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Secretariat: Faculty of Veterinary Medicine, Universitas Airlangga
Kampus C Unair, Jl. Mulyorejo Surabaya 60115 Indonesia
Ph +62 31 5993016; Fax +62 31 5993015 www. h.unair.ac.id
Email : vmic@ h.unair.ac.id
Identification Of *Staphylococcus Aureus* As Mastitis Causes In Crossbreed Etawa Goats In Polewali Mandar.

Ichwani Syam Mustapa*, Lucia Winata Muslimin*, Muhammad Fauzih Asjikin*
*Program Study of Veterinary Science, Faculty of Medicine, Hasanuddin University

**Abstrak**

Mastitis is an inflammation of the udder are divided into discovered subclinical mastitis without clinical symptoms and clinical mastitis that have clinical symptoms of the udder and decreased milk production and quality. *Staphylococcus aureus* is one of the species of pathogens causing mastitis in goats Peranakan Etawa. This research aims to detect *Staphylococcus aureus* as a cause mastitis at Crossbreed Etawa Goats in Polewali Mandar. Milk samples obtained from mastitis testing method California Mastitis Test (CMT) as many as 10 samples. Bacterial detection is done using culture method using four media namely *Natrium Agar* (NA) and *Baird Parker Agar* (BPA), *Mannitol Salt Agar* (MSA), *Muller Hinton Agar* (MHA) gram stain, catalase test, test *Mannitol Salt Agar* (MSA) and Novobiocin test. The results showed that all the samples of milk containing the bacteria *Staphylococcus aureus*.

**Key Word**: *Staphylococcus aureus*, Crossbreed Etawa Goats, Mastitis,
Identification Of *Staphylococcus aureus* As Mastitis Causes In Crossbreed Etawa Goats In Polewali Mandar

Ichwani Syam Mustapa,\textsuperscript{a} Lucia Winata Muslimin\textsuperscript{b}, Muhammad Fauzih Asjikin\textsuperscript{c}

\textsuperscript{a}Program Study of Veterinary Science, Faculty of Medicine, Hasanuddin University, Makassar, Makassar 90245, Indonesia.
\textsuperscript{b}Program Study of Veterinary Science, Faculty of Medicine, Hasanuddin University, Makassar, Makassar 90245, Indonesia.
\textsuperscript{c}Program Study of Veterinary Science, Faculty of Medicine, Hasanuddin University, Makassar, Makassar 90245, Indonesia.

**ABSTRACT**

Mastitis is an inflammation of the udder are divided into discovered subclinical mastitis without clinical symptoms and clinical mastitis that have clinical symptoms of the udder and decreased milk production and quality. *Staphylococcus aureus* is one of the species of pathogens causing mastitis in on Crossbreed Etawa Goats. This research aims to detect *Staphylococcus aureus* as a cause mastitis at Crossbreed Etawa Goats in Polewali Mandar. Milk samples obtained from mastitis testing method California Mastitis Test (CMT) as many as 10 samples. Bacterial detection is done using culture method using four media namely Natrium Agar (NA) and Baird Parker Agar (BPA), Mannitol Salt Agar (MSA), Muller Hinton Agar (MHA) gram stain, catalase test, test Mannitol Salt Agar (MSA) and Novobiocin test. The results showed that all the samples of milk containing the bacteria *Staphylococcus aureus*.

Keyword : *Staphylococcus aureus*, Crossbreed Etawa Goats, Mastitis,
breeding district in West Sulawesi is Polewali Mandar Regency. Where in 2015, goat population in Polewali Mandar regency reaches 104,622 heads, consisting of 27,367 goats and 63,487 goats female (Dinas Pertanian dan Peternakan Polman, 2014).

Identification of the causative agent of mastitis is a major factor as one of the steps in the treatment and determination of therapy on mastitis cases. By knowing the agent causes mastitis then handling mastitis will be easier to do.

RESEARCH METHODOLOGY
Time and place
This research took place from September to October 2016. While the research was conducted in Wonomulyo Polewali Sub-district Polewali Mandar District for sampling of Crossbreed Etawa Goats Breast Milk and Identification of bacteria that will be done in Microbiology Laboratory of Veterinary Medicine Program of Hasanuddin University.

Research Materials
Samples and Sampling Techniques
The milk samples were from the female Crossbreed Etawa Goat, obtained from one of the breeders. By using purposive sampling, milk taken from goats that have the criteria of udder must be swollen and postpartum. Samples taken as many as 25 samples and conducted CMT testing. Positive results were determined based on the scoring system on CMT testing.

Determination of Mastitis
Determination of positive results of mastitis was performed based on the level of viscosity when CMT reagents with milk mixed. The higher the viscosity that occurs the higher the positive height. The CMT test values consist of trace, positive 1 (+), positive 2 (++) and positive 3 (+++).

Figure 1. The test results of CMT (A) trace, (B) is weak, (C) being, and (D) is strong

Research Methods
Test Mastitis with CMT
Testing was done by taking 2 ml of milk placed in paddle then reacted with 2 ml CMT reagent. The mixture is shaken in a horizontal circle for 10-15 seconds, then observed. This reaction is characterized by the presence or absence of changes in milk viscosity.

Sampling
The milk samples to be tested in the Laboratory taken from milk which has been tested for CMT ± 20 ml and direct fit into a sterile enclosed tube and has been labeled, then stored in the cool box contains ice pack, so that the temperature is stable at 5-10°C to avoid the proliferation of bacteria, to arrive at the laboratory.

Isolation and Identification of Bacteria
- Samples of milk which has been tested with reactant CMT next performed a dilution 10⁻¹ to 10⁻⁴. Furthermore, the milk that has been diluted grown on media NA and BPA each 1 ml and incubated during 18-24 hours in temperatures of 37°C. Furthermore, the colonies that formed after 24 hours, observed the shape, color, size and elevation. The colonies were observed in microscopic observations with certain coloring Gram and continued with several other trials.

- Colonies grow on media NA used to calculate the Total Plate Count (TPC). Colonies of *Staphylococcus aureus* on BPA discrete circular colonies, slick/smooth, convex, 2 to 3 mm diameter, gray to blackish, round the edges of the colony. The colony has the consistency of fatty and gooey when taken with a needle and inoculated. Furthermore, the colonies of BPA media in cultured using the MSA. Colonies of *Staphylococcus aureus* on the MSA has a characterized yellow (BSN, 2011).

- Test identification with Staining Gram. Object glass melted aquades or NaCl 1 drop of bacterial suspension was placed on glass objects in the fixation on top of bunsen. The preparations have been in fixation and then melted with Crystal Violet and then silenced for 1 – 2 minutes. The rest of the color substance disposed of, then rinsed with running water. Throughout preparations be dripped with lugol's solution and leave it for 30 seconds. Discard
the solution and rinse with lugol's water flow. Preparations in 96% alcohol rinse until all substances the smudging of colors, and immediately wash with running water. Squirt with Fuschin color substance, leave on for 2 minutes then rinse under running water and then left to dry, observed under the microscope with a 100 x objective magnification emersi wear. Gram-positive bacteria have characterized the cocci and Gram-negative bacteria while the huddle has characterized the rod-shaped.

- The catalase test is carried out by dripping a 3% Hydrogen Peroxide solution above the glass object and with the ose wire taking some colonies and in touching the liquid it waits for a while until a reaction is marked by bubbles.

- Novobiocin test. The novobiocin test is done by first taking the colony and planted in 0.9% NaCl or aquades until it reaches the turbidity of 0.5 McFarland. The suspension that has been standardized according to McFarland standard is then carried out swab on MHA media using cotton buds that have been dipped into the colony that has been in accordance with the standard. After swabs, then put the Novobiocin disk to MHA media and incubated at 37°C. The existence of clear areas around The disk shows a positive result of Staphylococcus aureus and for subsequent measurements of the clear zone using a sliding range.

RESULT AND DISCUSSION
Examination of Mastitis
This study begins by examining mastitis Crossbreed Etawa Goats with criteria such as the occurrence of swelling on the udder as happened in the picture and the lactating goat. 25 samples examined obtained 10 samples of mastitis positive goat milk.

Figure 2. The ungodly criteria of the Crossbreed Etawa Goats taken by milk for CMT testing (Up) and Results of milk testing using CMT which experienced a change of viscosity (Down)

Isolation and Identification of Staphylococcus aureus
Bacterial Isolation
Isolation was done by planting bacteria on Baird Parker Agar (BPA) and Nutrient Agar (NA) media. Aseptically dilution starts from 10⁻¹ to 10⁴. For dilution 10⁻³ and 10⁻⁴ are inserted in a cup of 1 ml and Nutrient Agar (NA) medium is poured and homogenized by wiggling as number 8. While 10⁻² dilution is inserted in 1 ml cup then Baird Parker Agar (BPA) Poured and homogenized by wiggling like the number 8. The cup was incubated for 24 - 48 hours at 37°C.

The results of research on 10 milk samples are cultured on two media namely NA media and BPA media, each media produces the growth of different colonies. Colonies grown on Nutrient Agar medium were used to calculate Total Plate Count (TPC), while colonies growing on Baird Parker Agar medium were grown on Mannitol Salt Agar (MSA) medium.

Figure 3. Baird Parker Agar (BPA) Media with Colonies
Table 1. Calculation results of Total Plate Count (TPC) in NA media.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample Code</th>
<th>Total Plate Count (TPC)</th>
<th>Standard</th>
<th>Info</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample 1</td>
<td>1 x 10^6</td>
<td>1 x 10^6</td>
<td>&gt;BMCM</td>
</tr>
<tr>
<td>2</td>
<td>Sample 2</td>
<td>5.8 x 10^5</td>
<td>1 x 10^6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sample 3</td>
<td>3.2 x 10^7</td>
<td>1 x 10^6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sample 4</td>
<td>5 x 10^6</td>
<td>1 x 10^6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Sample 5</td>
<td>1.08 x 10^6</td>
<td>1 x 10^6</td>
<td>&gt;BMCM</td>
</tr>
<tr>
<td>6</td>
<td>Sample 6</td>
<td>1.8 x 10^6</td>
<td>1 x 10^6</td>
<td>&gt;BMCM</td>
</tr>
<tr>
<td>7</td>
<td>Sample 7</td>
<td>9.2 x 10^5</td>
<td>1 x 10^6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Sample 8</td>
<td>3.3 x 10^7</td>
<td>1 x 10^6</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Sample 9</td>
<td>2.5 x 10^6</td>
<td>1 x 10^6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Sample 10</td>
<td>5.9 x 10^7</td>
<td>1 x 10^6</td>
<td></td>
</tr>
</tbody>
</table>

The isolated milk will be followed by an identification test which includes observation of colony characteristics, Gram staining, mannitol fermentation test on Mannitol Salt Agar (MSA) media, catalase test, and Novobiocin test. Isolation was carried out on Baird Parker Agar (BPA) medium which is a selective medium for Staphylococcus because of the sodium pyruvate content that stimulates Staphylococcus growth. In this study, the milk samples used were derived from samples that had been previously diluted. The dilution used for the CPA preparation is 10^-2 dilution. Colonies that grow on CPA media show very mixed results. Colonies of Staphylococcus aureus in BPA are characterized by round, smooth, convex, 2 to 3 mm diameter, gray to blackish, around the edges of clear colonies. All colonies of suspected Staphylococcus colonies were then separated for further identification of bacteria by several tests (Fardiaz, 1989).

Tests of bacterial identification were then followed by a mannitol fermentation test with bacterial culture on Mannitol Salt Agar (MSA) media taken from existing colonies on CPA media. High sodium chloride (NaCl) content in MSA medium. Therefore, this medium becomes a selective medium against Staphylococcus aureus. Staphylococcus aureus bacteria can produce coagulase enzymes and can ferment mannitol in MSA Media, so the pink media color can turn golden yellow because golden Staphylococcus aureus colony.

Figure 4. The results of the Mannitol Salt Agar (MSA) medium extracted from colony planting in Baird Parker Agar (BPA)

Further identification test is by catalase test. The catalase test was used to determine the catalase activity in the bacteria tested. Most bacteria, especially the bacteria of the genus Staphylococcus sp. Producing catalase enzymes that can break down Hydrogen Peroxide (H2O2) into water (H2O) and oxygen (O2) so that if bacterial colonies mixed with H2O2 will produce gas bubbles.

Figure 5. Result of catalase test (positive marked with gas bubble)

The next stage is Gram staining. Gram staining aims to distinguish groups of Gram positive and negative bacteria, but also to distinguish the morphology of bacteria in the form of coccus and basil. Gram staining results showing purple bacteria (gram-positive bacteria). The principle of Gram staining is the ability of the cell wall against the basic dye (Crystalline violet) after 96% alcohol washing.

The Novobiocin test aims to look at the sensitivity of the bacteria Novobiocin or the susceptibility of a bacteria to a microbial substance such as antibiotics. Novobiocin is also known to be widely used for the treatment, control, prevention, condition and
symptoms of disease caused by *Staphylococcus aureus* bacteria (Gradwohls et al, 1980). In addition, staphylococcus aureus is known to be still sensitive to Novobiocin to be used as one of the tests for *Staphylococcus aureus*. Based on the result of the research, the result of activity test of Novobiocin to *Staphylococcus aureus* got the lowest inhibition zone diameter in samples 9 and 10 that is 21 mm where the antibacterial inhibition response is still sensitive. The largest resistor zone is in sample 1 which is 35 mm. Novobiocin is said to be resistant if the inhibit zone is <17 and it is said to be sensitive if if its inhibition zone is > 20. Based on the Novobiocin test results, all samples are still sensitive to Novobiocin and may be a reference to confirm the presence of *Staphylococcus aureus* in tested milk samples.

Table 2. Novobiocin Test Results

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Diameter of the clear zone of Novobiocin (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample 1</td>
<td>35 mm</td>
</tr>
<tr>
<td>2</td>
<td>Sample 2</td>
<td>31 mm</td>
</tr>
<tr>
<td>3</td>
<td>Sample 3</td>
<td>21 mm</td>
</tr>
<tr>
<td>4</td>
<td>Sample 4</td>
<td>25 mm</td>
</tr>
<tr>
<td>5</td>
<td>Sample 5</td>
<td>30 mm</td>
</tr>
<tr>
<td>6</td>
<td>Sample 6</td>
<td>23 mm</td>
</tr>
<tr>
<td>7</td>
<td>Sample 7</td>
<td>22 mm</td>
</tr>
<tr>
<td>8</td>
<td>Sample 8</td>
<td>28 mm</td>
</tr>
<tr>
<td>9</td>
<td>Sample 9</td>
<td>21 mm</td>
</tr>
<tr>
<td>10</td>
<td>Sample 10</td>
<td>21 mm</td>
</tr>
</tbody>
</table>

Figure 6. Novobiocin Test

**CONCLUSIONS**

Based on the results of research on Crossbreed Etawa Goats milk that has mastitis in Polewali Mandar regency, it can be concluded that 10 samples of on Crossbreed Etawa Goats milk are identified the cause is *Staphylococcus aureus*.

**REFERENCES**


