Somatic Cell Count and California Mastitis Test as a Diagnostic Tool for Subclinical Mastitis in Ewes*

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ABSTRACT

Background: Infectious mastitis has been described as one of the main diseases affecting animals during lactation. The disease in sheep has been studied for many years in countries where mastitis has an economical importance. However, the interest in mastitis studies in animals raised for food production has increased, because the disease may cause a reduction in weight and an increased mortality in lambs. In this study, Somatic Cells Count (SCC) and California Mastitis Test (CMT) were related to bacterial isolation for mastitis diagnosis in Corriedale sheep.

Material, Methods & Results: Twenty nine (29) ewes, varying from 2-5 years of age, with different numbers of lactation and born lambs and never before machine-milked were used. Milking was done from October to November, once a week in the morning period, with oxytocin application. Four milk collections were made, at biweekly intervals for bacteriologic, SCC and CMT test, which were analyzed considering each gland as a sampling unit. Low incidence of subclinical mastitis (9.66%) was observed, with the majority (15/17) caused by coagulase negative Staphylococcus. There was no change in milk production related to SCC or bacteria isolation. However changes in milk components occurred in the presence of subclinical mastitis. A poor relation (k = 0.115) was determined between the results obtained in the bacteriological test and the SCC, with low sensitivity (13.33%) and an increased number of false negative results (13%). Comparing the SCC and CMT results as the diagnostic method for subclinical mastitis, a low (r = 0.231) but significant (P = 0.0209) correlation was observed, as well as a poor concordance (k = 0.152). Using bacterial isolation as the standard test, it was determined that the CMT has low sensitivity (28.57%) as the diagnostic method of mastitis in ewes.

Discussion: Mastitis has been considered an economically important disease in the production of sheep for meat and wool. According to reports, the frequency of its clinical occurrence may range from zero to 50%. In meat-producing herds, a low weight gain in lambs has been associated with subclinical mastitis and the study of mastitis in Corriedale sheep is justified since this is considered a breed of meat sheep with the best milk production. This breed is being crossed with milk-producing breeds, such as Lacaune, to form milk-producing herds. Bacterial isolation has been adopted as the diagnostic method of mastitis in all livestock breeds. Similarly to the observation made by this study, Staphylococcus and, in a few cases, Streptococcus, have been the microorganisms most frequently involved in subclinical mastitis in sheep. The milk from ewes with mastitis tends to have a lower fat and lactose content than that of healthy ewes, due to the affected secretory function of these animals. The SCC of milk ewes has not yet been established, but its count in a healthy udder may reach up to 1.5x10^6 cell.mL^{-1}. Similarly, the CMT score to be used in sheep is still controversial, but the maximum score (+++) is adopted to indicate mastitis. The high number of false-negative and false-positive reactions observed in SCC and CMT tests means that healthy and ill animals are incorrectly identified and that no preventive and curative measures are adopted. Since the utilization of only one diagnosis method in sheep mastitis, without confirmation by bacteriologic test is not conclusive, the SCC and CMT should be used cautiously in sheep mastitis diagnosis.

Keywords: mastitis, sheep, milk production, diagnosis, milk composition.
INTRODUCTION

The milk production of small ruminants is based on three closely related aspects: adequate nutritional, sanitary and productive management.

Infectious mastitis has been described as one of the main diseases affecting animals during lactation. The most used methods to determine the health of the udder of lactating ewes are: California Mastitis Test (CMT), also known as indirect method, whose principle is the utilization of a detergent that acts on the external membrane of the cells (lipoprotein membrane), exposing the gel-like DNA and, for that reason, the higher its consistency the higher will be the somatic cell count (SCC) which, in turn, is known as direct method, in which an electronic device, through optical and infrared filter systems, determines the quantity of somatic cells and other components in the milk, as well as the causal agent of mastitis.

Ovine mastitis has been known and studied for many years in countries where the production of ewe’s milk is economically important [34]. The interest in mastitis studies has also increased in relation to meat-producing herds, because the disease may lead to a weight reduction and an increase in mortality of lambs [12,14].

The milk production in meat sheep breeds can be considered an important source of animal protein. We therefore aimed at characterizing the CMT and SCC tests as diagnostic methods of subclinical mastitis in Corriedale sheep, comparing them to bacterial isolation and variations in milk composition of these animals suffering from this disease.

MATERIALS AND METHODS

The experiment was carried out at Centro Agropecuário da Palma/UFPEL, in the town of Capão do Leão, RS, Brazil (31º 52’ S and 52º 29’ W) between October and November 2007. The herd was composed of Corriedale and Texel sheep specimens for the production of lambs. Twenty-nine Corriedale ewes were randomly selected, having their date of parturition as selection factor. The ewes were between 2 and 5 years old, with different number of lactations and number of born lambs, and none of them had been mechanically milked before. They were milked once a week in the morning, upon application of oxytocin [27]. The ewes would be separated from their herd the night before, remaining approximately 15 h apart from their lambs. After being milked, the ewes and their lambs would remain together in the same fenced meadow. Consequently, the milk production and composition data presented and discussed in this study refer to a 15 h period.

Considering that the purpose of the herd was the production of lambs, the milk was collected 60 days after birth, when the lambs no longer depended on the ewe’s milk. Four milk collections were made every 15 days, when the end of milk production was verified. The udders were cleaned with water and dried with paper towels. Once dried, the udders were physically examined [25] in order to detect clinical mastitis, which is characterized by visible signs, such as edema and increased sensitivity and temperature of the mammary halves [29], fever, inappetence, dehydration, etc. Then the teats were disinfected using cotton wool soaked in alcohol 70ºGL. The first jets of milk were discarded onto a paddle in order to perform the California Mastitis Test (CMT) [18,28]. Then individual milk samples from each teat were aseptically collected [28], of which 0.01 mL were streaked onto 5% sheep blood agar and incubated at 37ºC during 24 to 48 h. After incubation, the colonies were counted and characterized with regard to their morphology and staining characteristics [26] and then maintained at -20ºC in brain heart infusion added by 20% glycerol, until the moment of their identification [19]. Samples with a growth of 5 or more identical colonies [10] were considered positive for subclinical mastitis. The growth of two or more morphological types (>5 UFC per type) was considered as contamination and the result was excluded from the analysis [2,15].

After milk collection of bacteriological examination, the udders were emptied and the milk was homogenized. One sample per animal was produced in order to analyze fat, raw protein, lactose and total solid contents through infrared absorption, using the Bentley 2000® device and the somatic cell count technique, which was carried out by an electronic counting device based on flow cytometry, at Embrapa Terras Baixas’ milk analysis laboratory. Samples presenting a mastitis-positive counting higher than 1,000,000 cells mL⁻¹ [4] were considered.

The statistical analysis was made using the software GraphPad Prism 4.0, applying a significance level of 5%. The CMT, SCC and bacteriological data were analyzed considering each gland as a sample unit [23]. For the purpose, bacterial isolation was
considered in at least one of the teats and the highest CMT result was considered as the result of the animal. The sensitivity, specificity, as well as the positive and negative predictive values of the SCC and CMT were determined by the Fischer’s exact test and kappa index, using bacterial isolation as the standard test. The association between the CMT and SCC was calculated by the McNemar test and by the Kappa index and the correlation was calculated by the Spermann’s test. The correlation index was classified as high ($r > 0.7$), medium ($0.5 < r < 0.7$) and low ($r < 0.5$) [9]. The value scale of the kappa index was used [1]. The comparisons of production medians were made by the T-test and those of milk constituent medians, with or without mastitis, were made by the Mann-Whitney’s test.

RESULTS

This present study did not show evidence of females whose symptoms were compatible with those of clinical mastitis. One hundred seventy-six individual samples were collected from each teat, and subclinical mastitis was detected [10] in 17 (9.66%) samples. Coagulase-negative *Staphylococcus* was identified in 15 samples, *Streptococcus* sp. was identified in one sample and a Gram-negative bacteria was identified in another. This latter was not identified because it could not be retrieved after being frozen.

Among 29 females examined, bacteria could be isolated from 6 in at least one collection and from 4, in two or more collections. Only two ewes had subclinical mastitis in the two mammary halves at the same time.

There was no significant difference in the production of animals with subclinical mastitis (0.4750 L), in comparison to those without bacterial growth in their milk (0.4370 L).

With regard to milk constituents, a significant increase in the levels of protein ($P = 0.0299$), total solids ($P = 0.0106$) and fat ($P = 0.018$) was verified in animals with subclinical mastitis (Table 1).

Considering that animals which showed bacterial growth in at least one teat had subclinical mastitis,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With mastitis</th>
<th>Healthy udder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>0.47a</td>
<td>0.44b</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.63a</td>
<td>4.60a</td>
</tr>
<tr>
<td>Total Solids (%)</td>
<td>17.69a</td>
<td>15.77b</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>6.59a</td>
<td>5.03b</td>
</tr>
</tbody>
</table>

Different letters in the same line indicates a significant statistic difference ($P < 0.05$). There was no significant difference ($P = 0.2176$) in the SCC median of animals with (5.1 $\log_{10}$) and without (4.8 $\log_{10}$) mastitis (Table 2).

It was verified that at least six females had a cell count above one million (1,250,000 to 9,342,000 cel.mL$^{-1}$) in one single collection. For the purpose of comparison between the SCC /other diagnostic methods and the milk constituents and production, samples with a SCC > 500,000 cel.mL$^{-1}$ were considered positive in the diagnosis of mastitis. A cell count above this number was observed in 10 females and, of them, only one had a SCC count above 500,000 cel.mL$^{-1}$) in three collections.

A poor relation ($k = 0.115$) was determined between the results obtained in the bacteriological test and the SCC. Comparing the results of the bacterial isolation and of the SCC, it was observed concordance in 83 and discordance in 17 observations, resulting in a high number of false negative results (13%). Considering bacterial isolation as the standard method, its was determined that the SCC, as the diagnostic method of mastitis in sheep, had low sensitivity (13.33%) and a Positive Predictive Value (33.33%), and high specificity (95.29%) and Negative Predictive Value (86.17%).

Furthermore, there was no significant difference ($P = 0.6121$) in the milk production (0.4371 L) of animals with high SCC (> 500,000 cel.mL$^{-1}$) and other animals (0.4684 L). However, the protein ($P = 0.0278$), lactose ($P < 0.0001$), total solids ($P = 0.0056$) and fat ($P = 0.0009$) values were significantly different (Table 3). This study used score $\geq 2$ (++ or ++++) in the CMT [18] as the presumptive diagnosis of mastitis in this species.

Comparing the SCC and CMT results as the diagnostic method for subclinical mastitis, a low ($r = 0.2319$) but significant ($P = 0.0209$) correlation and
poor concordance (κ = 0.152) were observed between the two diagnostic methods of mastitis in ewes. Even though 78 of 99 results observed correspond to the CMT and the bacterial isolation, there was no association between the results of these two methods in the mastitis diagnosis in sheep. Considering bacterial isolation as the standard method, it was determined that the CMT has low sensitivity (28.57%) and a Positive Predictive Value (26.67%), and high specificity (87.06%) and Negative Predictive Value (88.10%). It was observed that the number of false positive and false negative results was similar.

**DISCUSSION**

Mastitis has been considered an economically important disease in the production of sheep for meat and wool [22]. According to reports, the frequency of its clinical occurrence may range from zero to 50% [20]. In sheep herds in Spain [2], mastitis was observed in only 0.4% of the samples analyzed and a higher number of subclinical mastitis was observed. On the other hand, in Brazil, subclinical mastitis was observed in Santa Inês females (11%) [35] during the first lactation week and clinical mastitis (6%) was observed at birth; in Hampshire Down ewes [13], a clinical mastitis outbreak was observed in 10% of a herd. However, the diagnostic methods used in each record must be considered, using the bacterial isolation in most cases. In meat-producing herds, a low weight gain in lambs has been associated with the presence of subclinical mastitis [12]. Differently in milk-producing breeds, mastitis has been observed more frequently, affecting up to 41% of the samples analyzed [2]. The study made with the Corriedale breed is justified since this is considered the breed of meat sheep with the best milk production [11] and, for that reason, it has been crossed with milk-producing breeds, such as Laucane, to form milk-producing herds.

Bacterial isolation has been adopted as the diagnostic method of mastitis in all livestock breeds. Similarly to the observation made by this study, *Staphylococcus* and, in a few cases, *Streptococcus*, have been the microorganisms most frequently involved in subclinical mastitis in sheep [2,24]. Mastitis caused by Gram-negative microorganisms is not frequent and the fact that the sample isolated in this study could not be recovered is supported by the scientific literature, where there is a loss of up to 40% of bacterial samples during the laboratorial identification process [5] or after being frozen [32].

The fact that a higher mastitis frequency was observed in the right teat could be explained by the number or offspring, considering that nine females with subclinical mastitis gave birth to one lamb and only two gave birth to multiple lambs. Since the lambs were kept with their mothers during the experiment, there is a tendency of the lamb to suck more frequently from the same teat in case of single births, due to

### Table 2. Somatic Cell Count (SCC) in ewes with and without subclinical mastitis, considering bacterial isolation as the diagnostic method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With mastitis</th>
<th>Without mastitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>123,000</td>
<td>62,000</td>
</tr>
<tr>
<td>Minimum</td>
<td>5,690</td>
<td>1,400</td>
</tr>
<tr>
<td>Maximum</td>
<td>6,697,000</td>
<td>9,342,000</td>
</tr>
<tr>
<td>Average</td>
<td>860,573</td>
<td>317,098</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1,859,287</td>
<td>1,154,792</td>
</tr>
</tbody>
</table>

### Table 3. Median values of Corriedale ewes’ milk in the presence (infected udder) or not (healthy udder) of Somatic Cell Counts (SCC) indicating mammary infection (>500,000 cel.mL⁻¹).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infected udder</th>
<th>Healthy udder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>5.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Solids (%)</td>
<td>17.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>6.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters in the same line indicates a significant statistic difference (P < 0.05).
physiological and anatomical characteristics. As a consequence, residual milk may occur in the teats, leading to the proliferation of microorganisms.

With regard to the milk components, it was observed a tendency for an increase in the fat percentage in ewes with subclinical mastitis [16] and for the reduction of these contents in experimental clinical mastitis [29]. Animals with mastitis would tend to have a lower fat and lactose content than that of healthy ewes, due to the affected secretory function of these animals [4].

In ewes with infectious mastitis, a high percentage of protein was observed in their milk in comparison to healthy ewes, and the fact can be explained by the presence of blood cells in the milk, which remains with the same production volume [33].

In addition to the hygiene issue, the economical aspect of mastitis is important due to the reduction it causes in the milk production [29,30]. However, this fact was not observed in this study. Similarly, an increase in somatic cells [30], which was not observed in this study, has been reported.

The microbiology of the milk is associated with the SCC, which is increased in pathological and inflammatory processes of the mammary gland, reducing the fat, casein and total solids contents and increasing the total nitrogen, non-protein nitrogen and milk protein contents [3]. In milk-producing cows, the SCC has been widely used as the diagnostic method of mastitis, being employed as a qualitative milk evaluation method, also with regard to the price paid to the producer. However, in small ruminants, there is a considerable epithelial desquamation of the mammary apparatus, i.e., presence of cytoplasmic corpuscles resulting from the apocrine milk secretion process [7], thus increasing the amount of SCC. Differently from the observation made in this study, a reduction has been noticed in the production of ewe’s milk with a high SSC count [20].

SCC values in healthy sheep (4.86 Log_{10}), which are lower than the SCC values observed in ewes with mastitis (5.9 Log_{10}) [2], similarly as observed in this study, have been reported. However, the somatic cell count (SCC) of a healthy udder in milk-producing ewes has not yet been established. Studies shows that the SCC levels vary greatly, reaching a count up to 1.5x106.mL^{-1} in a healthy udder [20]. However, a count limit above 250,000 cells.mL^{-1} [17,21] or below 500,000 cells.mL^{-1} [4] has been suggested for healthy udders. On the other hand, ewes with mammary infec-

tion will have a SCC above 1 million cells, in at least two consecutive samplings [4].

Other method to diagnose mastitis widely used in bovines is the CMT. This test is used worldwide to diagnose subclinical mastitis and, additionally, it can be carried out in the herd when the animals are being milked. The interpretation of the CMT is based on the visual inspection of the milk after the reagent is mixed in. The reaction takes place between the reagent and the genetic material from somatic cells found in the milk, forming a gel whose concentration is proportional to the number of somatic cells [6]. However, in small ruminants, this test still generates controversies, because the amount of physiological somatic cells from these animals is very large, causing a false-positive result.

The CMT score to be used in sheep is still controversial. A CMT score of 1 [14] is recommended for the subclinical mastitis diagnosis, and the maximum score (+++) is recommended for the diagnosis of infectious mastitis of sheep [8]. The high number of false-negative and false-positive reactions observed in diagnostic tests means that healthy and ill animals are incorrectly identified and that no preventive and curative measures are adopted.

The economical losses caused by mastitis in small ruminants are extremely high, particularly in milk-producing herds. A reduction in the milk production due to clinical and subclinical mastitis, as well as a reduction in the milk quality and the consequences it has on animals and on food safety are aspects associated with said losses. For that reason, it is necessary to study and develop strategies to control this disease [24] in sheep.

CONCLUSIONS

A low occurrence of subclinical mastitis (9.66%) was observed, and the majority (15/17) was caused by coagulase-negative Staphylococcus. There was no alteration in the production of milk associated with the SCC or bacterial isolation. However, there was an alteration in the milk constituents when subclinical mastitis was present. SCC and CMT have low sensitivity when compared to the bacterial isolation technique and, as such, they must be carefully used in the diagnosis of mastitis in sheep.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
REFERENCES


