Spanish Journal of Agricultural Research
13(4), e0504, 8 pages (2015)
eISSN: 2171-9292
http://dx.doi.org/10.5424/sjar/2015134-7482
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Mastitis diagnosis in ten Galician dairy herds (NW Spain) with automatic milking systems

Angel Castro, Jose M. Pereira, Carlos Amiama and Javier Bueno

University of Santiago de Compostela, Campus Universitario, Department of Agroforestry Engineering. 27002 Lugo, Galicia, Spain.

Abstract

Over the last few years, the adoption of automatic milking systems (AMS) has experienced significant increase. However, hardly any studies have been conducted to investigate the distribution of mastitis pathogens in dairy herds with AMS. Because quick mastitis detection in AMS is very important, the primary objective of this study was to determine operational reliability and sensibility of mastitis detection systems from AMS. Additionally, the frequency of pathogen-specific was determined. For this purpose, 228 cows from ten farms in Galicia (NW Spain) using this system were investigated. The California Mastitis Test (CMT) was considered the gold-standard test for mastitis diagnosis and milk samples were analysed from CMT-positive cows for the bacterial examination. Mean farm prevalence of clinical mastitis was 9% and of 912 milk quarters examined, 23% were positive to the AMS mastitis detection system and 35% were positive to the CMT. The majority of CMT-positive samples had a score of 1 or 2 on a 1 (lowest mastitis severity) to 4 (highest mastitis severity) scale. The average sensitivity and specificity of the AMS mastitis detection system were 58.2% and 94.0% respectively being similar to other previous studies, what could suggest limitations for getting higher values of reliability and sensibility in the current AMSs. The most frequently isolated pathogens were *Streptococcus dysgalactiae* (8.8%), followed by *Streptococcus uberis* (8.3%) and *Staphylococcus aureus* (3.3%). The relatively high prevalence of these pathogens indicates suboptimal cleaning and disinfection of teat dipping cups, brushes and milk liners in dairy farms with AMS in the present study.

Additional key words: automatic milking system; mastitis detection; pathogen.

Abbreviations used: AMS (automatic milking system); CMT (California Mastitis Test); DIM (days in milk); FAR (false alert rate); FN (false negative); FP (false positive); NPV (negative prediction value); PPV (positive prediction value); ROC (receiver operation characteristics); SCC (somatic cell count); SR (success rate); TN (true negative); TP (true positive).

Citation: Castro, C.; Pereira, J. M.; Amiama C.; Bueno, J. (2015). Mastitis diagnosis in ten Galician dairy herds (NW Spain) with automatic milking systems. Spanish Journal of Agricultural Research, Volume 13, Issue 4, e0504, 8 pages. http://dx.doi.org/10.5424/sjar/2015134-7482.

Received: 03 Feb 2015 **Accepted**: 03 Nov 2015

Funding: The authors are grateful for the financial support granted by the Autonomous Government of Galicia through the Directorate General for Research & Development (PGIDT/PGIDIT Project, Ref: 07MRU013291PR).

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Angel Castro: angel.castro@usc.es

Introduction

Bovine mastitis can be classified into sub-clinical, clinical and chronic forms, depending on the presence and duration of symptoms and the macroscopic appearance of the milk. It is associated to the causative pathogen, and the animal´s age, breed, immunological status and lactation stage (Viguier *et al*., 2009). The most commonly used diagnostic method for mastitis detection is to observe the visible indications during milking. Sub-clinical mastitis is most prevalent and it is commonly underdiagnosed due to the absence of symptoms. Furthermore, clinical mastitis may also go unnoticed in cows milked with an automatic milking system (AMS), where the farmer is not present during milking and no specific mastitis diagnostic methods are employed (Hogeveen *et al*., 2010). Quick mastitis detection in AMS is very important in order to avoid a decrease in milk quality and economic losses (Fröhling *et al*., 2010). A self-monitoring program significantly reduced the somatic cell count (SCC) of the bulk tank, an indicator of mastitis, by helping farmers to detect cows with abnormal foremilk at the start of automatic milking and work with the California Mastitis Test (CMT) and individual cow SCC from monthly Official Milk Recording (Rasmussen *et al*., 2001). During au-

tomatic milking reliable and sensitive methods are necessary (Viguier *et al*., 2009) and farmers need mastitis detection systems that produce a low number of false positives and negatives (Mollenhorst *et al*., 2012). For AMS, abnormal milk detection system must provide accurate alerts, related with the occurrence of the event (Hogeveen *et al*., 2010). To avoid false-positive alerts the AMS needs high specificity (Steeneveld *et al*., 2010b). Some studies developed mastitis detection models for AMS using different techniques (Kamphuis *et al*., 2010; Sun *et al*., 2010. A sensitivity of 70% and specificity of least 99% have been mentioned as minimum requirements for a reliable mastitis detection system (Steeneveld *et al*., 2010b). The International Standard ISO/FDIS 20966 describes a minimum sensitivity of 80% combined with specificity higher than 99%, but these recommendations are, however, still under discussion (Hogeveen *et al*., 2010). Evaluating the performance of automated mastitis-detection systems with respect to their practical value on a farm will allow farmers to compare different mastitis-detection systems sensibly and fairly before investing (Kamphuis *et al*., 2013).

Different methods of mastitis diagnosis are used (Viguier *et al*., 2009). The CMT is one of the oldest and best known. It is based on the principle that the addition of a detergent to a milk sample with a high cell count will lyse the cells, release nucleic acids and other constituents and lead to the formation of a 'gel-like' matrix (Kamphuis *et al*., 2010). Although the interpretation can be subjective, and this might result in false positives and negatives (Viguier *et al*., 2009), the CMT- score is a quick, easy and cheap test for pointing out quarters with clinical mastitis (Rasmussen, 2001; Lam *et al*., 2009) and subclinical mastitis (Fouz *et al*., 2004). Test results have a high correlation with composite milk SCC, even more so than electrical conductivity tests (Davis & Reinemann, 2002). However, to identify mastitis-causing microorganisms it is necessary to use culture techniques, considered the gold standard but, labor-intensive and expensive (Viguier *et al*., 2009). Notwithstanding, bacteriological culturing is commonly used as a diagnostic tool to solve mastitis cases (Lam *et al*., 2009) and a key tool in mastitis control programs as it allows to identify the causative agents (Ruegg, 2003).

Mastitis pathogens are typically classified as environmental or contagious organisms (National Mastitis Council, 1987). Historically, the most common contagious mastitis pathogens have been *Streptococcus* spp. and *Staphylococcus aureus* (Barrett *et al*., 2005). However, the adoption of modern milking practices has resulted in a considerable decline in the prevalence of these organisms in many modern US dairy herds (Makovec & Ruegg, 2003). Machine malfunctions, inappropriate milking practices and the presence of carrier cows in the herd, are aspects that contribute to the risk of mastitis (Barrett *et al*., 2005). Although there have been many studies in which the prevalence of mastitis pathogens in dairy herds has been investigated (*e.g.* Ferguson *et al*., 2007; Olde Riekerink *et al*., 2008; Lam *et al*., 2009; Nam *et al*., 2010; Hertl *et al*., 2011; Oliveira *et al.,* 2013), hardly any have been conducted in herds with AMS. However, Hovinen & Pyörälä, (2011) highlighted the importance of proper AMS management, to avoid transmission of infections between cows. The distribution of pathogens isolated from clinical mastitis samples differs between studies and housing or milking systems. In Canada, for example, *S. aureus* is the most frequently isolated bacteria, associated with tie-stall barns, followed by *Escherichia coli* (Olde Riekerink *et al*., 2008; in New York State, *Streptococcus* spp. were the most frequently isolated bacteria in cows milked in herringbone parlors (Gröhn *et al*., 2004). On farms with AMS there is also a relationship between high SCC values and high proportion of cows with dirty teats before milking (Dohmen *et al*., 2010) since pathogens such as *Klebsiella* spp. can be associated with cow and udder hygiene (Munoz *et al*., 2008).

The purpose of this study was to describe the distribution of mastitis pathogens in milk samples collected from dairy herds with AMS in Galicia (NW Spain) and to identify the operational reliability and sensibility of mastitis alerts from AMS.

Material and methods

Study herds and milking protocols

Data for this study were collected from 10 dairy farms with 13 AMS. Three different AMS systems were used in study farms including Lely Astronaut (Lely, Rotterdam, Netherlands), DeLaval VMS (DeLaval, Tumba, Sweden) and Galaxy (Insentec, Marknesse, Netherlands). The mastitis detection systems of these AMS are based on sensors of electrical conductivity, milk yield and colour.

An initial characterization of the herds studied with respect to hygienic and productive variables was carried out. On all farms the cows were kept indoors and they had free access to a total mixed ration and in the AMS gained access to additional concentrate. Table 1 shows the number and types of AMS installed on each farm, the number of cows observed, linear score, days in milk, milk yield per cow and day, hygiene score for udders, thighs and legs and body condition score. Depending on the brand of AMS, the milking routines

Table 1. Characterization of ten dairy herds (852 cows in total) milked with automatic milking systems (AMS) based on mean values of descriptive variables

Herd[1]	No. of AMS	Teat cup disinfection system[2]	Herd size	No. of cows observed[3]	Hygiene score[4]			Body condition score[5]	Milk yield/ cow/day (L)	LS[6]	DIM[7]
					Udder	Thighs	Legs				
A	1	HS	46	44	1.4	1.8	2.2	2.8	29.1	3.9	188
B	1	WW	59	59	1.2	1.8	2.2	2.8	24.0	4.3	210
C1	2	DP	109	83	1.1	1.4	2.0	2.8	35.0	2.6	158
C2	2	DP	99	64	1.2	1.5	1.8	3.0	35.7	2.6	186
D1	1	DP	77	54	1.3	1.5	1.9	3.0	28.1	3.5	210
D2	1	DP	60	49	1.2	1.4	1.8	3.1	29.5	2.8	175
E	1	HS	59	55	1.8	2.3	2.2	3.0	28.6	3.7	191
F1	1	HS	70	40	1.3	1.8	2.3	3.0	30.5	3.0	126
F2	1	HS	71	64	1.5	1.9	2.3	2.9	31.8	3.1	179
G1	1	WW	70	69	1.2	1.5	2.0	3.0	28.4	3.4	122
G2	1	WW	77	47	1.2	1.3	2.2	3.2	27.4	3.8	186
H	1	WW	56	49	1.6	2.0	2.4	3.3	28.0	3.1	215
I	1	WW	56	56	1.7	2.1	2.5	3.2	28.0	3.1	215
J	2	HS	79	72	1.8	2.3	2.3	3.3	34.6	3.4	158
Mean					1.4	1.8	2.1	3.0	28.8	3.0	180

[1]Ten different dairy herds (different letters) but fourteen visits, one to 6 farms and two to the other 4 farms (different subscript). [2]HS: heated steam; WW, warm water; DP, disinfection with peracetic. [3]It is almost the whole herd in each farm because some cows could not be seen due to the cows being in continuous movement in the free stall barn. [4]Range 1 to 4: 1 = completely clean or has very little dirt, 2 = slightly dirty, 3 = mostly covered in dirt, and 4 = completely covered, caked in dirt. [5]Five-points scale, from 1 = thin, to 5 = fat. [6]The linear score is a mathematical way of converting the raw somatic cell count (SCC) based in base 2 log score. [7]DIM, days in milk.

differed. Teats were cleaned with teat dipping cups or rotating brushes prior to milking. Milking cups were automatically attached immediately after and detached following milking and the teats were sprayed with a post-dipping bactericidal product. Teat cups were rinsed with warm water followed by water with or without peracetic acid or water vapour depending on the circumstances after each milking (Table 1).

Mastitis diagnosis and data collection

Fourteen visits were made, one to 6 farms and two to the other 4 farms (Table 1). We visited the farms as close to the date of the monthly Official Milk Recording test (OFMRT) as possible (within 2 to 7 days). This way we could use the monthly test SCC data of each cow to validate other mastitis detection tests used in the study. At the beginning of each visit we collected the report of the milk quality alarms from the AMS mastitis detection software. These alarms, in general, provide alerts per cow and quarter of milk quality based on deviations of conductivity, colour, temperature (Hogeveen *et al*., 2010). With these two reports, we generated a list of all the animals with a possible infection around the time of the visit. In total 228 cows were analysed (912 milk quarters) and 176 were cows marked by AMS sensors with a milk quality problem. We used 52 cows with SCCs from all four quarters of approximately 1 million cells/mL as positive controls.

The CMT was performed on the 228 cows selected and was considered the gold-standard test for mastitis diagnosis. Samples were classified into 5 categories: when the mixture was visually normal (no gel-formation) it was scored as 0, no infection. If the reaction was weak, with traces that dissolved, the score was 1. In these cases we repeated the test to confirm the weak reaction. A weak thickening was scored as 2. A more severe thickening of the mixture but still able to spill by turning the paddle was a 3. And the formation of a gel such that the mixture stuck to the paddle was 4. All tests were performed by the same person. Postpartum cows were tested but the results were not included if less than 5 days had passed after calving because the results obtained from postpartum cows are difficult to interpret (Fouz *et al*., 2010).

Finally, milk samples were aseptically collected for bacteriological study from CMT-positive cows and were analysed at the Animal Health and Production Laboratory of Galicia, Lugo. Milk samples were cultured on Columbia agar plates containing 5% lamb blood, using a disposable sterile loop that seeded approximately 0.01 mL of milk using a laminar flow cabinet. Samples were incubated for 48 h at 37±2ºC and cultures were examined 24 and 48 h after incubation. Bacterial species were identified using biochemical API (Vitek 2, Biomerieux). A sample was considered contaminated if >2 bacterial species were isolated. When we suspected the presence of *S. aureus* the sample was subsequently cultured in selective BD Baird-Parker agar media. If after at least 72 h of incubation no microorganism was observed this sample was classified as having no bacterial growth.

Data analysis

The comparison of the milk quality alerts given by the AMS sensors with the CMT results was assessed using a classification model. If the CMT tested positive and was also classified by the AMS sensors as positive then it was considered a true positive (TP). When the AMS sensors and the CMT were negative the result was considered a true negative (TN). A false positive (FP) classification was a CMT negative quarter classified by the AMS sensors as positive. Finally, a false negative (FN) classification was a CMT positive quarter that was classified by the AMS sensors as negative. Using these four classifications, the detection of mastitis by AMS can be evaluated as follows: firstly the sensitivity as the fraction of CMT positive quarters classified as positive for mastitis by the AMS [Sensitivity (%) = 100 × TP / (TP + FN)]. And secondly, the specificity was defined as the fraction of CMT negative quarters classified as negative for mastitis by the AMS [Specificity (%) = 100 × TN / (TN + FP)]. The relationship between the benefits of AMS sensors (TP) and costs of a detection system (FP) was analyzed by a receiver operation characteristics (ROC) graph or Sensitivity *vs*. (1 – Specificity) plot (Kamphuis *et al*., 2010). The 14 predictions for the herd visits were plotted in the ROC graph. These allowed us to classify the herds based on their prediction methods.

Moreover, the success rate (SR) or predictive positive value was also calculated as SR = TP/(TP + FP). This represents the probability of the AMS correctly identifying a quarter as having mastitis. In addition we calculated the false alert rate as FAR = 1000 × FP/Total cows milked. Descriptive statistics were calculated for all variables.

Differences in sensitivity and prevalence between CMT score groups were contrasted by a one-way ANOVA with a Scheffe mean comparison. For categorical variables, distributions were analyzed using frequency tables. All data were processed using IBM SPSS 19.0.0 for Windows (SPSS, 2008).

Results

Percentage of CMT and AMS positive quarters and AMS sensitivity and specificity

Descriptive statistics for mastitis prevalence and the performance of AMS sensors used in the analysis are listed in Table 2. For 912 quarter milks used in the analysis 322 (35.3%) of them were infected quarters, determined by CMT test. The visual appearance of these 322 infected quarter milks on the CMT test, were different depending on the level of infection or SCC level. The frequency of abnormal milk with a CMT score of 1 was 40.1% with a variation throughout the 14 visits between 0 and 77.8% of infected quarter milk. A CMT score of 2 appeared in 36% of cases ranging from 13.6 to 90%. Variations within visits with a CMT score of 3 were also large with percentages ranging from 0 to 57.9% and a total frequency of 15.8%. The least common CMT score was 4 with a frequency of 7.8%. The prevalence rate of clinical mastitis varied from 0.04 to 0.14 per farm with a total of 0.09 (Table 2).

The AMS classified as positive mastitis cases 210/912 (23.0%) quarters but 36 of these were CMT negative and considered false positives (Table 2). Among the remaining 702 AMS negative quarters,

Table 2. Descriptive statistics of the variables studied for determining the reliability of the quality milk alarms in the automatic milking systems (AMS)

	Farm mean	Min	Max	SD
Cows checked	16.0	10.0	31.0	5.5
CMT-positive (%)	35.3	17.9	47.7	8.5
AMS-positive (%)	23.0	9.7	39.6	9.1
Prevalence of clinical mastitis (%)	9.0	4.0	14.0	3.0
Sensitivity (%)	58.2	18.2	100	20.2
Specificity (%)	94.0	82.6	100	6.2
Positive predictive value	0.86	0.53	1.00	0.13
Negative predictive value	0.80	0.58	1.00	0.10
False alert rate	37.8	0.0	112.9	38.9

Table 3. Sensitivity (Se) of automatic milking systems (AMS) with respect to California mastitis test (CMT) and prevalence (Prev) in different degrees of infection of milk quarters studied.

Descriptive measure	CMT1 (n=129)		CMT2 (n=116)		CMT3 (n=51)		CMT4 (n=25)		CMT total		*p*-value	
	Prev	Se	Prev	Se	Prev	Se	Prev	Se	Prev	Se	Prev	Se
Mean	13.4[b]	40.9	13.2[b]	61.4	5.9[ab]	60.9	3.6[a]	73.2	9	57.9	<0.010	0.104
Minimum	0	0	5.0	16.7	0	0	0	0	0	0		
Maximum	31.0	100	30.0	100	18.0	100	23.0	100	31.0	100		
SD	9.9	25.4	7.7	28.0	5.4	27.9	6.2	42.5	8.5	31.7		

CMT1, the reaction was weak, with traces that dissolved; CMT2, a weak thickening; CMT3, a more severe thickening of the mixture but still spill able by turning the paddle; CMT4, a formation of a gel such that the mixture stuck to the paddle.

16.2% were CMT positive and therefore false negatives. So average sensitivity of the mastitis detection systems of the AMS studied was 58.2% and the specificity was 94%. The positive prediction value (PPV) ranged from 0.53 to 1 with a mean of 0.86, while the negative prediction value (NPV) was of 0.80. The average FAR was 37.8% of the cases (Table 2). The specificity in all cases is greater than 80% but only on three of the visits was the sensitivity above 70%. Most false negative cases (52.7%) are associated with a CMT score of 1 (Fig. 1) however, differences in sensitivity in the CMT 1, 2, 3 and 4 categories were not statistically significant ($p = 0.104$) (Table 3). However, the prevalence values were significantly different between categories of CMT ($p = 0.008$).

Pathogen profile

The total samples analyzed were 181. The percentage of quarters with specific mastitis pathogens identified was 31.5% (57/181) Pathogens were classified as environmental (18%) and contagious (13.8%) of which 10.5% were secondary contagious pathogens (Table 4). However, the most prevalent pathogen isolated, *Streptococcus dysgalactiae* (28.1%), was contagious. *Streptococcus uberis* was found in 26.3% of samples with isolated pathogens (8.3% of all samples) followed by *S. aureus* (10.5%). No bacteria were isolated in 30.9% of samples and 18.8 % of them were considered to be contaminated (Table 4).

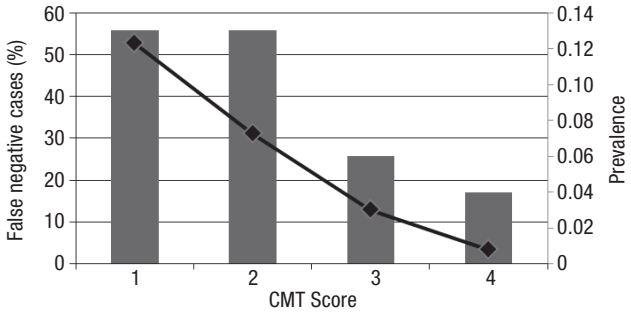

Figure 1. Prevalence (■) and false negative cases (–♦–) depending on CMT-score of abnormal milk.

Table 4. Distribution of mastitis pathogens in 181 milk samples of ten dairy farms with automatic milking systems

Pathogens isolated	% of Samples	% of Isolates
Main contagious pathogens[1]	3.3	10.5
Staphylococcus aureus	3.3	10.5
Secondary contagious pathogens	10.5	33.4
Streptococcus dysgalactiae	8.8	28.1
Staphylococcus chromogenes	1.1	3.5
Staphylococcus epidermidis	0.6	1.8
Environmental pathogens	18.0	56.4
Streptococcus uberis	8.3	26.3
Streptococcus bovis I	2.2	7.0
Prototheca zopfii	1.7	5.3
Klebsiella pneumoniae ssp.	1.7	5.3
Lactococcus garvieae	1.1	3.5
Arcanobacterium pyogenes	0.6	1.8
Enterococcus ssp.	0.6	1.8
Morganella morganii	0.6	1.8
Escherichia coli	0.6	1.8
Enterococcus faecium	0.6	1.8
No bacterial growth[2]	30.9	–
Contamination[3]	18.8	–
Culture-negative[4]	18.8	–

[1]*Staphylococcus aureus* and *Streptococcus agalactiae* are traditionally considered to be classical contagious while another contagious pathogens can be considered as minor mastitis pathogens (Pitkälä *et al*., 2004). [2]No bacterial growth after at least 72 h of incubation. [3]If there were more of two organisms on the sample. [4]Specific culture for detecting *S. aureus*. These analyses were made on those farms or individual animals where the farmers told us their experience about clinical history.

Discussion

Mastitis control with AMS

There are few studies estimating the prevalence of mastitis at milk quarters level instead of cow level. The prevalence of quarters with mastitis on the present work with AMS was 9%, lower than that in Dimitar & Metodija (2012) study, where prevalence was also analysed at milk quarter level (15%). Although in terms of mastitis detection, the minimum recommendations are a sensitivity of 80% and a specificity of 99% (Hogeveen *et al*., 2010), our AMS mastitis detection system did not reach these figures and our results do not agree with other researchers who reported higher values for specificity and sensitivity in both conventional milking systems (using single quarter samples) (Lam *et al*., 2009) and in AMS (Steeneveld *et al*., 2010a). However, these minimum levels for specificity and sensitivity are still under discussion (Hogeveen *et al*., 2010). Results suggest that the performance of mastitis detection systems is similar to other regions with the same milking system (Steeneveld *et al*., 2010b) where these authors claim that a sensitivity of 43% and an specificity of 97% are the clinical mastitis detection characteristics of current AMSs, like it was found from visual inspections of all milk samples obtained during three days on three Dutch commercial dairy farms (Mollenhorst & Hogeveen, 2008). Some authors consider that it is impossible for AMS systems to have a sensitivity of 100% (Kamphuis *et al*., 2010). An increase in the SR and a decrease in the FAR were confirmed when in-line SCC information was added to a detection model of AMS using electrical conductivity information (Kamphuis *et al*., 2008). According to our study half of the false negative cases were in milk quarters classified with the lowest CMT. This could mean that either lowest levels of infection are difficult to detect by the sensors installed in the AMS, or that there may not have been a real infection and that the error was in our interpretation of the CMT test. CMT interpretation can be subjective (Polat *et al*., 2010), and this might result in false positives and negatives (Viguier *et al*., 2009) when not being executed correctly (Lam *et al*., 2009). Some authors argue that quarters with low CMT-scores in foremilk probably do not have clinical mastitis (Rasmussen, 2001) or they could be subclinical cases. However, more severe mastitis cases are less frequent but easier to detect by the AMS sensors. If the reaction was weak, with "traces" that dissolved (scored as 1) even we had doubts about a possible mastitis because they could be cows with high days in milk (DIM) with a high cell count which can react with the CMT due to cell flaking (Fouz *et al*., 2004).

Pathogen profile

The contaminated samples together with the negative results and the ones in which no microorganisms were isolated represent more than half of the samples. This high percentage highlights the importance of a proper procedure when taking samples. These data coincide with that shown in a study, which also took place in Galicia but, in dairies with conventional milking systems (Cundins *et al*., 2010). However, the percentage of contaminated samples was higher than that of Olde Riekerink *et al*. (2008) study. Similar data were shown, with respect to the lack of bacterial growth in CMT-positive milk, in Makovek & Ruegg (2003) and Oliveira *et al*. (2013). The high rate of samples without results or contaminated samples was caused by the difficulty in sampling, due to the situation in which it is performed. To effectively use bacteriological culturing as a diagnostic tool, milk samples have to be collected from the correct cows and quarters at the correct point in time (Lam *et al*., 2009). In other cases it is possible that the time needed for bacterial growth was higher than the time allowed in the present study. It can also occur that the culture media used was not suitable for a specific pathogen as shown for *S. aureus* (Fouz *et al*., 2004).

S. aureus can be the pathogen with the greatest incidence in mastitis cases (Olde Riekerink *et al*., 2008), nonetheless, in our study it was found in only 3.3% of total samples. In many other studies the most common mastitis-causing agents were the coagulase-negative staphylococci (Ferguson *et al*., 2007; Nam *et al*., 2010; Schwarz *et al*., 2010). Although environmental mastitis pathogens were the most common causative agents, it was *S. dysgalactiae* which was the most particular pathogen in this study, being a contagious pathogen. Transfer of bacteria by the AMS pre-milking teat-cleaning device was suspected to be one cause for increased infections (Hovinen & Pyörälä, 2011). However, in this study the level of hygiene may be considered adequate since the percentage of pathogens related to udder hygiene such as *Klebsiella* spp. and *E. coli* was very low. In fact one Finnish study using AMS had a greater prevalence of these pathogens compared to the present study (Hovinen *et al*., 2005). Moreover *S. uberis* had a relatively high prevalence in our study and these bacteria can be considered as being both a contagious and an environmental agent.

In conclusion, dairy farms with AMS in this study had a similar prevalence of mastitis and pathogen profile as farms with conventional milking systems. The majority of the bacteria isolated from these herds were environmental pathogens and special attention needs to be placed on the prevention and control of environmental and contagious mastitis pathogens as all of the

cows are milked with the same machine and with AMS, milk cups are not disinfected between cows. Results suggest that the performance of an AMS mastitis detection system was similar to that in other regions with the same milking system, and lower than the CMT. More severe mastitis cases are less common but easier to detect with AMS mastitis detection systems.

Acknowledgements

The authors are grateful to the dairy farmers who facilitated access to their herds and data.

References

Barrett DJ, Doherty ML, Healy AM, 2005. A descriptive epidemiological study of mastitis in 12 Irish dairy herds. Irish Vet J 58: 31-35. http://dx.doi.org/10.1186/2046-0481-58-1-31

Cundíns A, Hernádez M, Castro A, Pereira JM, 2010. Parámetros de funcionamiento en 67 instalaciones de ordeño de la zona norte de Lugo y su relación con el estado sanitario de los rebaños. I Jornadas Técnicas sobre Calidad de Leche, Ribadeo (Spain), Oct 22-23, pp: 64-72.

Davis A, Reinemann DJ, 2002. Milking performance and udder health of cows milked robotically and conventionally. ASAE An. Int. Meeting. Chicago, ILL, USA, July 28-31, No 02-3112.

Dimitar N, Metodija T, 2012. Udder quarter risk factors associated with prevalence bovine clinical mastitis. Mac Vet Rev 35: 55-64.

Dohmen W, Neijenhuis F, Hogeveen H, 2010. Relationship between udder health and hygiene on farms with an automatic milking system. J Dairy Sci 93: 4019-4033. http://dx.doi.org/10.3168/jds.2009-3028

Ferguson JD, Azzaro G, Gambina M, Licitra G, 2007. Prevalence of mastitis pathogens in Ragusa, Sicily, from 2000 to 2006. J Dairy Sci 90: 5798-5813. http://dx.doi.org/10.3168/jds.2006-903

Fouz R, Corrales JR, Fernández G, Yus E, 2004. Manual: Programa de mellora da calidade do leite: control das mamites bovinas. Instituto de Investigación e Análises Alimentarias. Unidad de Epidemiología y Sanidad Animal, Lugo. ISBN: 84-609-2116-6.

Fouz R, Yus E, Sanjuán ML, Diéguez FJ, 2010. Statistical evaluation of somatic cell counts in bovine milk at calving, during lactation and at drying-off (by oficial recording). Livest Sci 128: 185-188. http://dx.doi.org/10.1016/j.livsci.2009.10.010

Fröhling A, Wienke M, Meierhöfer SR, Schlüter O, 2010. Improved method for mastitis detection and evaluation of disinfectant efficiency during milking process. Food Bioprocess Tech 3: 892-900. http://dx.doi.org/10.1007/s11947-010-0366-9

Gröhn YT, Wilson DJ, González RN, Hertl JA, Schulte H, Bennett G, Schukken YH, 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. J Dairy Sci 87: 3358-3374. http://dx.doi.org/10.3168/jds.S0022-0302(04)73472-4

Hertl JA, Schukken YH, Bar D, Bennett GJ, González RN, Rauch BJ, Welcome FL, Tauer LW, Gröhn YT, 2011. The effect of recurrent episodes of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on mortality and culling in Holstein dairy cows. J Dairy Sci 94: 4863-4877. http://dx.doi.org/10.3168/jds.2010-4000

Hogeveen H, Kamphuis C. Steeneveld W, Mollenhors H, 2010. Sensors and clinical mastitis. The quest for the perfect alert. Sensors 10: 7991-8009. http://dx.doi.org/10.3390/s100907991

Hovinen M, Aisla AM, Pyörälä S, 2005. Visual detection of technical success and effectiveness of teat cleaning in two automatic milking systems. J Dairy Sci 88: 3354-3362. http://dx.doi.org/10.3168/jds.S0022-0302(05)73019-8

Hovinen M, Pyörälä S, 2011. Invited review: Udder health of dairy cows in automatic milking. J Dairy Sci 94: 547-562. http://dx.doi.org/10.3168/jds.2010-3556

Kamphuis C, Sherlock R, Jago J, Mein G, Hogeveen H, 2008. Automatic detection of clinical mastitis is improved by in-line monitoring of somatic cell count. J Dairy Sci 91: 4560-4570. http://dx.doi.org/10.3168/jds.2008-1160

Kamphuis C, Mollenhorst H, Heesterbeek JAP, Hogeveen H, 2010. Detection of clinical mastitis with sensor data from automatic milking systems is improved by using decision-tree induction. J Dairy Sci 93: 3616-3627. http://dx.doi.org/10.3168/jds.2010-3228

Kamphuis C, Dela Rue B, Mein G, Jago J, 2013. Development of protocols to evaluate in-line mastitis-detection systems. J Dairy Sci 96: 4047-4058. http://dx.doi.org/10.3168/jds.2012-6190

Lam TJGM, Olde Riekerink RGM, Sampimon OC, Smith H, 2009. Mastitis diagnostics and performance monitoring: a practical approach. Irish Vet J 62: 34-39. http://dx.doi.org/10.1186/2046-0481-62-S4-S34

Makovec JA, Ruegg PL, 2003. Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. J Dairy Sci 86: 3466-3472. http://dx.doi.org/10.3168/jds.S0022-0302(03)73951-4

Mollenhorst H, Hogeveen H, 2008. Detection of changes in homogeneity of milk: Internal report. Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrech University, Utrech, The Netherlands.

Mollenhorst H, Rijkaart LJ, Hogeveen H, 2012. Mastitis alert preferences of farmers milking with automatic milking systems. J Dairy Sci 95: 2523-2530. http://dx.doi.org/10.3168/jds.2011-4993

Munoz MA, Bennett GJ, Ahlström C, Griffiths HM, Schukken YH, Zadoks RN, 2008. Cleanliness scores as indicator of *Klebsiella* exposure in dairy cows. J Dairy Sci 91: 3908-3916. http://dx.doi.org/10.3168/jds.2008-1090

Nam HM, Kim JM, Lim SK, Jang KC, Jung SC, 2010. Infectious aetiologies of mastitis on Korean dairy farms during

2008. Res Vet Sci 88: 372-374. http://dx.doi.org/10.1016/j.rvsc.2009.12.008

National Mastitis Council, 1987. Reasons for negative culture results. http://www.nmconline.org. [10 October 2014].

Olde Riekerink RGM, Barkema HW, Kelton DF, Scholl DT, 2008. Incidence rate of clinical mastitis on Canadian dairy farms. J Dairy Sci 91: 1366-1377. http://dx.doi.org/10.3168/jds.2007-0757

Oliveira L, Hulland C, Ruegg PL, 2013. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. J Dairy Sci 96: 7538-7549. http://dx.doi.org/10.3168/jds.2012-6078

Pitkälä A, Haveri M, Pyörälä S, Myllys V, Honkanen-Buzalski T, 2004. Bovine mastitis in Finland 2001. Prevalence, distribution of bacteria, and antimicrobial resistance. J Dairy Sci 87: 2433-2441. http://dx.doi.org/10.3168/jds.S0022-0302(04)73366-4

Polat B, Colak A, Cengiz M, Yanmaz LE, Oral H, Bastan A, Kaya S, Hayirli A, 2010. Sensitivity and specificity of infrared thermography in detection of subclinical mastitis in dairy cows. J Dairy Sci 93: 3525-3532. http://dx.doi.org/10.3168/jds.2009-2807

Rasmussen MD, 2001. Automatic milking. How to define a threshold for dumping mastitic milk? Proc. 2nd Int. Symp. on Mastitis and Milk Quality, AABP & NMC, pp: 401-404.

Ruegg, PL, 2003. Investigation of mastitis problems on farms. Vet Clin North Am Food Anim Pract 19: 47-73. http://dx.doi.org/10.1016/S0749-0720(02)00078-6

Schwarz D, Diesterbeck US, Failing K, König S, Brügemann K, Zschöck M, Wolter W, Czerny CP, 2010. Somatic cell counts and bacteriological status in quarter foremilk samples of cows in Hesse, Germany-A longitudinal study. J Dairy Sci 93: 5716-5728. http://dx.doi.org/10.3168/jds.2010-3223

Steeneveld W, van der Gaag LC, Ouweltjes W, Mollenhorst H, Hogeveen H, 2010a. Discriminating between true-positive and false-positive clinical mastitis alerts from automatic milking system. J Dairy Sci 93: 2559-2568. http://dx.doi.org/10.3168/jds.2009-3020

Steeneveld W, van der Gaag LC, Ouweltjes W, Mollenhorst H, Hogeveen H, 2010b. Simplify the interpretation of alert lists for clinical mastitis in automatic milking systems. Comput Electron Agric 71: 50-56. http://dx.doi.org/10.1016/j.compag.2009.12.011

Sun Z, Samarasinghe S, Jago J, 2010. Detection of mastitis and its stage of progression by automatic milking systems using artificial neural networks. J Dairy Res 77: 168-175. http://dx.doi.org/10.1017/S0022029909990550

Viguier C, Arora S, Gilmartin N, Welbeck K, O'Kennedy R, 2009. Mastitis detection: current trends and future perspectives. Trends Biotechnol 27: 486-493. http://dx.doi.org/10.1016/j.tibtech.2009.05.004