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## Bacteriological survey of bulk tank milk from dairy farms in Montero, Santa Cruz, Bolivia

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### Abstract

**Background and Aim:** Recently, bacterial surveys for mastitis-causing pathogens in bulk tank milk (BTM) have been conducted in several countries worldwide. However, no such surveys have been reported from Bolivia. Therefore, the present study aimed to estimate the prevalence of mastitis pathogens in BTM from dairy farms in Montero, Santa Cruz, Bolivia.

**Materials and Methods:** Between July 2016 and August 2017, a total of 43 BTM samples were collected from 3264 cows to determine bulk tank somatic cell counts (BTSCC) and identify mastitis-causing bacteria. BTSCC was classified as follows:  $<100 \times 10^3$ ,  $100-500 \times 10^3$ ,  $500-1000 \times 10^3$ , and  $>1000 \times 10^3$  cells/mL.

**Results:** Mastitis-causing pathogens identified by agar medium cultures included *Bacillus* spp., coagulase-negative staphylococci (CNS), coliforms, *Staphylococcus aureus* (SA), streptococci, and other species. The proportions of BTSCC of  $<100 \times 10^3$ ,  $200-500 \times 10^3$ ,  $500-1000 \times 10^3$ , and  $>1000 \times 10^3$  cells/ml were 0%, 37%, 51%, and 12%, respectively. The proportions of coliforms, streptococci, CNS, *Bacillus* spp., SA, and others detected in BTM were 33%, 30%, 16%, 7%, 2%, and 16%, respectively.

**Conclusion:** Although the herd prevalence of contagious mastitis-causing pathogens, such as SA, in BTM was low, increased BTSCC were identified in Montero, Santa Cruz, Bolivia.

**Keywords:** bacterial survey, Bolivia, bulk tank milk, dairy farm, mastitis.

### Introduction

Several studies have estimated the prevalence of major mastitis-causing pathogens in bulk tank milk (BTM) and surveyed bulk tank somatic cell counts (BTSCC) worldwide [1-11]. Assessing the bacterial counts of mammary pathogens in BTM and BTSCC samples have been reported as a useful monitoring tool for identifying major mastitis-causing pathogens in dairy herds [2,6,9]. In particular, *Staphylococcus aureus* (SA) is the most important mastitis-causing contagious bacteria in BTM [4,12]. Moreover, payment programs based on milk quality are used in South America, including Bolivia, as an incentive to improve milk quality [3]. The specific payment program currently implemented in Bolivia is shown in Table-1.

In Brazil, located next to Bolivia, BTSCC for dairy farms has remained high, with no recent improvement

despite the existence of payment programs based on milk quality [3]. High BTSCC was correlated with high levels of contagious mastitis, such as that caused by SA [7]. Moreover, mastitis caused by contagious pathogens is controlled differently than that caused by environmental pathogens [13-16]. Therefore, improving milk quality depends on investigating the association between BTSCC and BTM bacterial infections. However, there are currently no reports on the prevalence of major mastitis-causing pathogens in BTM or on the association between these bacterial infections and BTSCC in Bolivia.

Therefore, the present study aimed to estimate the prevalence of mastitis-causing pathogens in BTM and to determine the association between these pathogens and BTSCC in dairy farms in Montero, Santa Cruz, Bolivia.

### Materials and Methods

#### Ethical approval

No live animals were used in the present study. Samples were collected for routine milk testing from BTM by a clinical veterinarian. Therefore, no ethical approval was needed for the current study.

#### Study area

The study was conducted in Montero city, which is located to the East of Bolivia and 50 km North of

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**Table-1:** Additional payment rate based on BTSCC for each class in Bolivia.

| Class | BTSCC×10 <sup>3</sup> cells/mL | Payment addition (%) |
|-------|--------------------------------|----------------------|
| A     | 0-500                          | +7                   |
| B     | 500-1000                       | 0                    |
| C     | 1000-2000                      | -10                  |
| C1    | 2000-3000                      | -20                  |
| C2    | >3000                          | -40                  |

BTSCC= Bulk tank somatic cell count

Santa Cruz de la Sierra, the second city of Bolivia. The city is located at 17.3433 S'latitude, 63.2556 W'longitude, and 300 m altitude.

#### BTM sample collection

BTM samples were collected from 48 randomly selected dairy herds from July 2016 to August 2017 and used to determine BTSCC and identify mastitis-causing bacteria. Five samples were excluded due to missing BTSCC data. Therefore, 43 samples from 3264 cows were included in the study. Cow breeds included Holstein-Friesian, Brown Swiss, Jersey, Gyr (Gir), and respective mixes.

Sampling was performed according to a standardized protocol. Briefly, BTM was mixed thoroughly for a minimum of 5 min before collection, following which a sample was taken from the top of the bulk tank using gloves and a sterile disposable plastic syringe.

#### Bacterial isolation and identification of mastitis-causing pathogens

Mastitis-causing bacteria identified using agar medium cultures included *Bacillus* spp., coliforms, staphylococci, streptococci, and other species. Briefly, a loopful of milk sample (approximately 10 µL) collected from each bulk tank was inoculated onto Trypticase soy agar (Hardy Diagnostics, Santa Maria, CA, USA) enriched with 5% defibrinated sheep blood and MacConkey agar (Hardy Diagnostics). The inoculated plates were then incubated under aerobic conditions at 37°C for up to 72 h and examination of growth, morphology, and hemolytic features, Gram staining, catalase test, and coagulase test were performed.

*Bacillus* spp. were identified based on growth characteristics, hemolysis type, and catalase test positivity from Gram-positive Streptobacilli (rod-shaped). Coliforms were identified based on growth characteristics and lactose fermentation on MacConkey agar from Gram-negative rods. Staphylococci were identified based on positive catalase reaction, hemolysis type, and growth characteristics from Gram-positive cocci. Specific identification of staphylococci was performed using the tube coagulase test (Eiken Chemical, Tokyo, Japan). Streptococci were identified based on negative catalase reaction and growth characteristics. To differentiate between enterococci, catalase-negative and Gram-positive cocci were tested on *Streptococcus faecalis* (SF) media. The inoculated SF test tube was incubated under aerobic conditions at

37°C for 24–48 h [6,17-20]. Unidentified organisms were recorded as other bacteria. The growth characteristics and hemolysis type of each bacterium are shown in Table-2.

## Results

#### BTSCC proportions

The proportions of BTSCC for <100×10<sup>3</sup>, 100-500×10<sup>3</sup>, 500-1000×10<sup>3</sup>, and >1000×10<sup>3</sup> cells/mL were 0%, 37%, 51%, and 12%, respectively (Table-3). According to the classification used in Bolivia, the proportion of Class A was 37%, Class B was 51%, Class C was 12%, and Class C1 and C2 were 0%, respectively.

#### Prevalence of mastitis-causing pathogens in BTM

The prevalence of coliforms, streptococci, coagulase-negative staphylococci (CNS), *Bacillus* spp., SA, and others in BTM was 33%, 30%, 16%, 7%, 2%, and 16%, respectively (Table-4). Coliforms, CNS, streptococci, and others were detected in Class A; *Bacillus* spp., coliforms, CNS, streptococci, and others were detected in Class B; and SA, streptococci, and others were detected in Class C, respectively (Table-3).

The streptococci identified in this study were not *Streptococcus agalactiae* but other streptococci because beta-hemolysis was not detected in all streptococci (Tables-2-4).

## Discussion

BTM analysis is a useful monitoring tool, which has been used for many years to assess milk quality and screen mastitis-causing pathogens, mainly because the sample analysis is less expensive, more convenient, and faster than testing milk samples from individual animals or groups [1,2,6,9, 21-23].

In the present study, 37% of BTSCC were classified as Class A. However, there were no farms with BTSCC of <100×10<sup>3</sup> cells/mL, which is the level recognized as no risk of milk yield loss [24,25]. Therefore, increasing the rate of obtaining BTSCC of <100×10<sup>3</sup> cells/mL classified as Class A is the main strategy to improve BTM quality in Bolivia. Furthermore, 51% of BTM was classified as Class B, which is not associated with an additional payment. Therefore, increasing the rate of obtaining Class A is important for the farmers to increase their gain for the same milk yield. Although Class C BTM represented a proportion of 12%, the fact that it included SA, a contagious pathogen, represents a problem and highlights the need for therapeutic countermeasures against SA for improving BTM quality and preventing the spread of infection in Montero.

In the present study, only Class C BTM presented contagious SA. However, other contagious pathogens, such as *Mycoplasma* spp., could not be excluded from all classes due to methodological limitations. Although SA is reported as the most common contagious pathogen detected in BTM [6,9], all contagious pathogens should be investigated not

**Table-2:** Growth characteristics and hemolysis type of each bacterium for agar culture identification.

| Bacteria             | Colony size       | Colony color        | Growth time (days) | Hemolysis type |
|----------------------|-------------------|---------------------|--------------------|----------------|
| <i>Bacillus</i> spp. | Moderate to large | Yellow              | 1                  | β              |
| Coliforms            | Large             | Gray, Grayness      | 0.5-1              | -              |
| CNS                  | Moderate          | Yellow, Gray, White | 1                  | α, γ           |
| SA                   | Moderate          | Yellow, Gray, White | 1                  | α, αβ, β       |
| Streptococci         | Small             | Gray, Transparent   | 1                  | -              |

CNS=Coagulase-negative staphylococci, SA=*Staphylococcus aureus*

**Table-3:** Proportion and number of BTSCC.

| BTSCC×10 <sup>3</sup> cells/ml | Number of samples (tanks) | Proportion of samples (%) | Detected bacteria (number of tanks)  |
|--------------------------------|---------------------------|---------------------------|--|
| <100                           | 0                         | 0                         | 0  |
| 100-500                        | 16                        | 37                        | Coliforms (6)<br>Streptococci (OS) (6)<br>Others (2)<br>CNS (1)                            |
| 500-1000                       | 22                        | 51                        | Coliforms (8)<br>CNS (7)<br>Others (4)<br>Streptococci (OS) (4)<br><i>Bacillus</i> spp.(2) |
| >1000                          | 5                         | 12                        | Streptococci (OS) (3)<br>Others (2)<br>SA (1)  |

BTSCC=Bulk tank somatic cell count, OS=Other streptococci, CNS=Coagulase-negative staphylococci, SA=*Staphylococcus aureus*

**Table-4:** Proportion of bovine mastitis-causing pathogens in 43 BTM samples.

| Bacterial growth     | Number of samples (tanks) | Proportion of samples (%) |
|----------------------|---------------------------|---------------------------|
| Coliforms            | 14                        | 33                        |
| Streptococci (OS)    | 13                        | 30                        |
| CNS                  | 7                         | 16                        |
| <i>Bacillus</i> spp. | 3                         | 7                         |
| SA                   | 1                         | 2                         |
| Others               | 7                         | 16                        |
| NG                   | 3                         | 7                         |

BTM=Bulk tank milk, OS=Other streptococci, CNS=Coagulase-negative staphylococci, SA=*Staphylococcus aureus*, NG=No growth

only by cultural methods but also by genetic testing. Measures such as incorporated post-milking teat dipping, hygienic milking procedures, and strategic use of antibiotic therapy control the spread of contagious mastitis-causing bacteria [13].

In Classes A and B, most detected bacteria were environmental microorganisms (Table-3), which highlights the need for countermeasures against environmental pathogens as a strategy to decrease BTSCC in Montero. Important aspects in the prevention of environmental mastitis-causing pathogens include udder health maintenance; in particular, the application of foremilk is typically recommended and well established in mastitis control programs [14-16].

The present study had some limitations: Cultural methods in agar medium were used due to maneuvering limitations in lacking of the equipment. In future, we hope to use polymerase chain reaction (PCR) or real-time PCR to detect mastitis-causing pathogens

including *Mycoplasma* spp. Moreover, we could not perform the drug susceptibility test due to lacking of the facility; thus, an antibiotic therapeutic strategy for the detected mastitis-causing bacteria could not be established.

### Conclusion

The present study determined the prevalence of mastitis-causing bacteria in BTM and BTSCC in Montero, Santa Cruz, Bolivia. The herd prevalence of SA in BTM was low. Further studies using genetic testing, such as PCR and drug susceptibility test for mastitis-causing bacteria in BTM are needed for implementing an adequate therapeutic strategy.

### Authors' Contributions

YM and YT contributed to the conception and design of the study. YM conducted the sample collection, processing of samples in the laboratory, and participated in the data analysis. MK provided the technical support of the sample analysis. YM and YT prepared the first draft and participated in the correction of the manuscript. YT and MK supervised the research. All the authors have read and approved the final manuscript.

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## Competing Interests

The authors declare that they have no competing interests.

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