Prevalence of bovine mastitis, its therapeutics and control in Tatton Agriculture Park, Egerton University, Njoro District of Kenya

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This study evaluated the prevalence of bovine mastitis, use of therapeutics and its control in Tatton Agriculture Park (TAP), Egerton University, Njoro District of Kenya. Egerton University is located at an altitude of 1890.0-2190.0 M above sea level. The area experiences a temperature in the range of 14.9-21.9°C and an annual rainfall range of 850.0-1100.0 mm. The California Mastitis Testing (CMT) kit was used to identify presence of sub clinical infection. Causative agents were investigated through culture and identification then subjected to commonly used antimicrobials to determine their sensitivity. Milk from 41 lactating cows (Friesian and Ayrshire) were randomly sampled. Of all the samples 34.1% were CMT positive for sub-clinical mastitis while 82.9% yielded bacterial growths. When cultured, 21.4% of the CMT positive were without growths while 85.2% of the CMT negative yielded bacterial growths. The most prevalent bacterial species were Staphylococcus aureus (58.8%). The study showed that the causative organisms were most sensitive to augumentin, gentamycin and cortrimaxazole while resistant to cotroxin. There was 82.9% mastitis prevalence. The CMT reagent may not fully detect sub-clinical mastitis thus further diagnosis by bacteriological tests may increase accuracy. It is concluded that the Staphylococcus species were the most prevalent and augumentin, gentamycin and cortrimaxazole were more effective in the treatment and therefore are the drugs of choice.

Keywords: Antibiotics, California Mastitis Testing Kit, Dairy cows, Sub-clinical mastitis.

INTRODUCTION

Kenya’s dairy sector accounting for 3.5% of the country’s Gross Domestic Product (GDP) is an important source of livelihood Omore, (1997). Farmers raise various dairy cattle breeds (Bebe, 2003) and produce milk, milk products and other intangible benefits (Karanja, 2003, EPZ, 2005). However, the dairy sector is besieged by technical, economic and institutional challenges. Mastitis is a major disease of dairy cattle globally (Omore et al., 1996; Staal, 1998). While clinical mastitis presents with clinical signs, sub-clinical mastitis does not and this is more devastating for most farmers. Numerous agents can cause mastitis in dairy cows but Staphilococcus aureus is the most common etiological agent of bovine mastitis (Bramely and Dodd, 1984; Schukken et al., 1993). S. aureus is highly resistant to antibiotic therapy since it causes granulomas in the mammary gland tissues and easily becomes resistant to antibiotics and requires special treatment and control.

High mastitis prevalence is due to absence of surveillance on control program, poor hygiene during milking and infrequent preventive measures. Mastitis occurs when the protective mechanisms of the host against bacterial infection are deficient. Somatic cell count (SCC), California Mastitis Test (CMT), enzymatic method and conductometry have been mainly used for the diagnosis of mastitis in dairy cattle (Hideyuki et al., 2001). The SCC involves the count of populations of lactic gland epithelial cells, neutrophils, eosinophils, macrophages and plasmocytes in milk. High SCC mainly reflect poor hygiene and a long time-lag between milking and sale of the milk (Mwangi et al., 2000). The average production loss per lactation is about 250 dollars for one infected quarter, other losses are due to discarded abnormal milk, milk withheld from cows treated with antibiotics, cost of early replacement due to culling, reduced sale value of culled cows, cost of drugs and
veterinary service and increased labor cost. Mastitis prevalence is normally high during the long rainy season, and there is a need for control and prevention of mastitis at this time. In the endeavor to find the best management practices for control of mastitis in TAP, there was need to investigate the incidence rate, the specific causative agents and effective drugs for the treatment of mastitis. Losses from mastitis may be enormous- from loss of mammary functions, cost of treatment, spoilt milk, genetic potential due to high culling rates, antibiotic resistance and zoonoses (Arimi et al., 2000). It is therefore, essential to diagnose mastitis at the initial stage of infection to initiate the treatment as early as possible before the bacteria is anchored in the mammary gland (Hideyuki et al., 2001). The objective of this study was to detect the presence of mastitis, isolate and identify the mastitis causing bacteria and their drug sensitivity to the commonly used antimicrobials in Kenya.

MATERIALS AND METHODS

Cow management

Cows were grazed on paddocked Rhodes (Chloris gayana) grass pastures with dairy meal supplementation during morning (0600hrs) and evening (1700hrs) milking time. Each cow was given 2kg of the dairy meal, and during the day they are accessible to clean water and mineral salt ad libitum. A random sample of forty one lactating cows which were in their mid lactation was selected from a herd of eighty six lactating cows.

The CMT Procedure

The test is very simple, can be performed at milking time, gives instant results and is economical. It is a four-compartment paddle with one compartment used per quarter. One or two squirts of milk per quarter are collected in each paddle compartment after foremilk is removed. The paddle is tilted to allow most of the milk to run out leaving about 1 to 2 teaspoons (5 to 10 mL) in each compartment. The CMT reagent is added to each compartment in equal volume to the retained milk. The milk-reagent mixture is swirled in a circular motion with presence of gel (David et al., 2005) or slime being recorded for each quarter. It is the CMT reagent reacting with the DNA of the leukocytes that produces the measurable response in the paddle. Reaction score results are given as somatic cell count or gel formation (David et al., 2005). The CMT score is as follows: None = no gel; trace= very mild gel; 1= mild gel; 2= moderate gel and 3=heavy gel.

Milk sample collection

Milk samples were collected ascetically in labeled sterile bojour bottles. Teats were washed in sanitizing solutions, dried with clean disposable towels dipped in 70% ethyl alcohol. The first two squirts of milk were discarded from each teat then two squirts of milk were collected from each quarter and the bottles immediately closed before removing from beneath the teats. Samples were transported in ice box before being refrigerated at 4°C for laboratory processing.

Culturing of samples

Blood agar culturing

Using a sterile disposable loop, 1ml of milk was streaked on half plate of blood agar (Becton Dickinson and company, USA) supplemented with 5% ovine blood agar (oxoid) and incubated aerobically at 37°C. The plates were examined for bacterial growth at 24 and 48hrs. Pure cultures were further examined for morphological, staining, cultural characteristics and biochemical reactions. Cases with no growth were re-incubated at 37°C for another 24hrs while for mixed growths, a new half sample was re-examined. Bacteria were identified using standardized procedures (NMC, 1999). The quarters were identified as not infected (NT) if no organisms were isolated, or infected with major pathogens (MAP) if infective species e.g. Staphilococci spp, Streptococci spp, Staphilococcus aureus, or Escherichia coli were isolated.

Total viable counts

The pour plate method as described by Shearer and Haris, (2005) was used. Serial dilutions of the samples were made using distilled water to 10⁻⁵. Thereafter, 0.05 ml of dilutions 10⁻⁵ - 10⁻² were used to inoculate molten agar at 45°C. The inoculated medium was poured into sterile petri dishes, allowed to cool and incubated at 37°C for 18hrs. The colonies were counted and the number multiplied by the corresponding dilution factors.

Gram staining and sensitivity test

Bacterial colonies were identified according to colony and cultural characteristics hence reported as heavy pure growth, heavy mixed scanty growth, moderate mixed and no growth. Gram staining was done for the samples according to Gram (1884) procedure. Later, the Bauer-
Table 1. Type of mastitis and its occurrence (%)

<table>
<thead>
<tr>
<th>Type of Mastitis</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute mastitis</td>
<td>8.8</td>
</tr>
<tr>
<td>Chronic mastitis</td>
<td>5.6</td>
</tr>
<tr>
<td>Sub-acute mastitis</td>
<td>3.6</td>
</tr>
<tr>
<td>Gangrene</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Table 2. Presence and distribution of bacterial growth

<table>
<thead>
<tr>
<th>Test used</th>
<th>No. of samples</th>
<th>Growth (%)</th>
<th>No growth (%)</th>
<th>(%) of total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT positive</td>
<td>56</td>
<td>44 (78.6)</td>
<td>12 (21.4)</td>
<td>34.1</td>
</tr>
<tr>
<td>CMT negative</td>
<td>108</td>
<td>92 (85.2)</td>
<td>16 (14.8)</td>
<td>65.9</td>
</tr>
<tr>
<td>Total</td>
<td>164</td>
<td>136 (82.9)</td>
<td>28 (17.1)</td>
<td>100.0</td>
</tr>
</tbody>
</table>

CMT positive (+): gel formation
CMT negative (-): no visible change

Table 3. Bacterial species prevalence

<table>
<thead>
<tr>
<th>Microorganism(s)</th>
<th>Number (n)</th>
<th>% prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>80</td>
<td>58.8</td>
</tr>
<tr>
<td>Mixed (Staphylococci spp., E. coli and Streptococci spp.)</td>
<td>28</td>
<td>20.5</td>
</tr>
<tr>
<td>Streptococcus aureus</td>
<td>16</td>
<td>11.8</td>
</tr>
<tr>
<td>E. coli</td>
<td>12</td>
<td>8.9</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4. Sensitivity (Kirby-Bauer) test and ranking

<table>
<thead>
<tr>
<th>Drug (effective rank)</th>
<th>Disc code</th>
<th>R (mm)</th>
<th>I (mm)</th>
<th>MS (mm)</th>
<th>S (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin (1)</td>
<td>GM</td>
<td>13</td>
<td>14-15</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>Nitrofurazole (5)</td>
<td>NF</td>
<td>9</td>
<td>10-12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Nallidixic acid (4)</td>
<td>NA</td>
<td>9</td>
<td>9-10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Noroflaxin (6)</td>
<td>NF</td>
<td>8</td>
<td>10-11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Cotrimaxazole (3)</td>
<td>CM</td>
<td>10</td>
<td>12-13</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Cotroxin (7)</td>
<td>CT</td>
<td>6</td>
<td>9-10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Augmentine (2)</td>
<td>AG</td>
<td>12</td>
<td>13-16</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Disc code= is the code given to a specific drug; R (mm)= Resistant strain showing little or no clear zone; I (mm)= intermediate with a small clear zone; MS (mm)= moderate with a moderate clear zone; S (mm)= sensitive or susceptible showing the largest clear zone

Kirby Disk-Diffusion Method (Bauer and Kirby, 1959) sensitivity test was done.

RESULTS

The assessed animals showed different degrees of infection as shown in Table 1. The largest (8.8%) proportion of cows exhibited acute type of mastitis. Chronic and sub-acute mastitis was shown by 5.6% and 3.6% of the animals, respectively. The lowest infection was the gangrene type, which is the severest where there is tissue destruction.

Table 2 presents the results of the presence and distribution of bacterial growths from the milk samples. Out of the total 164 samples, 56 (34.1%) were CMT positive. Of these, 44 had growths and 12 showed no growths. The other 108 (65.9%) samples were CMT negative of which 92 and 16 showed growth and no growth, respectively. The 92 CMT negative samples were false negative.

The mastitis causing microbial species prevalence is presented in Table 3. The most prevalent microorganisms were the Staphylococcus aureus that presented 80 counts or 58.8%. The mixed species of Staphylococci, E. coli and Streptococci presented 28 cases or 20.5% occurrence. While Streptococcus aureus was the second least with a count of 16 (or 11.8%), Escherichia coli was least in occurrence exhibiting only a count of 12 or 8.9% prevalence rate.

The sensitivity test results presented in Table 4 revealed the highest sensitivity was for...
Infections are the most frequent, accounting for 8.9% of the samples. It progresses into being chronic. Once infected, it is difficult to treat. The results are associated with kidney damage and other complications. Bacterial isolation is essential, thereby releasing DNA responsible for the gel clumping test (GCT).

The unreliability of CMT reagent has been highlighted. According to the study of Leslie et al. (1998) and Barbano (1989), this test is not reliable in detecting infections. In a study of 41 samples, 14 were CMT negative, while 27 were CMT positive. Gentamicin led to the highest recovery against others. They found that gentamicin led to the highest recovery against others. These results are corroborated with those of Tufani et al. (2012) who tested the drugs in vivo. They found that gentamicin led to the highest (84.21%) recovery against others. The current test drugs are the available ones in the Kenyan market and therefore are the commonly used therapeutics. In a recent investigation, the incidence of mastitis was found to be 13.3 per 100 cow-years at risk. According to Omoro et al. (1998) the prevalence of bacteria pathogens is high, with Staphylococcus aureus the most common (22.1% of all samples) but the infectious agents only modestly associated with increases (highest increase of 5% for S. aureus) in milk yield. This led to the conclusion that mastitis is not currently an important constraint to productivity despite the high prevalence of infectious agents. However, it is

**DISCUSSION**

Mastitis infections could be controlled by using the common intramammary infusions of antibacterial agents as a dry-cow therapy. More modern methods like the external and internal teat sealers described by Huxley et al. (2002) have not been tested in Kenya. An increase in somatic cell count in milk leads to the release of lipolytic (lipases) and proteolytic (plasmin) enzymes which can degrade the triglycerides of milk fat and casein contents of the milk (Saeman et al., 1988; Barbano, 1989). This leads to poor quality milk in the mastitis-affected animals. The CMT, enzymatic method and conductometry enable mastitis diagnosis from the middle stage to the later stage after bacterial infection. When the udder is invaded by bacteria, leukocytes with phagocytotic bacterialcidal activity accumulate in the milk to interrupt bacterial proliferation.

Current milk handling and safety regulations in Kenya are derived from models in industrialised countries. While the strip cup identifies the clinical mastitis by showing milk changes in terms of colour, smell and consistency, the sub-clinical infections escape to be detected since there is no detectable physical changes in the milk in sub-clinical cases. The reagent-based CMT then is resorted to. The CMT is an indirect cow-side chemical test for estimating somatic cell counts or numbers (Leslie et al., 2002). The CMT chemical reagents rapture the cells thereby releasing DNA responsible for the gel formation and viscosity (Schalm et al., 1971). The ability of the test to predict presence of bacteria depends on the level of somatic cell in the quarter. The CMT helps to indicate the presence of subclinical mastitis, which is the presence of an intramammary infection without apparent signs of local inflammation or systemic involvement. Although transient episodes of abnormal milk or udder inflammation may appear, these infections are for the most asymptomatic and, if the infection persists for about 2 months, it transits into being chronic. Once established, many of these infections persist for entire lactations or the life of the cow. Detection is best done by examination of milk for somatic cell counts (predominantly neutrophils), which at the farm level is tested using the California Mastitis Test. According to the results shown in Table 1 above, out of 41 samples, 14 were CMT positive of which 21.4% were false positive. Of the 27 CMT negative samples, 85.2% were false negative. This scenario occurs due to low SCC that may result in undetection since somatic cell counts are positively correlated with the presence of infection. It could also occur due to the unreliability of CMT reagent as indicator of bacterial presence. Out of the 41 samples 34 showed growth, which represents 82.9% of the cases analyzed. The CMT negative samples that showed growth may have escaped detection due to single sampling. This concurs with the results of Randy et al (2003) who tested the CMT on bacteriological culture of single milk samples. This emphasizes the need for further tests on CMT cases like bacterial isolation. Identifying quarters with higher CMT scores increase the probability of getting a positive culture. Quarters with a CMT of “3” are three times more likely to yield a positive culture than a CMT of 1. Conversely, CMT tests that result in “trace” (200,000 to 400,00 cells/mL) are quarters that are likely to be infected, but may be difficult to detect. Thus, the accuracy of CMT or somatic cell counts to predict infection is low. Studies have suggested that a single CMT or somatic cell count may only detect 60 to 80% of infected quarters. Multiple tests increase the sensitivity of detecting infections, and may be acceptable within several days after calving. Thus, decisions for treatment or mastitis management programs should be made with a combination of somatic cell testing, cultures, and cow and herd history.

The bacterial isolates in this case are important pathogens associated with mastitis in dairy production (Wilson et al., 1995). Staphylococcus ssp account for 58.8% of the mastitis cases in TAP while Streptococcus ssp accounts for 11.8% and mixed infections of Staphylococcus ssp. Streptococcus ssp and E. coli accounts for 20.5%. E. coli accounts for 8.9% of the cases of infections. In Kenya, diarrhoea is one of the commonest diseases of children caused by a variety of pathogens including E. coli (Sang et al., 1992). 0157: H7 strain isolates from the milk produce verocytotoxin and these toxins are associated with kidney damage and kidney failure and have been identified in Kenya (Arimi et al., 2000).

The sensitivity table shows that the most effective drugs to clear the mastitis infections are: augmentine, gentamycin, and cotrimaxazole. These results are corroborated with those of Tufani et al (2012) who tested the drugs in vivo. They found that gentamycin led to the highest (84.21%) recovery against others. The current test drugs are the available ones in the Kenyan market and therefore are the commonly used therapeutics. In a recent investigation Omoro et al., (1996), the incidence of clinical mastitis was found to be 13.3 per 100 cow-years at risk. According to Omoro et al., (1998) the prevalence of bacteria pathogens is high, with Staphylococcus aureus the most common (22.1% of all samples) but the infectious agents only modestly associated with increases (highest increase of 5% for S. aureus) in the natural logarithm of somatic cell counts. S. aureus was the only bacteria associated with a small decrease (3%) in milk yield. This led to the conclusion that mastitis is not currently an important constraint to productivity despite the high prevalence of infectious agents. However, it is
expected that mastitis will be a big problem once milk yields are increased, given the large proportion of cows with bacterial infections, the confined housing practised and the minimal mastitis control measures performed (udder disinfection or dry-cow therapy is rarely practised).

RECOMMENDATION

A well established surveillance programme of detecting mastitis can avert developing and spreading of herd mastitis. The TAP can use augmentin, gentamycin or contrimaxazole for therapeutics. For effective prevention and treatment of mastitis the use of conventional antibiotic drugs is discouraged. Principles of control of Staphylococcus aureus should be focused on preventing new infections, elimination of existing infections; and monitoring progress after implementation. The single most important step in preventing new infections is to dip every quarter of every cow after every milking with an effective teat dip as well as practicing dry cow therapy. Additionally, milking equipment should be hygienic and routinely use sound milking procedures.

Dry cow CMT scores also can be useful in the administration of dry cow treatments on a selective basis. Additionally, selecting infected cows for therapy with CMT is not foolproof because some infected cows may have low CMT scores, and likewise some non-infected cows may have high CMT scores. Considering the limitations of CMT testing, it is a quick, economical method of screening cows, and particularly quarters with elevated SCC, especially with trace and above CMT score. Maintenance of environmental hygiene can help to reduce the E. coli and Staphylococcus aureus infections.

CONCLUSION

The mastitis incidence rate of 82.9% is relatively high. The specific causative agents found were Staphylococcus aureus, Streptococcus agalactiae and Escherichia coli, as single or mixed type. These microorganisms were most sensitive to gentamycin, augmentin and contrimaxazole and least sensitive to cototrexin. The drug of choice for the treatment of the above causative agents are gentamycin, augmentin and contrimaxazole.

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