Relationship of mammary gland health status and other noninfectious factors with electrical conductivity of milk in Manchega ewes

G. Romero, A. Roca, M. Alejandro, R. Muelas, and J. R. Díaz
Dpto. Tecnología Agroalimentaria, Universidad Miguel Hernández (UMH), Ctra. de Beniel km 3.2, 03312 Orihuela, Spain

ABSTRACT

Measuring the electrical conductivity (EC) of milk during milking has been extensively studied in cattle as a low-cost mastitis detection method that can be easily automated. The aim of this work was to study the effect of the health status of the glands and several noninfectious factors (lactation stage, milking session, and lactation number) that affect the use of EC measurement of milk to detect mastitis in dairy sheep livestock. Likewise, we studied the relation between EC and milk composition (macrocomposition and mineral content) and between EC and somatic cell count (SCC). Finally, we evaluated the use of EC thresholds as a mastitis detection method. To this end, we monitored the glandular milk EC throughout 2 consecutive lactations, during which 42 and 40 ewes were controlled, respectively. We carried out 7 biweekly checks, analyzing the EC, SCC, composition, and mineral content of glandular milk at morning and evening milkings. Before the morning milking, samples were aseptically collected for bacteriological analysis, and the results along with the SCC were used to classify the glands according to their sanitary status (healthy, latently infected, or infected). Lactation stage, parity, milking (morning or evening), health status, and the interactions of parity with health status, lactation stage with health status, and parity with lactation stage all had a significant effect on SCC and EC of the milk. The correlation between EC and SCC was only significant when all the data were analyzed jointly \((r = 0.33)\) and for SCC \(\geq 600.000\) cells/mL \((r = 0.25)\). The changes in milk composition, mainly in fat content, largely explained the variation in EC \((R^2 = 0.69)\). For the same EC threshold, the specificity and sensitivity varied depending on the parity or the milking, with the negative predictive value obtained being higher than the positive predictive value at all times. We concluded that developing methods of detecting mastitis in sheep by milk EC readings would require consideration of noninfectious factors that also affect the gauging of EC. One option to consider would be individualized daily monitoring of the glands, as demonstrated in other species such as cattle and goat.

Key words: mastitis detection, electrical conductivity, dairy sheep, sensitivity, specificity

INTRODUCTION

Mastitis in dairy sheep, both clinical and subclinical, causes economic losses because of decreased milk production and cheese yield (Leitner et al. 2008). These problems are compounded by the expenses arising from treatment costs and losses from milk withdrawal periods following the treatments. In some cases, mastitis may even lead to animal death or the total loss of one or both mammary glands.

In sheep, production losses due to subclinical mastitis can reach 12.2% in herds with 75% of glands infected, whereas the losses in goat livestock are only 2.3% with the same percentage of infected glands (Leitner et al. 2008). The alteration of milk composition and the consequent decline in cheese yield is also more acute in sheep than in cattle or goats (Leitner et al. 2011). For this reason, the development of techniques that allow early and effective detection of mastitis cases in sheep and help minimize the associated economic losses is of prime importance.

Electrical conductivity (EC) of milk during milking has been widely studied in cattle as a mastitis detection method. The method can be automated, and in some cases it reaches 92% sensitivity and 93% specificity (Cavero et al., 2006).

In small ruminants, only a few reports have been published on the effect of mastitis on EC of milk, and some of the results are contradictory. In recent studies carried out in Murciano-Granadina goats, Díaz et al. (2011) observed that in addition to the glandular health status, milk EC is also affected by animals’ lactation stage and parity number and the farm from which the animals are sourced. In the same study, the authors set out a series of absolute thresholds for EC (5, 5.10, 5.20, 5.30, 5.40, 5.50, 5.60, 5.70, 5.80, 5.90, and 6.00 mS/cm)
for mastitis detection and found important variations in the sensitivity and specificity obtained for a threshold depending on the farm. Romero et al. (2014) proposed a series of algorithms for mastitis detection in goats based on the daily and individual measuring of the EC of glandular milk. The algorithms were able to classify all cases of clinical mastitis, although they obtained different values in subclinical cases. A higher sensitivity (58.3%) was obtained when cases were considered positive if the EC deviated over the moving average of the 4 previous days by at least 3 times the standard deviation. Specificity varied between 75 and 100%, according to the algorithm. In another goat study, Romero et al. (2012) obtained sensitivity of 70% and specificity of 50% with an EC threshold of 5.20 mS/cm, regardless of the milking fraction studied. Zaninelli et al. (2015) assessed the use of Fourier spectral analysis for online readings of glandular milk EC as a mastitis detection method. They concluded that the EC of milk from mastitic glands presented slower fluctuations and an irregular trend, and the frequency peaks obtained by the Fourier transformation could therefore be used as mastitis indicators and be included in the design of the algorithms for mastitis detection by means of online EC readings.

Very few studies are available in sheep. Peris et al. (1989) observed that mastitis caused an increase in milk EC and proposed 2 thresholds for mastitis detection. One threshold was 5 mS/cm for diagnosing glands with mastitis, which achieved 60.2% sensitivity and 91.4% specificity, with 87.9% of the samples classified correctly. The other threshold consisted of using a difference in milk EC between the 2 glands of the same animal of 0.3 mS/cm, which yielded better results (70% for sensitivity, 93% for specificity and 89.1% of samples properly classified). This latter threshold is similar to that obtained by Barth et al. (2008), who observed a difference in milk EC between glands of 0.1 mS/cm in healthy sheep and 0.4 mS/cm between glands of sheep with one infected gland. McDougall et al. (2002), despite finding no significant differences between the impedance (EC inverse property) of milk from healthy and infected glands, obtained a negative correlation between the impedance and SCC (r = −0.27), leading them to deduce that the increase in SCC must be related to an increase in EC. Caria et al. (2016) found a positive correlation (r = 0.31) between milk EC and SCC in Sarda sheep. In the same study, they achieved 73.08% sensitivity and 75.46% specificity, setting an EC threshold of 4.84 mS/cm, which was then applied in the evaluation of a prototype designed to detect subclinical mastitis by online gauging of the EC of the milk.

To determine the factors affecting the measuring of EC as a mastitis detection method, this investigation focused on the effect of different noninfectious (lactation status, milking type, and parity) and infectious factors on EC of glandular milk from sheep. Likewise, we also studied the relationship between EC and milk composition (macrocomposition and mineral content) and between EC and SCC.

### MATERIALS AND METHODS

#### Location and Animals Used

The investigation was conducted at the Small Ruminants Teaching Farm of the Escuela Politécnica Superior de Orihuela, which belongs to the Miguel Hernández University (Spain).

We used Manchega ewes, a native Spanish breed with average milk production for every sheep in the breed of 1.156 L/d (Arias et al., 2012). The milk of this mixed-use breed is mainly used in Manchego cheese manufacturing. Manchego cheese and Manchego lamb are 2 products of great value, and they are traded under the European guarantee labels of Protected Designation of Origin and Protected Geographical Indication, respectively.

The farming system in practice was intensive, with permanent stabling. The reproductive rate was 1 annual litter, with lambs weaned at birth and reared by artificial feeding. Postpartum, the ewes were milked twice a day (0800 and 1600 h) in a Casse low-line milking parlor 1 × 12 × 12 (number of platforms × number of places/platform × number of milking units/platform) with the following milking parameters: 36 kPa vacuum level, 180 pulsations/min rate, and 50% pulsation ratio.

Diet, which consisted of 2.5 kg daily mix of Unifeed and straw ad libitum, was the same throughout lactation.

#### Experimental Design

During 2 lactations, we monitored 42 (22 primiparous and 20 multiparous) and 40 (3 primiparous and 37 multiparous) ewes, respectively. We performed 7 biweekly samplings, the first at 2 wk postpartum, and the sampling lasted 3.5 mo. Sheep were sampled at morning (0800 h) and evening (1600 h) milkings.

Two samples were taken from each gland at the morning milking (5 and 100 mL, respectively) and one of 100 mL at the afternoon milking. The first sample from the morning milking was used for bacteriological analysis and was obtained aseptically by milking in sterile tubes after cleaning the teats with 70% ethanol and eliminating the first streams. Next, the glands were machine milked separately, collecting the milk into volumetric meters. The production was measured with a 500-mL...
test tube and a 100-mL sample representative of the total milk extracted from each gland was subsequently used to measure EC, SCC, macrocomposition, and mineral content.

**Variables Analyzed**

For bacteriological analysis, 20 μL of milk was seeded onto sheep blood agar plates (BioMérieux, Lyon, France) immediately after collection. The plates were then incubated at 37°C, and a bacterial growth count was performed at 24, 48, and 72h after seeding. To classify cultures as positive or negative, we followed the National Mastitis Council recommendations (Harmon et al., 1990); the culture was deemed positive if at least 5 identical colonies were observed, and negative if no growth had occurred by 72h after seeding. Identification of the bacterial genus was done by Gram stain and catalase test in colonies with morphology suggestive of gram-positive microorganisms. Finally, in the case of staphylococci, identifying the bacterial species was performed using the Apistaph kit (BioMérieux).

Electrical conductivity (mS/cm) was measured immediately after milk collection using a laboratory conductivity meter (GLP 32, Crison, Alella, Spain) with automatic temperature compensation to 25°C. After the EC of the milk was measured, an aliquot of 30 mL was taken and azidol was added for preservation. The SCC of the aliquot was measured (×10³ cells/mL) at the Valencia Community Interprofessional Milk Laboratory (Spain) by a fluorometric method (Fossomatic 5000; Foss, Hillerød, Denmark).

Macroscopic composition, fat, casein, serum protein, lactose, and ash were analyzed by infrared spectrosopy (Milko Scan FT 120; Foss), and the results were expressed as a wet matter percentage.

The sodium (Na⁺) and potassium (K⁺) contents (mg/L) were determined by flame photometry (PFP, Jenway, Staffordshire, UK). Mohr’s method adapted for milk (AOAC International, 1995) was used to determine the chloride (Cl⁻) content (mg/L), with the analysis always being performed by the same person to prevent operator effect.

**Glandular Health Status Definition**

The glands were classified by health status, according to the bacteriological analysis and SCC results. Glands with positive bacteriological analysis in 2 or more consecutive controls were classified as glands with infectious mastitis when the SCC was higher than 400 × 10³ cells/mL and glands with latent infection when the SCC remained below 400 × 10³ cells/mL. Glands in which the bacteriological analysis was negative were classified as healthy glands (all glands below 400,000 cells/mL had negative culture). For this classification, we agreed to set the threshold at 400 × 10³ cells/mL in accordance with the literature and the experience of our research group.

**Statistical Analysis**

To normalize the data distribution and apply statistical analyses, EC and SCC variables were transformed into base 10 logarithm (LEC and LSCC, respectively). The relationship of the LEC and LSCC variables with fixed effects was analyzed by a mixed linear model (Proc Mixed, SAS V. 9.2, SAS Institute Inc., 2012). Gland production (PROD) was included as a continuous covariate and the following fixed effects were considered: the health status of the glands (INFᵢ, with 3 levels: healthy, with infectious mastitis, or latent infection); milking session (ORDⱼ, with 2 levels: morning or evening); parity number (NLᵏ, with 2 levels: multiparous or primiparous); lactation stage (CONTᵢ, with 7 levels: every 2 wk up to 14 wk postpartum); and the interactions between the glandular health status and parity number, the glandular health status and lactation stage, and the parity number and lactation stage. The random effects considered were the year of investigation (1, 2) and the glands (right or left) nested to the ewe to model the covariance between observations of the glands within each ewe (Barkema et al., 1997). A “compound symmetry” type model of fit was used for the correlation of variance among repeated measurements from the same animal. The model using this hierarchical structure provided the best fit for the data at every studied variable in comparison with different models considering other covariance and hierarchical structures (as assessed using Bayesian and Akaike’s information criteria). Some samples were destroyed in transit to the laboratory for the SCC analysis, so the number of SCC cases is lower than that of EC.

The interactions of milking type with parity number and milking type with glandular health status were not significant, so they were not included in the final model.

The correlation of EC with SCC was analyzed (Proc Corr, SAS V.9.2. SAS Institute Inc., 2012) according to 4 SCC intervals (SCC < 200,000, 200,000 ≤ SCC < 400,000, 400,000 ≤ SCC < 600,000, and SCC ≥ 600,000 cells/mL). The relationship between the milk composition (fat, casein, serum protein, lactose, ash, Cl⁻, Na⁺, K⁺) and EC was also analyzed (Proc Reg, SAS V.9.2. SAS Institute Inc., 2012).

The evolution of sensitivity and specificity for detection of mastitis was calculated based on different EC
thresholds for the data set and according to the type of milking and parity of the animals. We defined sensitivity as the percentage of positive cases that the method was able to detect or the probability that a positive sample would be classified as such (true positives over the sum of true positives and false negatives). The specificity was defined as the percentage of negative cases that the method was able to detect or the probability that a negative sample would be classified as such (true negatives over the sum of true negatives and false positives). The positive predictive value (PPV) was defined as the probability that a gland was infected when the sample was classified as positive (true positives over the sum of true positives and false positives). Finally, the negative predictive value (NPV) was defined as the probability that a gland was not infected when the sample was classified as negative (true negatives over the sum of true negatives and false negatives).

**RESULT**

**Mastitis Incidence and Prevalence**

At the beginning of the first year of the investigation, 7 of the 81 glands were classified as unhealthy (8.64% prevalence), with 4 presenting infectious mastitis and the other 3 latent infection, with all the unhealthy glands belonging to multiparous ewes (Table 1). Throughout the investigation, one of the glands with infectious mastitis was cured and 10 new cases of unhealthy glands appeared: 5 of infectious mastitis and 5 of latent infection, with 1 case of each type being cured, so that by the end of lactation the overall prevalence was 17.28%. Of the new cases that appeared, 4 belonged to primiparous ewes (9.52% incidence in primiparous) and 6 to multiparous (18.75% incidence).

At the beginning of the second year, 9 glands presented infectious mastitis and another 9 cases had latent infection, so the prevalence was 23.08%. Throughout the investigation, 8 of the 9 glands that presented latent infection at the first control were cured, but 10 new cases appeared, of which 5 were cured at subsequent checks. As for infectious mastitis, 3 glands that were initially infected were cured and a new case appeared that was also cured spontaneously. In total, 11 new cases of unhealthy glands were recorded (18.33% incidence), but because some were cured, prevalence at the end of the second year (16.67%) was lower than at the start of the investigation.

The glands classified as having latent infection or infectious mastitis at the onset of lactation and were cured during lactation, did so spontaneously. No treatment was applied to any sheep.

Both infectious mastitis and latent infections presented subclinically, even those that were caused by novobiocin-sensitive pathogens.

**Pathogen Types Isolated During the Investigation**

Of the pathogens isolated, 97.4% were staphylococci, with *S. xylosus* and *S. lentus* being isolated in the majority of cases (Table 2). The higher SCC and EC were recorded for milk from glands infected by *S. aureus* (5.73 mS/cm and 15,601 × 10³ cells/mL), followed by the values associated with glands infected with *S. xylosus* (4.61 mS/cm and 2,989 × 10³ cells/mL) and *S. haemolytica* (4.48 mS/cm and 102 × 10³ cells/mL). The average milk EC of mastitis-free glands was 4.17 mS/cm.

**Factors Associated with EC and SCC in Milk**

All factors considered and their interactions were significant for both variables (Table 3), although they were not affected in the same way. For EC, milking time was the main factor responsible for the variations, followed by lactation stage and glandular health state. For SCC, the health status of the glands and the production caused the greater effect, followed by the type of milking. Both EC and SCC were affected by the production of the glands, with both variables increasing as production decreased.

The EC of milk from the morning milking was significantly higher (*P* < 0.001) than that from the evening milking (4.34 vs. 4.08 mS/cm, Table 4), whereas the SCC obtained at the evening milking (162 × 10³ cells/mL) was significantly higher (*P* < 0.001) than that of the morning session (129 × 10³ cells/mL).

The milk EC of multiparous ewes (4.37 mS/cm) was significantly higher than that of primiparous females (4.07 mS/cm) when all data were analyzed and the health status of the glands was considered. In all 3 cases (healthy glands or glands with infectious mastitis or latent mastitis) the EC was significantly higher for multiparous ewes than for primiparous females (Table 5). Both for multiparous and primiparous ewes, the EC of milk from infected glands was significantly higher than that from healthy or latently infected glands. However, the interaction of the state of infection with the parity was significant. In the primiparous ewes, the EC of milk from the glands with latent infection (3.77 mS/cm) was significantly lower than that from the healthy glands (4.08 mS/cm), whereas for the multiparous ewes this difference was not significant.

Similar to the outcomes for EC and with all data considered, SCC was significantly higher for multipa-
Table 1. Health status of glands and ewes at the beginning and end of lactation

<table>
<thead>
<tr>
<th>Group</th>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactation onset</td>
<td>Lactation end</td>
</tr>
<tr>
<td></td>
<td>Ewes</td>
<td>Ewes</td>
</tr>
<tr>
<td></td>
<td>Uni  Bil Glands</td>
<td>Uni  Bil Glands</td>
</tr>
<tr>
<td>Primiparous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>— 20+2²</td>
<td>— 19+2²</td>
</tr>
<tr>
<td>Infected</td>
<td>—</td>
<td>— 1</td>
</tr>
<tr>
<td>Latent infection</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>20+2²</td>
<td>20+2²</td>
</tr>
<tr>
<td>Multiparous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>13+1²</td>
<td>27+5³</td>
</tr>
<tr>
<td>Infected</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Latent infection</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>19+1²</td>
<td>39</td>
</tr>
<tr>
<td>Primi- and multiparous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>— 33+3²</td>
<td>71+5³</td>
</tr>
<tr>
<td>Infected</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Latent infection</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>39+3²</td>
<td>81</td>
</tr>
</tbody>
</table>

1 Uni = ewes with infectious or latent mastitis in one gland; Bil = ewes with infectious or latent mastitis in both glands.
2 Ewes with a single functional gland.
3 Healthy gland from ewe with unilateral gland infection.
4 Ewes presenting infectious mastitis in one gland and latent infection in the other.
rous than for primiparous ewes (205 × 10^3 and 102 × 10^3 cells/mL, respectively). The SCC was significantly higher for multiparous compared with primiparous ewes for healthy and infected glands, but no significant differences were obtained for latently infected glands (Table 5).

Lactation stage had a significant effect on both SCC and EC. The highest values for both variables were recorded in the first sampling, performed at 2 wk postpartum (276 × 10^3 cells/mL and 4.57 mS/cm). A significant drop in EC was observed at the second sampling (fourth week of lactation), and thereafter the values remained without significant differences until the end of lactation. The lowest EC value was observed in wk 10 of lactation (4.04 mS/cm), after which a slight increase was observed, albeit without a significant difference. For SCC, the maximum value was observed at the first sampling (wk 2 postpartum), after which it fell off significantly at the next control, with the lowest value being observed at 12 wk (95 × 10^3 cells/mL), which increased nonsignificantly at 14 wk (129 × 10^3 cells/mL).

The interaction between lactation number and lactation stage was significant for EC and SCC. In the case of EC, this interaction was barely relevant from the biological standpoint (Figure 1). For both primiparous and multiparous ewes, the highest EC values were registered at the first sampling (4.49 vs. 4.64 mS/cm, respectively), with no significant differences between the 2 types of animals. At the second sampling, the EC fell off significantly in both types of animal, with a greater decrease for primiparous ewes, leading to differences being observed between primiparous and multiparous females. These differences were maintained until the end of the investigation, although no further significant differences were observed between samplings.

The SCC in milk from the multiparous ewes was significantly higher than that from primiparous ewes throughout the investigation, except at the last sampling, when no significant differences were observed (Figure 2). In general, the progress of lactation caused a progressive decrease in SCC. The highest SCC values were recorded at the first sampling (385 × 10^3 and 197 × 10^3 cells/mL for multiparous and primiparous ewes, respectively); the minimum SCC value for primiparous ewes was obtained at 12 wk of lactation (57 × 10^3 cells/mL), and increased at the final sampling, although not significantly. For the multiparous ewes, the minimum value was reached at wk 14 of lactation (141 × 10^3 cells/mL).

The EC of milk from infected glands was significantly higher than that from healthy and latently infected glands throughout lactation, except at the first sampling (Figure 3). For glands with latent infection, a

Table 2. Electrical conductivity (EC) and SCC (mean ± SD) of milk per gland depending on type of pathogen causing infection

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>EC (mS/cm)</th>
<th>No. of samples</th>
<th>SCC (×10^3 cell/mL)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy glands</td>
<td>4.17 ± 0.43</td>
<td>1,882</td>
<td>304 ± 1,318</td>
<td>1,804</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>4.41 ± 0.68</td>
<td>68</td>
<td>452 ± 1,082</td>
<td>61</td>
</tr>
<tr>
<td>Staph. xylosus</td>
<td>4.61 ± 0.64</td>
<td>92</td>
<td>2,989 ± 5,953</td>
<td>85</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>5.73 ± 1.31</td>
<td>10</td>
<td>15,601 ± 9,075</td>
<td>9</td>
</tr>
<tr>
<td>Staph. caprae</td>
<td>4.21 ± 0.42</td>
<td>19</td>
<td>1,337 ± 1,775</td>
<td>14</td>
</tr>
<tr>
<td>Staph. lentus</td>
<td>4.14 ± 0.45</td>
<td>63</td>
<td>572 ± 2,839</td>
<td>66</td>
</tr>
<tr>
<td>Staph. haemolytica</td>
<td>4.48 ± 0.54</td>
<td>6</td>
<td>102 ± 39</td>
<td>5</td>
</tr>
<tr>
<td>Staph. sciuri</td>
<td>4.09 ± 0.28</td>
<td>6</td>
<td>163 ± 161</td>
<td>7</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>4.03 ± 0.16</td>
<td>7</td>
<td>228 ± 186</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3. Statistical analysis result (F-value and significance level) of electrical conductivity and SCC variables

<table>
<thead>
<tr>
<th>Effect</th>
<th>No. of classes</th>
<th>LEC (n = 2,138)</th>
<th>LSCE (n = 1,901)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td>6.65**</td>
<td>30.04***</td>
<td></td>
</tr>
<tr>
<td>Lactation stage</td>
<td>23.34***</td>
<td>7.99***</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>16.57***</td>
<td>10.32**</td>
<td></td>
</tr>
<tr>
<td>Milking</td>
<td>328.98***</td>
<td>23.68***</td>
<td></td>
</tr>
<tr>
<td>Health status</td>
<td>22.77***</td>
<td>39.88***</td>
<td></td>
</tr>
<tr>
<td>Parity × health status</td>
<td>3.57**</td>
<td>7.04***</td>
<td></td>
</tr>
<tr>
<td>Lactation stage × health status</td>
<td>6.09***</td>
<td>5.10***</td>
<td></td>
</tr>
<tr>
<td>Parity × lactation stage</td>
<td>5.36***</td>
<td>3.75**</td>
<td></td>
</tr>
</tbody>
</table>

1LEC = electrical conductivity logarithm; LSCE = log SCC; n = number of observations used.

*P < 0.05; **P < 0.01; ***P < 0.001.
lower milk EC was recorded than for healthy glands throughout lactation, but this difference was only significant at 8, 10, and 12 wk postpartum. The evolution throughout lactation varied between the infected glands and the others. The EC declined slightly for all types of health status at wk 4 of lactation, but without significant differences, and remained stable until wk 10. At this point, the EC of the milk from infected glands increased until wk 14 (reaching the maximum EC value of 4.96 mS/cm), whereas no change in trends was observed in the rest of the sanitary states.

As for SCC, from the first control, when significant differences were observed between the 3 health states, the SCC of milk from the infected glands was significantly higher than for the other health states throughout lactation, with no differences observed between healthy glands and those with latent infection as of wk 4 of lactation until the end of the investigation (Figure 4). The maximum SCC value of milk from the infected glands was obtained at 6 wk of lactation, and the minimum at the end of lactation.

The correlation coefficient of the EC with the SCC was higher when the data were analyzed together (r = 0.33) compared with when analysis was done by SCC intervals (Table 6). The correlation coefficient was only significant when SCC > 600 × 10^3 cells/mL and when all the data were analyzed together (P < 0.001).

When studying the relationship between EC and the chemical composition of milk, we obtained a high coefficient of determination (R^2 = 0.6901, Table 7). The variables that explained the higher EC variance were fat (R^2 partial = 0.2416), which was negatively related to EC, and the sodium and potassium content (R^2 partial = 0.1913 and 0.0921, respectively), which was positively related to EC. To a lesser extent, EC was also negatively related to lactose, serum proteins, and caseins and positively related to the chloride content.

### Mastitis Detection by Means of Absolute EC Thresholds

Based on all the data together, the point at which the specificity and sensitivity intersected stood at 4.2 mS/cm (Figure 5a), varying slightly depending on milking type (4.3 mS/cm in the morning and 4.1 mS/cm at evening milking, Figure 5b) or the lactation number of the ewes (4.2 mS/cm for multiparous and 4.3 mS/cm for primiparous, Figure 5c). In general, moderate
sensitivity and specificity values were obtained, and their variations stood out depending on the milking type or parity number of the ewes for the same EC threshold. The highest values obtained at the cutoff point of both curves were observed for primiparous ewes, with 63.64% sensitivity and 63.31% specificity for a threshold of 4.3 mS/cm (Figure 5c). In all cases, a reduction in the number of false positives was observed (increase in PPV) as the EC threshold increased, raising the specificity, but false negatives also increased, which diminished the sensitivity of the method. However, the PPV was very low; its maximum value (67.39%) was recorded for multiparous ewes with a threshold of 5 mS/cm, whereas the negative predictive value was no lower than 80% in any case (Figure 6).

DISCUSSION

Throughout the 2 lactations, no cases of clinical mastitis were found. This prevalence of clinical cases coincides with the results of Contreras et al. (2007), who concluded that the percentage of clinical mastitis in small ruminants was very low (less than 5%). The observed prevalence of subclinical mastitis (8.64% and 17.28% at the beginning and 23.08% and 16.67% at...
the end of the first and second years, respectively) was similar to the results reported by Seegers et al. (1997) and Leitner et al. (2001), 10.4% and 39.9%, respectively. All cases of glands with infectious mastitis or latent infection at the onset of lactation were in multiparous ewes. Likewise, Leitner et al. (2003) observed a higher percentage of healthy glands in primiparous ewes (70%) than in multiparous, with ewes in third lactation having the minimum (52%). Mastitis prevalence in a herd is related to many factors, as is its development throughout lactation. In this investigation, we observed an increase in prevalence in the first year, whereas in the second year the high percentage of spontaneous cures led to a drop in prevalence at the end of lactation. This finding coincides with that observed by McDougall et al. (2002), who reported that 93.8% of infected ewes were spontaneously cured. However, Leitner et al. (2001) noted that the health status of the glands remained stable throughout lactation in 90% of cases.

The majority of pathogens isolated were staphylococci (97.4%), with gram-negative bacteria found in only

<table>
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<th>Item</th>
<th>Statistic</th>
<th>Parameter</th>
<th>SE</th>
<th>Partial R²</th>
</tr>
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<td>Intercept</td>
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<td>10.3639</td>
<td>0.54156</td>
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<td>Fat (%)</td>
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<tr>
<td>Potassium (mg/L)</td>
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<td>0.0005</td>
<td>0.00042</td>
<td>0.0921</td>
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<td>Lactose (%)</td>
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<td>−1.2988</td>
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<td>Serum protein (%)</td>
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<td>Chloride (mg/L)</td>
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<td>0.1456</td>
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<td>Caseins (%)</td>
<td></td>
<td>−0.1413</td>
<td>0.0959</td>
<td>0.0070</td>
</tr>
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</table>

Model (n = 1,360)

F-value: 30.71
P-value: <0.001
R²: 0.06901

\(^1n = \text{number of observations.}\)

Table 7. Relationship between physical-chemical composition and electrical conductivity in milk

Figure 4. Means comparison results for SCC logarithm (LSCC) throughout lactation by health status of glands. Error bars indicate SE.

Figure 5. Sensitivity (Se) and specificity (Sp) of electrical conductivity (EC) for mastitis detection by proposed EC thresholds considering all the data (a), differentiating by milking (b), and differentiating by parity (c).
a small percentage of cases (2.6%). These results agree with those of several authors (Albenzio et al., 2002) and with McDougall et al. (2002), who observed that staphylococci caused 100% of subclinical mastitis cases. Regarding the reaction caused by the isolated pathogens, Ariznabarreta et al. (2002) and Gonzalo et al. (2002) proposed classifying staphylococci that caused mastitis in terms of their sensitivity or nonsensitivity to novobiocin. From the pathogens isolated in this investigation, *S. aureus*, *S. caprae*, and *S. haemolytica* would be in the group of pathogens sensitive to the antibiotic, which the authors proposing this classification reported as causing considerable increases in SCC. In our investigation, high EC and SCC values were observed when the pathogen was *S. aureus* (5.73 mS/cm and 15,601 × 10³ cells/mL, respectively). In the case of *S. caprae*, a high SCC was also recorded (1,337 × 10³ cells/mL), but the EC was lower (4.21 mS/cm). In contrast to the findings of Ariznabarreta et al. (2002) and Gonzalo et al. (2002), which were based on infection caused by *S. haemolytica*, the SCC registered was low (102 × 10³ cells/mL). Moreover, novobiocin-resistant staphylococci would be present, which the authors claimed are associated with a low SCC, typical of minor pathogens, such as *S. xylosus* and *S. lentus*. However, in this study milk from the glands in which *S. aureus* was isolated presented a high SCC and EC (2,989 × 10³ cells/mL and 4.61 mS/cm), which could be attributed to a depressed immune system, whereby the pathogen caused greater inflammation of the glands.

The significantly higher EC observed for milk from the glands with infectious mastitis compared with milk from healthy glands (difference of 0.30 mS/cm in primiparous and 0.53 mS/cm in multiparous) agrees with the findings of Peris et al. (1991) in sheep livestock in a comparison between glands with California Mastitis Test (CMT) 0 and CMT +: 0.54 mS/cm difference in the stripping fraction, 0.21 in the first jets, and 0.27 mS/cm in machine milk. In cattle, Kaşıkçı et al. (2012) also reported significant differences between EC of the milk from glands with CMT +, ++, or ++++, as did Norberg et al. (2004), who measured the EC in glandular milk every 2 s during milking using the “Mastitis Detector” prototype (S.A. Christensen, Kolding, Denmark), finding significant differences in EC among the different glandular health states (4.87, 5.37, and 6.44 mS/cm for healthy glands, those with subclinical mastitis, or those with clinical mastitis, respectively). In goats, Díaz et al. (2012) recorded an increase in EC in the 4 d following the onset of an intramammary infection when the disease presented bilaterally.

In glands with latent infection, significant differences were only observed compared with healthy glands in primiparous ewes, noting that the milk EC was higher for healthy glands. The EC for infected glands was higher than in the other 2 health states in both multiparous and primiparous ewes. This different behavior depending on the parity number was also observed in goats by Díaz et al. (2011), who found a significant increase in EC due to infection only in the glands of primiparous ewes. In the same study, they recorded higher EC for multiparous goats than primiparous overall, considering only healthy glands or those with bacterial mastitis, whereas no differences were observed in parity number when considering only glands with nonspecific mastitis. Ying et al. (2004) observed different behavior of EC for infected goat glands depending on the breed; in Saanen

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**Figure 6.** Positive predictive value (PPV) and negative predictive value (NPV) of electrical conductivity (EC) for mastitis detection by proposed EC thresholds considering all the data (a), differentiating by milking (b), and differentiating by parity (c).
goats, the EC increased for infected goats, whereas in Alpine breed goats, EC was reduced for the infected animals. As with EC, the SCC of milk from the glands with infectious mastitis was significantly higher than that from healthy glands or those with latent infection, in both primiparous and multiparous ewes. In this case, the SCC in the context of latent mastitis was not significantly reduced compared with the count for healthy glands in primiparous sheep. The SCC recorded for healthy glands and those with latent mastitis (61 × 10^3 cells/mL in primiparous ewes with latent mastitis and 99 × 10^3 cells/mL in healthy multiparous ewes) were similar to those obtained by Peris et al. (1991) in Manchega ewes (80 × 10^3, 72 × 10^3, and 130 × 10^3 sales/mL for CMT = 0 in the first jets of milk, machine milk, or stripping milk, respectively) and those of Martins et al. (2013) in healthy Santa Inés ewes (167.1 × 10^3 cells/mL).

The SCC in milk from the glands that had infectious mastitis was significantly higher both in primiparous and multiparous animals, being more marked in multiparous ewes. In goats, Díaz et al. (2011) observed a significant effect of the health status of glands and the parity number on the SCC, finding higher SCC for multiparous goats than for primiparous, although the interaction of both factors (health status and parity number) was significant in contrast to our observations.

The SCC diminished throughout lactation in accordance with the results of Gonzalo et al. (1994), who obtained the minimum SCC value in dairy sheep 75 d postpartum, whereas Fuertes et al. (1998) recorded the minimum value in the fifth week of milk production. Both works recorded an increase in SCC as lactation progressed, but in the case of Fuertes et al. (1998), this increase was very slight and could thus be attributed to a higher concentration of SCC in a smaller volume of milk rather than to a worsening of the glandular health status. In our investigation, an increase in SCC was only observed at the end of lactation for multiparous ewes and for glands with latent infection or infectious mastitis, so it may be attributed to worsening of the glandular health status.

Regardless of health status, the milk EC recorded for multiparous ewes (4.39 mS/cm) was higher than that obtained for primiparous ewes (4.05 mS/cm). These differences remained throughout lactation in line with reports by several authors in cattle (Holdaway et al., 1996; Zecconi et al., 2004). Those differences were attributed to greater permeability of the blood–milk barrier in multiparous cows due to damage that occurred over the course of several lactations.

The increase in milk SCC observed at the end of lactation coincides with that observed for goats (Díaz et al., 2011) and cattle (Holt, 1985). The authors of these studies concluded that as lactation progressed the blood–milk barrier permeability increased, causing an increase in Na^+ and Cl^- concentrations in milk. In Sarda ewes, Caria et al. (2016) also obtained a high correlation coefficient (r = 0.893) between the EC of the milk and its chloride content.

The milk composition explained a high variance in EC (R^2 = 0.69), with fat content exerting the greatest influence, followed by the Na^+ and K^+ contents. In contrast, Díaz et al. (2011) found that the inorganic salts exerted a greater influence on the EC of milk than its macroconstituents (fat, protein, and lactose).

The negative correlation of EC with the fat content of milk agrees with Caria et al. (2016), Prentice (1962), and Muchetti et al. (1994), who stated that fat globules increased the actual distance in the migration of ions that tend to cross over between the 2 electrodes of the conductometer. Albenzio et al. (2002), Leitner et al. (2004), Bianchi et al. (2004), and Santos et al. (2007) observed a decline in the fat content of milk from ewes with mastitis, which along with the ion concentration would explain the increase in EC observed in the milk from the infected glands. Castillo et al. (2008) noted that the shorter the milking interval became, the more the fat content of the milk increased, so milk from the evening milking had a higher fat percentage than milk from the morning. Thus, along with the negative relation of fat content with the observed EC, this difference explains why the EC of milk from the evening milking was lower than from the morning milking. The negative relationship between lactose content and EC is in agreement with the results published by Caria et al. (2016) in sheep and Hamann and Zecconi (1998). They showed that lactose is the most important lactic component in the regulation of milk osmotic pressure, and its content is inversely proportional to the concentration of Cl^- for the same osmotic pressure. Thus, the passage of chloride ion to the alveolar lumen from the bloodstream, which is enhanced by the deterioration of the mammary gland epithelial cell membranes, is counteracted by a decrease in lactose, while at the same time deterioration in lactocytes also causes a decrease in lactose synthesis (Shuster et al., 1991). In accordance with the preceding observations, intramammary infection may cause a drop in the amount of lactose, ranging from the 3% observed by Bianchi et al. (2004) to the 25% obtained by Leitner et al. (2004).

Regarding the correlation between EC and SCC, when the data were considered together, we obtained a positive but moderate correlation (r = 0.33). McDougall et al. (2001) found a correlation of −0.37 between the SCC and impedance (inverse of EC) and stated that the impedance would not be a good indicator of mastitis in sheep. Peris et al. (1991) observed that the

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correlation between EC and SCC was significant in the first few jets of milk and in stripping milk (0.53 and 0.52, respectively) but not in the machine milk fraction. The overall correlation found by Díaz et al. (2011) in goats was similar to that observed in this work (0.38), only being significant when the SCC was low (<500 × 10^3 cells/mL) or high (>2,000 × 10^3 cells/mL). In the majority of cases (80%), the SCC was below 200 × 10^3 cells/mL because of the low prevalence of infectious mastitis recorded.

Although sensitivity and specificity values at the cutoff point (4.2 mS/cm) were not high (56.73% and 55.29%, respectively), we observed a high NPV (87.16%), so the use of absolute EC thresholds for mastitis detection would be more accurate and reliable for ruling out healthy glands than for detecting mastitic glands. For glands in which the threshold is exceeded, other methods would have to be used to confirm the disease and to avoid the cost of unnecessary treatments of a gland that is not actually infected. Peris et al. (1989) achieved better results than those in this work. With a threshold of 5 mS/cm, they managed to correctly classify 87.9% of samples in sheep, obtaining a sensitivity of 60.2% and a specificity of 91.4%. However, they also observed that when taking the difference in EC between both glands of the same animal into account, the outcomes improved considerably (70% sensitivity, 93% specificity, and 89.1% of samples classified correctly for a difference between glands greater than 0.3 mS/cm). The results obtained by Caria et al. (2016) were also better than those obtained in this work; they achieved sensitivity and specificity of 73.08% and 75.46%, respectively, setting an EC threshold of 4.84 mS/cm.

These results show that for EC to be used as a method for detecting mastitis in sheep, as Díaz et al. (2011) also noted, we should develop detection methods that would not be affected by the variations arising in milk EC due to intrinsic animal factors (parity number, milking session, and lactation stage) and not be limited to setting absolute thresholds.

CONCLUSIONS

Several factors affect EC in sheep livestock in addition to the glandular health status. Factors such as lactation stage, parity number, and milking session (morning and evening) have proven to be significant, so it is necessary to consider them when using EC as an indicator of the health status of the gland. Therefore, all these factors must be taken into account when developing EC-based methods of detecting mastitis in sheep. One option worthy of consideration is individualized daily monitoring of glands, as reported in other species such as cattle and goats. Further studies are necessary to determine the effect of the onset of mastitis on EC of the milk and the detection method to apply to optimize the sensitivity and specificity results.

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REFERENCES


Electrical Conductivity of Ewe Milk


