counties			
County	Infection positive	Infection negative	Total
Meru	40(59%)	27	67
Embu	50(58%)	36	86
Nyeri	86(54%)	71	157
Total	176(57%)	134	310

Bacterial isolates

The results from this study revealed that Coagulase Negative Staphylococcus was the most prevalent 28% (176/620), followed by *Staphylococcus aureus* 13.5% (84/620), Streptococcus 7.4% (46/620)   *Streptococcus agalactiae* 1.4% (9/620), *Escherichia coli* 3% (19/620) *Micrococcus* 4% (24/620) *Corynebacterium* 1 (7/620) *Pseudomonas* 0.1% (1/620).(Table 3)

Table 3: Bacteria isolated from the goat milk samples from Meru, Nyeri and Embu counties, separately and collectively

Bacteria		Meru N=136		Embu N=172		Nyeri N=312		Combined Meru, Embu, Nyeri N=620	
		No.	%	No.	%	No.	%	No.	%
Staphylococcus	Total isolated	60	44.1	81	47	119	38.1	260	41.9
	CPS	12	8.09	26	15	46	14.7	84	13.5
	CNS	48	35.5	55	31.9	73	23.4	176	28.3
Streptococcus	Total isolated	12	8.8	17	9.8	26	8.2	55	8.8
	S. Agalactiae	6	4.4	1	0.5	2	0.6	9	1.4
	S. species	6	4.4	16	9.3	24	7.6	46	7.4
Micrococcus		7	5	14	8.14	3	1	24	3.89
Escherichia coli		7	5	8	4.6	4	1.2	19	3
Corynebacterium		3	2.2	0	0	4	1.2	7	1.3
Pseudomonas		1	1	0	0	0	0	1	0.1

Antibiotic sensitivity

Majority of *Staphylococcus aureus* (87.8%), CNS (82.5%) Micrococcus (76%) and *Escherichia coli* (75%) were susceptible to most antibiotic tested. Of all the pathogen tested moderate resistance was recorded in tetracycline (28%) and kanamycin (30%) antibiotics. Streptococcus (56%) and *Escherichia coli* (25%) showed moderately high resistance to most of the antibiotics

tested. Most of the organisms 82% were sensitive to Norflaxacin, chloramphenical (77%) and

Amoxycycline (77%) (Table 4)

Table 4 Antimicrobial Resistance Profiles of Mastitis Pathogens to Different Antimicrobials						
Bacteria isolates	Tetracycline	Kanamycin	Amoxyclin	Chloramphenical	Norflaxacin	Means
S.A	27(85%)	28(88%)	24(85%)	26(81%)	30(94%)	87.8
E.coli	5 (50%)	7(70%)	7(80%)	9(90%)	10(100%)	75%
CNS	35(81%)	31(90%)	29(67%)	35(81%)	35(81%	82.5%
Streptococcus	7(41%)	7(42%)	10(58%)	11(65%)	10(59%)	54%
Micrococcus	6(86%)	5(71%)	5(71%)	4(57%)	6(85%)	76%
Total n=110	80(72%)	78(70%)	85(77%)	85(77%)	91(82%)	76.8%

Discussion

The CMT-positive and culture-negative samples could be partly explained in that the udder

could be injured and was recovering from infection or that the infection was not due to a

bacterial pathogen. It could also be due to an organism such as mycoplasmas, which requires

special media and cannot be identified in the routine bacterial isolation techniques. Of the CMT

negative which yielded bacterial growth on culture this may be due to less pathogenic bacteria

that do not induce detectable levels of somatic cell counts. This result was consistent with the

reports of Ndegwa *et al.,* 2000 who isolated bacteria from 22.5% of 568 CMT negative milk

samples and Wamukoya *et al.,* 2006. They both indicated that these bacteria may cause latent

infection or they do not stimulate detectable increase in somatic cell counts.

The results of the study showed that the overall mean prevalence of subclinical mastitis in Mount Kenya region based on CMT results was 61% while a prevalence of 57% was estimated based on culture results.

The finding in this study was in close agreement with results reported in Palestine, 52% (Adwan *et al.,* 2005*),* Tanzania 51.5% (Swai *et al*., 2008) and Nigeria (Adekeye *et al.,* 1995) 56%.The results recorded in this study were higher than those recorded in other countries. Kenya 28.7% (Ndegwa *et al.,* 2000), Ethiopia 18.0% (Gebrewahid *et al*., 2012), South Ethiopia 15.5% (Megersa *et al*., 2009) and Vermont (USA) 27.3% (McDougall *et al*., 2002), However the results of the study were lower than those recorded in Tanzania 76.7% by Mibilu *et al.,* 2007. The high prevalence recorded in this study might be due to the differences in host and management risk factors that influence intra-mammary infection of goats also due to lack of standard milking procedure.

The present study showed that the most prevalent pathogen causing subclinical mastitis in dairy goats was *Staphylococcus species* (41.9%) followed by *Streptococcus species* (8.8%), Micrococcus spp 4%, *Escherichia coli* 3%, Corynebacteria 1.3% and Pseudomonas 0.1%. Of the Staphylococcus, CNS was more prevalent (28.3%) while *Staphylococcus aureus* had a prevalence of 13.5%.

These results were in agreement with results from studies done in other countries. CNS are the most prevalent pathogens causing subclinical mastitis in dairy ruminants.

Contreras *et al*., (1999) investigated bulk tank milk from commercial dairy goats in the USA and found that most of the pathogen isolated was *Staphylococcus* spp. (95.7%) with Coagulase negative *Staphylococcus* as the predominant species (66.7%). Ndegwa (1999) reported that milk samples from small-scale dairy goat farms in Kenya The most prevalent bacteria was

Staphylococcus spp. which were 78% with Coagulase Negative *Staphylococcus* having a prevalence of 71%. Foschino *et al.,* (2002) reported that CNS were found in 90% of samples milk samples collected from ten farms in the Bergamo area, Italy. *Staphylococcus aureus* was found in 43% of samples. (Gebrewahid *et al.,* 2012), reported most prevalent pathogen was CNS in Ethiopia, Kyozaire *et al*.,(2005) South Africa reported CNS 85.7% of the infected udder halves and 14.3% of the infection was due to *S. aureus*. (Adwan *et al*., 2005*),* 35.6% reported that CNS are the most prevalent organisms detectable on udder skin, inside the streak canal and in mammary glands of dairy goats and also humans hands and can easily be transmitted during unhygienic milking procedures (Kalogridou-Vassiliadou, 1991). Various CNS species are commonly detected in goat milk and these microorganisms can frequently cause subclinical infections persisting for several months, (Moroni *et al*., 2005a). Therefore this explains why CNS are most prevalent in dairy goats.

According to Koops *et al*., 2009 CNS should be seen as major pathogens, given their potential to significantly increase SCC and decrease milk yield.

Staphylococcus aureus was the second most prevalent in this study with a prevalence of 23% this was in accordance with reports and literature by Gebrewahid *et al*., 2012, *Staphylococcus aureus* (27.7%), in Ethiopia and Beheshti *et al*., 2010 in Iran , Foschino *et al.,* (2002), Italy *Staphylococcus aureus* was found in 43%,.

This may be due to contamination from milker's hands which are considered as the main tool in the distribution of microorganisms from teat to teat and from animal to animal just like in cattle.

The other pathogens isolated in the study were Streptococcus species (8.8%), Micrococcus spp 4%, *Escherichia coli* 3%, Corynebacteria 1% and Pseudomonas 0.1% which are also important causes of subclinical mastitis in dairy goats although there prevalence is usually low compared to

that of Staphylococcus spp. The presence of *Escherichia coli* and environmental Streptococcus could have been due to poor hygiene as most of these pathogens are found on the animals environment (Gebrewahid *et al*., 2012). The results of prevalence of streptococcus (13%) in these study was in contrast with finding by many researchers Ndegwa *et al*., 2000, Contreras *et al.,* 1997, Kalogridou –Vassiliadou, 1991.

The results of this study showed that the pathogens were generally more sensitive to most of the antibiotic tested. Most pathogens showed high sensitivity to gentamycin and norfloxacin and tetracyclines. The rest of the drugs showed moderately sensitivity.CNS which is the main cause of mastitis in dairy goats showed high sensitivities in gentamycin at 80%, followed by kanamycin and tetracycline both at 79%.This sensitivity pattern is similar to the trend reported by Ndegwa (1999) and this means that this drugs are still effective for treatment of mastitis in dairy goats.

The finding of this study were also in agreement with studies by Wakwoya *et al.,* 2006 in Ethiopia who reported that majority of *Staphylococcus aureus* (92.5%), CNS (88.2%), Corynebacterium (91.6%), and were susceptible to the antimicrobials tested.

Results of this study were also consistent with the reports of Egwu *et al*., 1994 which indicated the presence of drug resistance to bacterial pathogens, including coliforms and streptococci spp. isolated from mastitic goats in Nigeria.

These results were in contrast with finding by Malinowski *et al.,* 2002 who reported that most *Staphylococcus aureus* species have developed multi-resistance to most antibiotics used. The results of this present study shows high prevalence mastitis, Coagulase Negative Staphylococcus is still the most prevalent pathogen causing subclinical mastitis followed by *Staphylococcus aureus* and that most of the pathogen are still susceptible to most antibiotics.

Conclusion

- The prevalence of the subclinical mastitis was high which had negative impact in dairy goat production and hence proper management practices should be instituted to curb the disease.

- Most antibiotic can be used in treatment of subclinical mastitis as there was minimal evidence of antibiotic resistance

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