Goat mastitis detection using daily records of milk conductivity: comparative results of different algorithms

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ABSTRACT: Milk electrical conductivity is employed for mastitis detection in cows due to its automation, low cost, and infection detectability at early stage. Nevertheless, the number of publications about its use in dairy goats is scarce. The aim of this study was to check and compare the detectability of goat mastitis (sensitivity and specificity) using different algorithms, constructed with individual daily conductivity data from glands, in order to improve the know how about the potential of this variable for goat mastitis detection. A total of 18 goats (8 primiparous and 10 multiparous) free of mastitis were used, and gland milk conductivity was daily monitored. After 16 days of monitoring, some unfavourable situations for gland health were simulated in order to increase the cases of infection. Once infection was established (9 goats and 12 glands got infected), the experiment continued for further 16 days. A total of 19 different algorithms that employed conductivity data from gland were designed; they were tested using gland milk conductivity (EC) and ratio of EC of collateral glands in the same goat (RAT_{EC}). The algorithms were tested in all the animals and intramammary infection detection ability characteristics (sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), and negative predictive value (NPV)) were recorded. All clinical cases were detected (n = 2, 100% SENS) with all the algorithms. Best global SENS (clinical and subclinical, 33.3–58.3%) and SPEC (77.8–100%) were similar to results reported in previous studies in cows, and obtained with algorithms ARIMA and Rule 1 (3 standard deviations of data). The best algorithms to use in mastitis detection depend on the prevalence and type of mastitis. EC ARIMA and Rule 1 algorithms detected the most severe cases on-line and quickly, with a low proportion of false positives.

Keywords: intramammary infection; gland measurements; on-line; sensitivity, specificity; ARIMA

INTRODUCTION

Milk electrical conductivity (EC) has been used for cow mastitis detection for early subclinical and clinical cases because it can be automated in the milking parlour and gives early results (on-line) (Nielen et al. 1992). There are several factors other than mastitis related to EC, including differences between animals, so that, in dairy cows, methods that employ an absolute EC threshold for all the animals for mastitis detection are not accepted, even if EC is measured in the complete milking or at gland level. Studies in dairy goats (Ying et al. 2002; Diaz et al., 2011, 2012; Romero et al. 2012) have shown significant effects of parity, lactation stage, farm, and the analyzed fraction, in addition to mammary infection.

Most studied methods for cow mastitis detection using EC are based on processing data from EC sensors located at short milk tube or claw (also at gland level) and applying algorithms that consider the comparison of gland EC with the moving average of previous milkings (Lansberger et al. 1994; Mele et al. 2001; Biggadike et al. 2002; Zecconi et al. 2004; Cavero et al. 2007; Kamphuis et al. 2008a) and the comparison of EC of collateral glands (Maatje et al. 1992, 1997; Lien et al. 2005). In all these studies, specificity (SPEC) was around 90%,

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but sensitivity (SENS) was lower (different results were obtained depending on the study and type of mastitis: clinical, subclinical or somatic cell count (SCC) increases, from 25 to 89%). Other methods employ two or more variables in addition to EC that can be registered automatically. The Neural Net method includes several variables in addition to EC, such as yield, days in milk or milk flow. Nielen et al. (1995b) used this algorithm with best results of 77% SENS and 100% SPEC in clinical mastitis. In subclinical mastitis, Nielen et al. (1995a) reported 54% SENS and 92% SPEC. Cavero et al. (2008) reported 84.2% SENS and 51.1% SPEC for SCC > 100 000 cells/ml detection and 78.6% SENS and 74.9% SPEC for SCC > 400 000 cells/ml detection. The fuzzy logic method uses EC combined with another variable; Kamphuis et al. (2008b) employed EC combined with SCC and reported 80% SENS in clinical mastitis with a low true positive value (32%); Cavero et al. (2006) reported 83–92% SENS and 75–93% SPEC combining EC, yield, and milk flow variables in the algorithm.

The best SENS-SPEC level for a farm or species will depend of the type and prevalence of mastitis. In farms with high mastitis prevalence, especially if it is clinical, high SENS will be required in order to reduce mastitis effects on farm economy; but if prevalence is low and mastitis is subclinical, like Contreras et al. (1995) published for goats in the studied region (18% of glands), a high SPEC will reduce unnecessary treatments, and their associated costs.

For goat mastitis detection, there are no published studies about the employment of algorithms using on-line EC measurements like those used for cows (previously referenced), probably due to the fact that EC sensors to be included at the milking machine, for gland level measurements, are not commercially extended for small ruminants parlours. Tangorra et al. (2010) compared daily EC between healthy and infected glands with a mixed model in a pilot study, showing significant differences, and our research group has reported significant effects of mammary infection and mastitis at studies carried out monthly (Diaz et al., 2011), in an individual sampling day (Romero et al. 2012), and daily (Diaz et al. 2012) with mixed models.

The aim of this study was to check and compare the ability for goat mastitis detection (sensitivity and specificity) of different algorithms, that consider individual daily milk conductivity data from glands, in order to improve the know how about the potential of this variable for goat mastitis detection.

MATERIAL AND METHODS

Animals and management. The experiment was carried out in the Murciano-Granadina goat herd of the Miguel Hernández University of Elche (Spain). The management system was intensive, permanent stabling, one parturition per year, and mechanical milking once a day (in the morning). Milking parameters were: rate of 90 pulsations per min, vacuum level of 40 kPa, and a 60% pulsation ratio. Animals were fed a commercial mixture (unifeed system) for high production goats which maintained constant for the whole study (quantity and quality).

Experimental design and analyzed variables

Animal enrolment criteria. Fifty-six goats in their third month of lactation were observed for a 1-week period during which the following data were obtained from both glands: bacteriological analyses of milk (aseptic sampling before milking), EC, and SCC of milk (representative samples of the whole milking). These data were used to identify 18 goats (8 primiparous and 10 multiparous) with no indications of mastitis (see Gland Health Status definition) that were enroled in the experiment.

Experimental phase. The experimental phase had two sub-phases. The aim in the first 16 days was to obtain daily information on the conditions of the studied variables before the possible establishment of mastitis: all the 18 selected animals were milked and variables were analyzed (as mentioned below). After this first experimental sub-phase, various unfavourable health situations (UHS) for the mammary gland were simulated during 5 days in all the goats, consisting of situations of the type that could occur on any commercial farm that might increase mastitis probability, such as: milking a healthy goat after a goat with intramammary infection (IMI), favouring a wet milking and inverse milk flow, increase of milking vacuum level to 44 kPa, 3 min of over-milking, and elimination of iodine teat dipping after the milking. These UHS continued until IMI was established in 9 goats (half of the considered animals). After IMI establishment, animals were milked over the following 16 days and variables were recorded. Animals that remained free of IMI were also monitored to obtain information about the behaviour of variables under healthy gland conditions and discard any effect of the day.

Variables were analyzed at gland level: bacteriology (samples aseptically collected before milking),

EC, and SCC (from a representative sample from the milking of the gland collected by the yield recording device (Metatron; Gea Westfalia Surge, Bönen, Germany)).

Electrical conductivity (mS/cm) was recorded daily using a laboratory conductivity meter GLP 32 (Crison Instruments s.a., Alella, Spain) equipped with a PT100 temperature probe and reading compensated at 25°C.

Bacteriology was tested weekly before UHS. After the beginning of UHS, one analysis was performed and then repeated every 2 days to confirm the presence and persistence of infection. Milk samples for bacteriological analysis (5 ml) were obtained aseptically, from teats carefully cleaned with 70% ethanol, discarding the first three streams of foremilk and placed into sterile tubes; these were kept at 4°C for a maximum of 12 h until analysis, used for the bacteriological analysis, and afterwards kept frozen until the end of the experiment. 20 µl of each sample were plated on blood agar plates (bioMérieux, Marcy l'Etoile, France). The plates were incubated aerobically at 37°C and examined at 24, 48, and 72 h. Cultures with five or more identical colonies were considered positive for IMI. Bacteria were identified according to the National Mastitis Council recommendations (Harmon et al. 1990). Presumptive identification of bacterial genera was done for positive samples: coagulase-positive staphylococci, coagulase-negative staphylococci, streptococci, Gram-negative *bacillus*, and other (*Corynebacterium*, etc.). Gram staining was done and the catalase test was run for Gram-positive microorganisms. For staphylococci, the bacterial species were identified using the apiStaph kit (bioMérieux).

SCC (× 1000 cells/ml) was analyzed on samples kept in azidiol, using fluoro-opto-electronic method (Fossomatic 500; Foss Electric, Hillerød, Denmark) in the Inter-Professional Dairy Laboratory of the Community of Valencia (LICOVAL, Spain). Prior to UHS, it was analyzed weekly until 3 days before UHS (3 analyses); then the frequency was daily until 11 days after establishment of the infection, and finally on days 13, 15, and 16 of the infection.

Gland health status definition. To determine the health status of the glands, both bacteriological analysis and SCC results were considered in addition to clinical observation, according to Diaz et al. (2011). A gland was defined as having bacterial mastitis (positive for IMI) when bacteriological analyses were positive. When the bacteriological analysis was negative and SCC was > 1 000 000 cells/ml on two or more consecutive sampling days and for non-physiological causes, it was considered unspecific (UNS). A physiological increase in SCC, for example due to oestrus (Christodoulopoulos et al. 2008) or acute stress (Mehdid et al. 2013) was defined when bacteriological analysis was negative and there was an increase of SCC in both glands for a maximum of 3 consecutive sampling days which was followed by SCC < 1 000 000 cells/ml in a subsequent analysis. A gland was considered free from mastitis with negative bacterial culture and SCC < 1 000 000 cells/ml or if the increase of SCC values was due to physiological causes. A case was considered clinical if changes in milk appearance were observed (clots or changes in colour); if no appearance changes were observed, the infection case was considered subclinical.

According to gland health status results obtained (only infective cases were recorded), and in order to analyze the progress of EC around IMI establishment, glands were classified into 8 levels that considered gland health status and its collateral in the same animal (gland classification: 1 = glands from udder with both glands healthy, 2 = subclinically infected glands of unilaterally infected udders, 3 = healthy glands of unilaterally subclinically infected udders (each gland 2 had its contralateral gland in the group of glands 3), 4 = clinically infected gland of unilaterally infected udder, 5 = healthy gland of unilaterally clinically infected udder (the same animal as gland 4), 6 = clinically infected gland of bilaterally infected udder, 7 = subclinically infected gland of bilaterally clinically infected udder (the same animal as gland 6), 8 = subclinically infected glands of bilaterally subclinically infected udder, both glands of each bilaterally subclinically infected udder).

Data treatment and statistical analysis. First, a preliminary analysis of EC records was run, consisting of calculating average and standard deviation by gland classification along periods of 4 days. Glands from animals free of IMI were also studied. An infected gland was assigned to every uninfected gland, and the same period was considered in the analysis.

After that, 19 algorithms were constructed for EC and the EC ratio of collateral glands in the same goat (RAT_{EC} = maximum EC/minimum EC) employing all data recorded prior to the day of the infection establishment ("infection day") for predicting the range of variation: Autoregressive

Integrated Moving Average – ARIMA procedure of SAS (Statistical Analysis System, Version 9.1., 2002); deviations exceeding 3 standard deviations (SD) (Rule 1), deviations exceeding 4 SD (Rule 2); deviations of 5, 10, 20, 30% of moving average (Rules 3, 4, 5, and 6, respectively), the moving average being calculated with 3 different data sizes recorded before the "infection day": 4, 8, and 14 days (MS Office – Excel, 2010).

All the algorithms tested use the past data of each gland (or animal if RAT_{EC}) to predict a range of variation of the future ones. If something affects the tested variable (like mastitis), then the next measured data are expected to be out of the predicted range.

It was checked if any of the 5 data recorded after the "infection day" were out of the predicted range (this period of 5 days was adopted because the highest EC increase was observed during the first 4 days after IMI, with a gradual EC decrease after that period). A "positive case" occurred if one of the 5 recorded data was over the predicted range. A "negative case" was registered if data were in the predicted range.

The IMI detection ability (sensitivity = SENS, specificity = SPEC, positive predictive value = PPV, and negative predictive value = NPV) was studied for each variable (EC and RAT_{EC}) and algorithm. SENS was defined as the probability of a truly infected mammary gland being classified as test positive (true positive/(true positives + false negatives)). SPEC was defined as the probability of a non-infected sample being classified as such (true negative/(true negative + false positive)). Additionally, the PPV was calculated, defined as the probability of the gland being truly infected when the sample is classified as positive (true positives/(true positives + false positives), as well as the NPV, defined as the probability of the gland not being infected when the sample is classified as negative (true negatives/(true negatives + false negatives).

RESULTS AND DISCUSSION

All mastitis cases recorded were infective, not recording any case of UNS mastitis. Prevalence and incidence of infection after UHS were 50% in goats and 33.3% in glands (a total of 12 infected glands: 3 goats were infected in both glands (bilateral infection), and 6 in one gland (unilateral infection), all in multiparous animals).

Genera and species were typical of IMI in Murciano-Granadina goats in south-eastern Spain (Contreras et al. 1997): *Staphylococcus* (*xylosus*, *n* = 4; *caprae*, *n* = 3; *aureus*, *n* = 1; *chromogenes*, *n* = 1; spp., *n* = 1), *Streptococcus* (*n* = 1), and *Enterobacteriaceae* (*n* = 1). Mastitis was subclinical in most cases; clinical cases (*n* = 2 glands) were caused by *S. aureus* (infecting one gland in a bilaterally infected goat) and by *Bacillus* Gram (infecting one gland in a unilaterally infected goat), according to Contreras et al. (1997) and Poutrel et al.

Table 1. Milk electrical conductivity (average ± standard deviation, mS/cm) of healthy, clinically and subclinically infected glands along different periods around the intramammary infection (IMI) establishment

Gland classification*	Glands (*n*)	Days around IMI establishment			
		8–5 days before	1–4 days before	1–4 days after	5–8 days after
1	18	5.38 ± 0.65	5.39 ± 0.66	5.34 ± 0.56	5.31 ± 0.56
2	5	5.56 ± 0.39	5.55 ± 0.46	5.62 ± 0.38	5.69 ± 0.35
3	5	5.53 ± 0.41	5.55 ± 0.45	5.55 ± 0.38	5.56 ± 0.39
4	1	5.09 ± 0.13	5.01 ± 0.11	5.52 ± 1.25	5.32 ± 0.09
5	1	5.21 ± 0.14	5.01 ± 0.11	5.71 ± 1.01	5.29 ± 0.12
6	1	5.58 ± 0.09	5.61 ± 0.03	8.61 ± 1.65	7.75 ± 1.05
7	1	5.52 ± 0.13	5.49 ± 0.19	5.59 ± 0.09	5.68 ± 0.08
8	4	6.13 ± 0.44	6.05 ± 0.33	6.05 ± 0.50	5.92 ± 0.49

*1 = glands from udder with both glands healthy, 2 = subclinically infected glands of unilaterally infected udders, 3 = healthy glands of unilaterally subclinically infected udders (each gland 2 had its contralateral gland in the group of glands 3), 4 = clinically infected gland of unilaterally infected udder, 5 = healthy gland of unilaterally clinically infected udder (the same animal as gland 4), 6 = clinically infected gland of bilaterally infected udder, 7 = subclinically infected gland of bilaterally clinically infected udder (the same animal as gland 6), 8 = subclinically infected glands of bilaterally subclinically infected udder, both glands of each bilaterally subclinically infected udder

(1997) who reported a low prevalence of clinical mastitis in goats.

Averages of EC around the mastitis establishment day by gland level (according to Materials and Methods classification) are shown in Table 1. The standard deviation average of data previous to infection of glands was 0.15 mS/cm (maximum SD = 0.57 mS/cm, minimum SD = 0.07 mS/cm). No relevant increase after IMI establishment was observed for subclinically infected glands (unilaterally and bilaterally infected ones) and their noninfected collateral glands. The highest increases were recorded in the 1–4 day period after infection for the clinically infected gland of a unilaterally infected udder (gland 4: from 5.01 mS/cm before infection to 5.52 mS/cm after infection), its collateral which remained healthy (gland 5: from 5.01 mS/cm before infection to 5.71 mS/cm after infection), and the clinically infected gland in a bilaterally infected udder (gland 6: from 5.61 mS/cm before infection to 8.61 mS/cm after infection); these increases dropped in 5–8 days after the IMI establishment period to values slightly higher than 1–4 days before infection, except for gland 6 and its collateral (gland 7: subclinically infected) where EC remained much higher than before IMI establishment, with a moderate tendency to keep increasing 5–8 days after the IMI establishment period.

All the clinical mastitis cases were detected with all the algorithms considered. The difference of results of algorithms was due to the differences in subclinical mastitis detection and SPEC (Table 2). The highest SENS results were obtained with EC data (33.3–58.3%, 4–7 glands detected from 12 infected): better algorithms were ARIMA (33.3%) and Rule 1 (58.3%). Algorithms that use RAT_{EC} achieved high SPEC (88.9–100%), but SENS was lower than that obtained with EC algorithms (except for ARIMA algorithm that was equal).

The main limitation of the tested algorithms was in not achieving a higher SENS at the same time as high SPEC. An oscillation of EC among consecutive days previous to infection was observed in some animals (SD = 0.22 and 0.57 mS/cm); this caused a wider prediction range for ARIMA and SD rules, and so a higher increase in EC was required to get positive cases. Related to this, when period

Table 2. Results of electrical conductivity (EC) and electrical conductivity ratio (RAT_{EC}) for IMI detection characteristics (%) using different algorithms, by number of days before establishment of infection considered in the predictive range (days)

Days	Algorithm	EC				RAT_{EC}			
		SENS	SPEC	PPV	NPV	SENS	SPEC	PPV	NPV
All	ARIMA	33.3	95.8	80.0	74.2	33.3	100.0	100.0	60.0
4	Rule 1 (3 SD)	58.3	75.0	53.8	78.3	44.4	88.9	80.0	61.5
	Rule 2 (4 SD)	33.3	87.5	57.1	72.4	33.3	100.0	100.0	60.0
	Rule 3 (Δ5%)	25.0	95.8	75.0	71.9	22.2	77.8	50.0	50.0
	Rule 4 (Δ10%)	16.7	95.8	66.7	69.7	22.2	100.0	100.0	56.3
	Rule 5 (Δ20%)	16.7	95.8	66.7	69.7	22.2	100.0	100.0	56.3
	Rule 6 (Δ30%)	16.7	100.0	100.0	70.6	22.2	100.0	100.0	56.3
8	Rule 1 (3 SD)	41.7	91.7	71.4	75.9	22.2	100.0	100.0	56.3
	Rule 2 (4 SD)	25.0	95.8	75.0	71.9	22.2	100.0	100.0	56.3
	Rule 3 (Δ5%)	25.0	91.7	60.0	71.0	44.4	88.9	80.0	61.5
	Rule 4 (Δ10%)	16.7	95.8	66.7	69.7	22.2	100.0	100.0	56.3
	Rule 5 (Δ20%)	16.7	95.8	66.7	69.7	22.2	100.0	100.0	56.3
	Rule 6 (Δ30%)	16.7	95.8	66.7	69.7	22.2	100.0	100.0	56.3
14	Rule 1 (3 SD)	33.3	91.7	66.7	73.3	22.2	100.0	100.0	56.3
	Rule 2 (4 SD)	25.0	95.8	75.0	71.9	22.2	100.0	100.0	56.3
	Rule 3 (Δ5%)	33.3	87.5	57.1	72.4	33.3	100.0	100.0	60.0
	Rule 4 (Δ10%)	16.7	95.8	66.7	69.7	22.2	100.0	100.0	56.3
	Rule 5 (Δ20%)	16.7	95.8	66.7	69.7	22.2	100.0	100.0	56.3
	Rule 6 (Δ30%)	16.7	95.8	66.7	69.7	22.2	100.0	100.0	56.3

SENS = sensitivity, SPEC = specificity, PPV = positive predictive value, NPV = negative predictive value, SD = standard deviation

to calculate moving average increased (from 4 to 14 days), SENS decreased for rules based on SD (Rules 1 and 2). This effect was not observed for rules based on percentage increases (Rules 3 to 6) that got lower SENS results (only detected clinical mastitis cases) for all the size periods considered. These rules are more affected by the tendency in the variable: if it is increasing, higher EC value should be recorded to get a positive case; opposite to this, if the tendency is to decrease, the average will be lower and a smaller increase will cause a positive case.

A cause of reducing SPEC to 95.8% at most EC algorithms was the fact that gland 5 (healthy gland of unilaterally clinically infected udder) caused a false positive, with high increase of EC due to the effect of the collaterally clinically infected gland. Although it was a false positive case, it helped detect an infected animal and it would have been a useful result at farm.

The SENS and SPEC levels obtained in this study are similar to those published in cows by Biggadike et al. (2002) (40–46 and 87–92%, respectively) using moving average models; Nielen et al. (1995a) (33–77% SENS and up to 100% SPEC, respectively, for clinical mastitis), Nielen et al. (1995b) (54–66% SENS and 80–92% SPEC, for subclinical mastitis) with neuronal nets; Maatje et al. (1992) (50–100% SENS for subclinical and clinical mastitis), Maatje et al. (1997) (53% SENS for subclinical and 95–100% for clinical mastitis), Lien et al. (2005) using the comparison of collateral glands for subclinical mastitis (47.2% SENS and 81.7% SPEC), and Lien et al. (2005) which used a global threshold (33.6 SENS and 79.6% SPEC).

The best published results in dairy cows were recorded for clinical mastitis or high SCC detection, like in this study. Generally, the models that increase SENS cause a decrease in PPV, due to the higher number of false positives; high SENS is published in some works, but SPEC is not published (Maatje et al. 1992, 1997), leaving the question of the proportion of false positive cases open. Other published results obtained higher SENS with low SPEC (Zecconi et al. 2004; Cavero et al. 2008). High SENS and SPEC have been published for clinical and subclinical mastitis in cows using the increases above moving average (Mele et al. 2001; Cavero et al. 2007), Tracking Signal Method (De Mol et al. 1999; Mele et al. 2001) or Fuzzy Models (Cavero et al. 2006) that combine one or more variables (for example, temperature, flow or yield) with EC.

CONCLUSION

The best algorithms to use in mastitis detection will depend on the prevalence and type of mastitis. When mastitis prevalence is low, especially clinical (like it is for goats), a high SPEC is required in order to avoid unnecessary treatments. A system enabling the farmer to detect the most severe cases on-line and quickly, with a low proportion of false positives, like obtained with EC ARIMA and Rule 1 algorithms, would be a useful tool for goat milk production systems. The next step should be to test the best algorithms resulted (ARIMA and Rule 1) at farm level. For this, specific sensors designed to measure on-line EC (during milking) at goat gland level, and specific software to analyze the data, must be used in order to manage properly the big amount of data that should be recorded.

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