ABSTRACT
California mastitis test (CMT), direct leukocytes counts and bacteriological examination were performed on 430 milk samples from apparently healthy mammary glands of dairy goats comprising a mixed population of German Alpine, Toggenberg, Saanen and Galla crosses to find the prevalence of subclinical mastisits. The prevalence of subclinical mastitis was 9.8% according to CMT, 9.7% according to direct leukocyte counts and 28.7% by bacterial isolation during a 3-month period. The proportion of the bacteriologically positive milk samples was significantly (P < 0.05) higher than that positive for CMT and direct leukocyte counts. There was a significant (P < 0.05) correlation between CMT and direct leukocyte counts. There was no significant direct relationship between bacterial isolation and CMT. Bacterial organisms were isolated in 22.5% of the 568 CMT-negative milk samples. The results suggest that bacterial organisms isolated from the CMT-negative milk samples were either latent infections or did not stimulate any significant increase in somatic cell counts that could be detected by either the CMT or direct leukocyte counts. The observations of this study indicate that the mere presence of bacteria in goat's milk does not mean that the udder is infected and so does not warrant antibiotic therapy.

Key words: dairy goats, Kenya, subclinical mastitis.

INTRODUCTION
In Kenya the dairy goat industry is becoming an important enterprise in high-potential areas where land fragmentation has rendered dairy cattle farming unsustainable due to insufficient feeds. This is also due to the realization of the economic benefits associated with this enterprise. Goats’ milk in these areas provides a small but all-year-round source of animal protein to the farmer1–3. Therefore, any factor(s) that would affect the quantity and quality of goats’ milk are of a great socioeconomic interest. Milk quality is mainly affected by bacterial contamination of the mammary gland, which may cause either subclinical or clinical mastitis.

There are various indirect methods that are used for diagnosing subclinical mastitis4,5. These include tests such as the California mastitis test (CMT); Whiteside test and Coulter count and direct microscopic counts of somatic cell counts. The indirect tests for determining somatic cell counts in does may be affected by cytoplasmic particles that are present in their milk6,7, which give higher readings even in normal milk8,9. Direct microscopic leukocyte count, although a time-consuming process, has been found to be the most accurate and sensitive test for diagnosing subclinical mastitis in goats10. Somatic cell counts of 1 x 106 cells/ml have been found to detect most of the infected doe milk samples11,12. However, the type of bacterial organism involved13 may influence the somatic cell count in the infected halves. Weakly pathogenic or non-pathogenic microorganisms have been found to stimulate low production of somatic cells in infected quarters14. Bacterial culture of milk samples with subclinical mastitis is performed to determine the organisms involved15. Owing to lack of a definitive diagnosis for subclinical mastitis in does, a combination of tests is often applied16,17.

Information concerning subclinical mastitis in Kenyan dairy goats is limited. This study was undertaken to determine the prevalence of subclinical mastitis in Kenyan dairy goats, and to determine the relationship between mammary gland bacterial infection and CMT and direct leukocyte count (DLC) in goats’ milk.

MATERIALS AND METHODS
The investigation was carried out in 7 dairy goat groups in Nyeri district in Kenya. All the does, in different stages of lactation from all the dairy goat groups, were sampled once a month for 3 months. On the day of sampling, each goat was examined clinically. Particular attention was given to the condition of the mammary glands. All the does used in this investigation were clinically normal and showed no mammary disorders. The numbers of does sampled were 130 in the 1st month, 108 in the 2nd month and 77 in the 3rd month. The does were mainly German Alpine crosses with a few Toggenberg, Saanen and Galla crosses. The mammary glands of the does were examined visually for any injuries and by palpation for consistency and warmth. The milk samples (20 ml) from each mammary gland half were aseptically collected into sterile bijou bottles and kept at 4 °C during transportation to the laboratory. In the laboratory the milk samples were tested for subclinical mastitis by CMT, DLC and bacterial culture. The results for CMT were recorded in 4 categories; 0 negative, traces +1, +2 and +3. The microscopic leukocyte count technique was used to determine the number of leukocytes in the milk samples. The leukocyte cells in the milk samples were prepared and examined by standard Gram’s staining techniques. The milk samples were then streaked on sheep blood agar and McConkey agar plates and incubated aerobically for 48 hours. A quarter was considered infected if 5 or more pure bacterial colonies of the same or different morphological characteristics were present on any of the plates at 24 and 48 hours18. The significant colonies at 24 and 48 hours were selected and subcultured for 24 hours, after which they were Gram-stained and biochemically tested and classified according to standard methods19. When no growth was evident after 72 hours, the sample was regarded as negative for bacterial growth.

Apart from a few cases, most organisms were classified to genus level. The data were analysed using Statistics (SX v4.0).

RESULTS
The results of quarter infection rate according to CMT readings and mammary gland halves positive for DLC are given in Table 1. A quarter was regarded positive if it had a CMT score of + 2. The proportion of mammary gland halves infected in the...
Bacteria were isolated from 22.5% (128) of bacterial isolation and CMT or DLC. No significant direct relationship between DLC for the 3-month period. There was that were positive according to CMT and higher than the number of milk samples positive was significantly ($P < 0.01$) higher than the number of milk samples bacteriologically period was 28.7% (181/630) (Table 2). The overall prevalence of subclinical mastitis for the 3 months was 9.7% (61/630). The overall prevalence of subclinical mastitis according to CMT score was 9.8% (62/630). With regard to the DLC, a mammary gland half was considered infected if it had a value of 8x10³ cells/ml. There were no significant differences in the halves positive for subclinical mastitis according to the DLC reading among the 3 groups. The overall prevalence of subclinical mastitis for the 3 months was 9.7% (61/630). There was a direct relationship between CMT and DLC, i.e. most of the mammary gland halves that were positive for mastitis on CMT score were also positive according to DLC.

The prevalence of bacterial organisms in the milk samples for the 3-month period was 28.7% (181/630) (Table 2). The number of milk samples bacteriologically positive was significantly ($P < 0.01$) higher than the number of milk samples that were positive according to CMT and DLC for the 3-month period. There was no significant direct relationship between bacterial isolation and CMT or DLC. Bacteria were isolated from 22.5% (128) of the 568 milk samples with negative CMT (5nil, 18trace and 105 +1 CMT). The main bacterial organisms isolated from these CMT-negative milk samples were coagulase-negative Staphylococcus (CNS) (71 = 55.5%), Micrococcus (32 = 25.0%), Actinomyces spp. (9 = 7.0%) coagulase-positive Staphylococcus (CPS) (4 = 3.1%), and mixed bacterial growths (12 = 9.4%). Of the 62 CMT-positive milk samples, 9 (14.5%) did not yield bacterial organisms. The most prevalent bacterial organism isolated from the CMT-positive milk samples was CPS (40), which accounted for 75.5% of the isolates. The other bacteria that were isolated from these milk samples were Actinomyces spp. (5 = 9.4%), CNS (3 = 5.7%), Streptococcus spp. (3 = 5.7%), and mixed bacterial organisms (2 = 3.7%).

**DISCUSSION**

The prevalence of subclinical mastitis was 9.8, 9.7 and 28.7% according to the CMT, DLC and bacterial isolation, respectively. There was a significant ($P < 0.01$) correlation between the results of CMT and those of the DLC. This was similar to the observation of Boscos et al. Both CMT and DLC detect somatic cells in the milk, hence the positive correlation between the 2 tests. However, owing to secretion of cytoplasmic particles in goats’ milk, the use of CMT and Coulter counts to predict presence of intra-mammary infections in dairy goats is dubious. Direct microscopic somatic cell count, on the other hand, is considered to be a more reliable test for intramammary infections than CMT and DLC, as this parameter is not influenced by the presence of cytoplasmic particles in the milk. Somatic cell counts of 5 x 10³ cells/ml detected most infected mammary gland halves in goats. The 28.7% prevalence of subclinical mastitis according to bacterial isolation was significantly ($P < 0.01$) higher than that obtained by CMT and DLC methods. Presence of bacteria in the udder of the does is presumed to stimulate production of somatic cells and hence an increase in CMT scores. By contrast, no relationship was found between CMT scores and the presence of bacteria in goats’ milk in this study. This is, however, similar to the observations reported by other workers. The type of bacterial organism involved may influence the level of somatic cell counts in infected quarters. For example, coagulase-negative staphylococci, the predominant bacterial organism (55.5%) isolated from the mammary gland halves with negative CMT, is generally considered to be non-pathogenic or of low pathogenicity to the mammary glands of domestic ruminants, and

**Table 1: California mastitis test (CMT) and direct leukocytes count (DLC) results for 3 sampling occasions.**

<table>
<thead>
<tr>
<th>Sampling month</th>
<th>Mammary gland quarter</th>
<th>Nil</th>
<th>Trace</th>
<th>+1</th>
<th>+2</th>
<th>+3</th>
<th>DLC positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st n = 130 (a)</td>
<td>Right</td>
<td>65</td>
<td>26</td>
<td>24</td>
<td>11</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>56</td>
<td>25</td>
<td>20</td>
<td>17</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>2nd n = 108 (a)</td>
<td>Right</td>
<td>64</td>
<td>14</td>
<td>23</td>
<td>6</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>62</td>
<td>16</td>
<td>21</td>
<td>6</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>3rd n = 77 (a)</td>
<td>Right</td>
<td>44</td>
<td>16</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>40</td>
<td>17</td>
<td>13</td>
<td>6</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Total n = 630 (b)</td>
<td></td>
<td>331</td>
<td>124</td>
<td>113</td>
<td>50</td>
<td>12</td>
<td>61 (9.7)</td>
</tr>
</tbody>
</table>

Number in brackets = percent of the number of the quarter milk samples and (b) of the total quarter milk samples.

**Table 2. Number of bacterial organisms isolated on 3 sampling occasions according to CMT scores.**

<table>
<thead>
<tr>
<th>Sampling month</th>
<th>Mammary gland quarter</th>
<th>Nil</th>
<th>Trace</th>
<th>+1</th>
<th>+2</th>
<th>+3</th>
<th>Number of bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st n = 130 (a)</td>
<td>Right</td>
<td>0</td>
<td>3</td>
<td>19</td>
<td>11</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>1</td>
<td>5</td>
<td>27</td>
<td>12</td>
<td>3</td>
<td>48</td>
</tr>
<tr>
<td>2nd n = 108 (a)</td>
<td>Right</td>
<td>2</td>
<td>3</td>
<td>16</td>
<td>5</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>2</td>
<td>4</td>
<td>19</td>
<td>6</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>3rd n = 77 (a)</td>
<td>Right</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>4</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0</td>
<td>3</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Total n = 630 (b)</td>
<td></td>
<td>5</td>
<td>18</td>
<td>105</td>
<td>44</td>
<td>9</td>
<td>5 (5.0)</td>
</tr>
</tbody>
</table>

Number in brackets = percent of the total bacterial organisms (181).
would thus stimulate low production of somatic cells in infected halves. The other main microorganisms isolated from the CMT-negative milk samples, Micrococcus (25.0 %) and Acinetobacter (7.0 %), are rarely associated with clinical changes in the udder, and often induce only a moderate somatic cell count (SCC) response. The presence of these organisms in the udder, however, has been credited with maintaining a higher than normal SCC, and with increasing the resistance of the colonised quarter to invasion by the major pathogens16,17. Bacterial organisms were not isolated from 14.5 % of the milk samples with positive CMT. This may have been due to the secretion of cytoplasmic particles16 into the milk, resulting in a positive CMT without the presence of bacteria in the udder. Coagulase-positive staphylococci (64.5 %) and Actinomyces pyogenes (14.5 %) were the major intra-mammary pathogens that were predominately isolated from the milk samples with positive CMT. These organisms, owing to their pathogenicity, are known to stimulate marked production of somatic cells in the udder18, hence their predominance in the CMT-positive milk samples.

The observation that bacterial organisms were isolated in 22.5 % of the milk samples that were negative for subclinical mastitis on CMT and DLC indicates that the mere isolation of bacteria does not mean that the udder is infected. This suggests that bacterial isolation has low sensitivity for detecting subclinical mastitis in lactating does, an observation that agrees with other reports19,20.

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REFERENCES

4. Haenlein G F W 1987 Cow and goat milk aren’t the same especially in somatic cell content. Dairy Goat Journal 65: 806
12. Manser P A 1986 Prevalence, causes and laboratory diagnosis of subclinical mastitis in the goat. Veterinary Record 118: 552–554
18. Upadhaya T N, Rao A T 1993 Diagnosis and threshold values of subclinical mastitis in goats. Small Ruminant Research 12: 201–210