ISOLATION AND IDENTIFICATION OF PATHOGENIC STAPHYLOCOCCUS AUREUS IN HARAMAYA UNIVERSITY GOAT FARM

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ABSTRACT

A cross-sectional study was conducted to investigate the prevalence of subclinical mastitis and isolate pathogenic Staphylococcus aureus from lactating dairy goats located in Haramaya University (HU) goat farm, Ethiopia, from November 2014 to June 2015. California mastitis test (CMT) was used to screen subclinical case of mastitis on 40 lactating doe. All CMT positive animals were further subjected to further microbiological analysis so as to isolate and identify S. aureus. CMT result showed that 23 (57.5%) animals had subclinical mastitis and pathogenic S. aureus was isolated from 11 (47.8%) of animals with subclinical mastitis. The present study indicated that pathogenic S. aureus is highly prevalent among sub clinically infected animals in the farm. Therefore careful milking practice (single towel for each caw, disinfecting hands before milking, and milking infected caws last, regular checkup and dry caw, ewes and does therapy should be followed.

Key words: Goat, Haramaya University, Staphylococcus aureus, Subclinical mastitis

LIST OF ABBREVIATIONS

CMT California mastitis test
FAO Food and agriculture organization
HU Haramaya University
MSA Mannitol salt agar
MSCC Mastitis somatic cell count
NMC National mastitis council
RBC Red blood cell
SCC Somatic cell count
1. INTRODUCTION

In Ethiopia cows represent the largest proportion of cattle population. According to the food and Agriculture Organization (FAO, 2001), 42% of the total cattle heads for the private holding are milking cows. Milk produced from these animals provides important dietary scour for the majority of urban and per urban population. However, milk production often doesn’t satisfy the country’s requirement due to multitude of factors contributing to disease of the mammary glands known as mastitis which is among the leading factors contributing to reduced milk production (Fekadu, 1995). Moreover, mastitis is incriminated as an important diseases constraint (Mohammed and Etana, 1996).

Mastitis, or inflammation of the mammary gland, may be diagnosis based on chemical changes in the milk and pathological changes in the glandular tissue. The most important changes in the milk include discoloration, the presence of clots and the presence of large numbers of leukocytes. There is swelling, heat, pain and edema in the mammary gland in many clinical cases. However, a large proportion of mastitic glands are not readily detectable by manual palpation nor by visual examination of the milk using a strip cup; these quarters represent subclinical infections. Because of the large numbers of subclinical cases, the diagnosis of mastitis depends largely on indirect tests (Radostits et al., 2000).

Mastitis usually results from an infectious agent. The majority of the cases are caused by only a few common bacterial pathogens, namely, Staphylococcus species, Streptococcus species, Coliforms and Actinomyces pyogenes (Du Preeze, 2000; Quinn et al., 2011). It may also be associated with many other organisms including Actinomyces pyogenes, Pseudaomonas aeruginosa, Nocardia asteroides, Clostridium perfringens and others like Mycobacterium, Mycoplasma, Pastuerella and Prototheca species and yeasts (Rodostits et al., 2000).

The most common pathogens involved in mastitis are contagious bacteria mainly Staphylococcus aureus and Staphylococcus agalatiae and environmental bacteria mainly coli forms and some Streptococci species that are commonly present in the environment (Radostits et al., 2000).

Intramammary infections caused by S. aureus warrant special attention because this bacterium is responsible for both acute clinical mastitis (gangrenous mastitis) and sub
clinical mastitis. *S. aureus* secretes several toxins contributing to the pathogenesis of mastitis and also plays a role in food borne disease, even with pasteurized milk because of the thermo-stable enterotoxins. These enterotoxins are produced not only by *S. aureus* isolates from clinical mastitis but also by isolates from sub clinical mastitis (De Santis *et al.*, 2005).

Pathogenic staphylococci are commonly identified by their ability to produce coagulase, and thus clot blood (Kloos and Musselwhite, 1975). This distinguishes the coagulase positive strains, *S. aureus* (a human pathogen), and *S. intermedius* and *S. hyicus*, which are animal pathogens, from the other staphylococcal species such as *S.epidermidis*, which is coagulase-negative.

In goats mastitis has been reviewed by Contreras *et al.* (2003), and Bergonier and Berthelet (2003) have reviewed the epidemiology and control of mastitis in sheep. Additionally, in their study, Paape *et al.* (2001) explored the feasibility of indirectly diagnosing mastitis in small ruminants by using MSCC, and Gonzalo (2004) recently discussed the analytical, health, productive and technological aspects of performing MSCC in sheep and goat milk. Because important differences exist among dairy ruminants, the approach to mastitis control in goats and sheep should be carefully made with a specific point of view, and not by generalizing results obtained from research on mastitis in dairy cows. Current knowledge of mastitis in small ruminants has been recently reviewed Bergonier *et al.* (2003), more specifically, the role of intramammary pathogens in mastitis.

Mastitis may render milk unsuitable for human consumption or provide a mechanism for the spread of diseases like tuberculosis, streptococcal intoxication, colibacillosis, streptococcal sore throat and brucellosis to human (Radostits *et al.*, 2000). Other public health hazards associated with mastitis is the consumption of antibiotic contaminated milk and milk products which leads to allergic responses, changes in intestinal flora and development of antibiotic resistant pathogenic bacteria (Thirapatsakun, 1999).

Mastitis is one of the major causes of economic loss in dairy cattle. *Staphylococcus aureus* is the main pathogenic species causing the sub clinical form of mastitis. This type of udder infection impairs alveolar function, reduces milk yield and has a deleterious effect on milk composition, including increased milk somatic cell count (SCC) (Gudding *et al.*, 1984; Nickerson, 1989). Staphylococcal mastitis is the commonest and economically the greatest concern wherever dairy farming is practiced. The chief reservoir of this bacterium is an infected udder. The organism is well adapted to survive
in the udder and usually establishes mild sub clinical infection of long duration. Bacteria are shed into milk from infected quarters (Tsegaye, 1988).

The prevalence of clinical and subclinical mastitis in Ethiopia ranges from 1.2-21.5 and 19-46.6%, respectively (Hussein, 1999; Kassa et al., 1999; Lemma et al., 2001; Workineh et al., 2002; Dego and Tareke, 2003). According to Lemma et al. (2001) of the major diseases of crossbred cows in Addis Ababa milk shed mastitis was the second most frequent disease next to reproductive diseases. Mungube et al. (2005) and Tesfaye et al. (2010) estimated the economic losses from mastitis in the urban and periurban areas of Addis Ababa, to be 58 and 78.65 US$ per cow per lactation, respectively.

In present day of Ethiopia, there is a national drive to alleviate the existing food deficit by devising different agricultural strategies including improvements of the productivity of livestock sector by controlling some of the major infectious disease of dairy animals through regular monitoring. However, mastitis, as a disease, has received little attention in Ethiopia, especially the sub clinical form which is mainly caused by S. aureus (Hundera et al., 2005; Mekonnen et al., 2005). In Ethiopia mastitis has been reported mainly in cattle. However, data on the occurrence of subclinical mastitis as well as the status of S. aureus in goats is limited. Haramaya University has many livestock farms and among these the dairy goat is the one which provides milk and milk products. This led to investigate and isolate pathological S. aureus bacteria from milk of the dairy goat farm and hence decision was made to study the case by considering those lactating goats.

Therefore, the objectives of the present study were:

- To investigate the prevalence of subclinical mastitis in lactating doe and
- To isolate and identify pathogenic Staphylococcus aureus in Haramaya university dairygoats.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted from November 2014 to June, 2015 in Haramaya University dairygoat farm. Haramaya University is located in Eastern Haramaya zone of Ethiopia,
approximately 505 km East of Addis Ababa. The vegetation that constitutes the available pasture lands in the area is predominately native grasses and legumes interspersed with open acacia shrub land. The elevation is approximately 2000 meter above sea level and the mean annual temperature and relative humidity are 18 °C and 65 °C, respectively. HU receives an annual rain full approximately 900 mm, with a bimodal distribution pattern peaking in mid-April and mid-August.

2.2. Study Animals

The study animals were lactating goats managed under Haramaya University goat farm. All were endogenous breed kept under intensive husbandry.

2.3. Study Design

A cross-sectional study was carried out from November 2014 to June 2015 to isolate and identify pathogens involved in mastitis in Haramaya University dairy goat farm.

2.4. Sampling method and Sample size

Purposive sampling method was employed to select lactating animals and relevant information about lactating goats in the farm was recorded on appropriate format (Annex I). Accordingly, 40 doe were included in the study.

2.5. Sample Collection and Laboratory Examination

2.5.1. Milk sample collection

Milk sample was collected before milking early in the morning to avoid contamination for culture. The milk sample was taken before the cow treated with either intramammary or systemic antimicrobial agent. For good collection of sample the teat was wiped thoroughly with disinfectants such as soap, water and 70% ethyl alcohol. The sterile collection bottle was used and the first stream of milk from each quarter was discarded to avoid contamination (NMC, 1999). Approximately 10ml of milk sample was taken; 3ml of the milk was used for CMT and the remaining sample for bacterial culture.
2.5.2. Methods of transportation and submission of samples

After collection milk sample, all samples were clearly labeled with appropriate identification the cow’s identification number, quarter using permanent marker on the test tube and all samples were transported with ice box to the laboratory without delay. In the laboratory, sample were cultured immediately or stored at 4 °C in any case of delay. Analysis of specified samples was performed on isolation and identification of pathogenic bacteria was conducted in microbiology, college of Veterinary Medicine, Haramaya University.

2.5.3. Detection of mastitis using CMT

The California mastitis reagent was used to screen cows with sub clinical mastitis. Milk sample collection was made according recommended by NMC (1999). Equal amount of milk and commercial reagent which contains 3% alky aryl sulfonate and bromocersol purple as PH level indicator are mixed in the cup on a paddle and gentle swirling was applied to the mixture in a circular motion the result of the test was indicated on the base of gel formation (Smith and Sherman, 2009). The interpretation grade of CMT was evocated and the result grades 0 for negative and trace, 1, 2 and 3, for positive (Quinn et al., 2011) (AnnexII).

2.5.4. Isolation and identification

Preparation of Culture Media

To prepare media for bacterial culture, the manufacture’s instruction should always be followed, besides few additional general points were included, all glasses wares used for the preparation of media were first sterilized using appropriate equipment like autoclave, hot air over, the appropriate amount of dehydrated media were weighed out using digital balance placed in a flask and the required amount of distilled water were added to the powder media. Dehydrated media containing agar are dissolved in a hot plate which incorporate magnetic stirrer until it boil, then the media were sterilized by autoclave at 121 °C for 15min holding time, and cooled in water bath at 50 °C since RBC are not tolerate high Temperature, adapted from Quinn et al.(2011). The media used during the study were blood agar and manitol salt agar.
Milk Sample Culture

Bacteriology was performed on isolated pathogenic *S. aureus* bacteria in laboratory of college of veterinary medicine, HU. All CMT positive milk sample were subjected to culture on blood agar in search of mastitis producing in standard of examination for mastitis. The bacteriological culture was performed following the standard microbiological technique (Quinn *et al.*, 2011) and microbiological procedures for the diagnosis of bovine mastitis infection (NMC, 1999). A loop full of milk was streaked on 7% sheep blood agar plates were checked for growth for primary culture every 24, 48 and up to 72 hours to rule out slow growing microorganisms. According to NMC (1999) the following assumption were considered for the results. A sample was considered negative if there was no growth after 48 hrs or 72 hrs and if there was profuse growth of environmental bacteria.

Primary Identification

For the primary isolation of pathogenic *S. aureus* microorganisms, colony size, shape, color, hemolytic characteristics, gram reaction, catalase, oxidase, O-F testes were considered. After these colonies were sub cultured to manitol salt agar to get a pure culture. Standards were used according Quinn *et al.* (2011).

Biochemical Tests

After the colonies were grow on primary and selective media, the colonies were subculture to nutrient agar for other biochemical test. Characterizations of isolated pathogenic *S. aureus* bacteria were done by different methods of biochemical tests such as, coagulase test, catalase test OF test. The procedures for the identified pathogens were adapted from Quinn *et al.* (2011) (Annex III).

2.6. Statistical analysis

The data was compiled and analyzed with SPSS statistical package version 16. Prevalence of mastitis and isolated pathogens was determined using standard formulae (i.e., the number of positive animals/samples divided by the total number of animals/samples examined).
3. **RESULT**

3.1. **Prevalence of Mastitis Based on CMT**

Out of total examined (n=40) lactating goats, 23 (57.5%) goats were positive under screening test using CMT (Table 1).

3.2. **Staphylococcus aureus Isolates**

Out of all CMT positive lactating goats examined, *S. aureus* was isolated from 11 (47.8%) animals (Table 1).

**Table 1:** CMT positive milk samples and Isolates of *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Animals</th>
<th>Total examined</th>
<th>Number of CMT positive animals (%)</th>
<th>Number of animals positive to <em>S.aureus</em></th>
<th>Prevalence of <em>S.aureus</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doe</td>
<td>40</td>
<td>23 (57.5)</td>
<td>11</td>
<td>47.8</td>
</tr>
</tbody>
</table>

3.3. **Microbiological Characteristics of Isolates**

The growth characteristics of bacterial isolates on different media and their morphological as well as biochemical reactions on different tests are indicated in table 2.

**Table 2:** Phenotypic characteristics of isolates

<table>
<thead>
<tr>
<th>Test conducted</th>
<th>Number of isolates which were Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Grape like appearance</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Catalase test</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Coagulase test</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Growth on blood agar</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Hemolysis on blood agar</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Growth on MSA</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Mannitol fermentation on MSA</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>
4. DISCUSSION

Analysis of the bacteriological examination of the milk samples was made to isolate and identify the main ethological agent involved in the disease process. The organism were identified on the base their culture, staining characteristics and biochemical reaction.

The isolation rate of pathogenic *S.aureus* from subclinical cases of mastitis in goat was 47.8%. The isolation rates of pathogenic *S. aureus* in the present study was higher than the finding of Geberewahid *et al.* (2011), who reported 27.7% in Kefeta Humera and Tanqua Abergelle District, while it was far higher than report in sheep (10.6%).

The present study finding was closely comparable with the finding of Werkneh *et al.* (2002) and Kerro and Tereke (2003) where *S.aureus* accounted for 39.2% and 40.5% isolate respectively in Addis Ababa and southern Ethiopia. However, the present finding was far higher than that of Gizat (2004), Bishi (1998) and Hussein (1999) who reported 17.8%, 9% and 10.69% in Addis Ababa respectively in cows.

The relatively high prevalence of pathogenic *S.aureus* in the study could be associated with the total absence of dairy doe therapy and post milking teat dipping the invariable hold milking practice, low calling rate of chronically infected does culling was usually due to ageing and reproductive problem limited knowledge of farm workers on segregation as control options.

5. CONCLUSIONS AND RECOMMENDATIONS

The present study indicated that subclinical mastitis is prevalent in haramaya university goat farm. More importantly, pathogenic *S. aureus* was isolated from large numbers of lactating animals and considered as threat to the dairy farm and the beneficiaries as goat farm in Haramaya University plays significant role to the society. Therefore careful milking practice (single towel for each caw, disinfecting hands before milking, and milking infected caws last, regular checkupand dry caw, ewes and does therapy should be followed.

Based on the above conclusions and available material, the following recommendation are forwarded:
The farm management practices need to be improved in term of milking and personal hygiene.

- The dairy, sheep and goat farms should seriously implement regular screening test for the detection pathogenic *S. aureus*.

- Avoid indiscriminate use of antibiotic in the dairy farm.

- Culling of chronically infected animals should be practiced to reduce reservoir of infection for the rest of the herd

**ACKNOWLEDGEMENTS**

First and for most, I want to thank the Almighty God for His everlasting mercy and protection. Next, my deepest gratitude goes to Dr. Fitsum Almayhu my academic advisor, for his guidance, valuable advice and devotion of time which has enabled me to have the correct path throughout my course of engagement.

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ANNEXES

Annex I: Data recording sheet

1. A recording sheet during sample collection
   1.1 Name of the farm ___________________
   1.2 Date of sample collection_________________
   1.3 Goat/Sheep Id No. ____________________
   1.4 Stage of lactation (early, middle, late)  □ □ □
   1.5 Clinical examination of under and teat
      1.5.1 Heat and/or udder lesion and tick infestation
      1.5.2 Inflammatory signs (swelling, hotness, pain etc…)
   1.6 Physical examination of milk (Normal, presence of clots discoloration, watery, Blood stained and other)

2. Screening test result (CMT), (-, trace, 1+, 2+, 3+)
   2.1 Presence or absence of mastitis ______________

3. Data recording sheet during bacteriological analysis
   3.1 Presence or absence of hemolysis on blood agar __________
   3.2 Colonial characters on blood Agar (size, shape, color) at 24, 48, and 72 hrs ______________
   3.3 Stain character Gram stain ______________
      3.3.1 Shape (cocci, rod, pleomorphic) ______________
      3.3.2 Staining reaction (Gram + ve or Gram –ve) __________
   3.4 Other media inoculated and the result

4. Biochemical test conducted and the result (catalase, oxidase, O.F)
   4.1 Coagulase ______________
   4.2 Catalase ______________

5. The organisms’ isolated. ______________
Annex II: Interpretation of CMT finding

<table>
<thead>
<tr>
<th>CMT</th>
<th>Meaning</th>
<th>Visible Reaction</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>Negative</td>
<td>Milk fluid and Normal</td>
<td>0-200,000 cell/mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-25% neutrophils</td>
</tr>
<tr>
<td>T</td>
<td>Trace</td>
<td>Slight precipitation But no gel formation</td>
<td>150,000-5000,000 cell/mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30-40 neutrophils</td>
</tr>
<tr>
<td>+1</td>
<td>Weak positive</td>
<td>Mixture thickness With gel formation</td>
<td>400,000-1000,000 cell/mg</td>
</tr>
<tr>
<td>+2</td>
<td>Distinct positive</td>
<td>Mixture thickness With gel formation</td>
<td>8000,000-5,000,000 cell/mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60-70% neutrophils</td>
</tr>
<tr>
<td>+3</td>
<td>Strong positive</td>
<td>Viscosity increases With gel formation</td>
<td>5,000,000-7,000,000 cell/mg</td>
</tr>
</tbody>
</table>

Annex III: Biochemical reactions of *S. aureus*

<table>
<thead>
<tr>
<th></th>
<th><em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Coagulase</td>
<td>+</td>
</tr>
<tr>
<td>Hmolysis</td>
<td>+</td>
</tr>
<tr>
<td>OF test</td>
<td>F</td>
</tr>
<tr>
<td>Growth on MSA</td>
<td>Grow and ferment Manitol (yellow)</td>
</tr>
</tbody>
</table>

+ = Positive; O= Oxidative; - = Negative;  F = Fermentative