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Prevalence of bovine milk pathogens in Azorean pastures: mobile versus fixed milking machines

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ABSTRACT

The aims of the present study were (1) to evaluate the influence of using mobile (n=47) or fixed (n=45) milking machines in Azorean herds on the apparent prevalence of several milk pathogens in bulk tank milk (BTM) and (2) to determine whether separated subclinical mastitic cows can serve, in real time, as predictors of milk pathogen prevalence for the remaining animals at the herd level. The use of a mobile or fixed milking machine influenced ($P \leq 0.05$) the prevalence of *Staphylococcus aureus* (72.3 per cent; n=34 v 51.1 per cent; n=23, respectively) and *Klebsiella* species (46.8 per cent; n=22 v 26.7 per cent; n=12, respectively). *S aureus* (95 per cent CI OR 1.1 to 6.0) and *Klebsiella* species (95 per cent CI OR 1.0 to 5.8) were 2.5 times more likely to increase in the BTM of herds using mobile milking machines. The prevalence of coagulase-negative staphylococci (100 per cent; n=92), *Escherichia coli* (75.0 per cent), *Corynebacterium bovis* (57.6 per cent), *Enterococcus* species (55.4 per cent), *Streptococcus dysgalactiae* (51.1 per cent), *Streptococcus uberis* (41.3 per cent), *Actinomyces pyogenes* or *Peptostreptococcus indolicus* (41.3 per cent) and *Streptococcus agalactiae* (32.6 per cent) in BTM remained similar among the herds. κ coefficients were always <0.70, indicating intra-herd disagreement of the prevalence of milk pathogens between BTM and separated milking cows. Milking hygiene should be improved in pastures, focusing specifically on herds that use a mobile milking machine. The segregated cows at milking time are not good predictors of milk pathogens in BTM.

INTRODUCTION

Microbiological milk quality on herd bulk tanks is primarily dependent on the subclinical mastitic milk produced by infected cows, which remains a major problem in dairy herds that use different production systems (Piepers 2014), and on bacterial contamination during milking procedures (Berry and others 2006, Bava and others 2011).

In many dairy herds on San Miguel Island (Azores), mobile milking machines and mobile bulk tank milk (BTM) were largely used by farmers to support the transhumance of dairy cows.

Cow and stall hygiene, milking procedures, the cleaning of milk handling equipment and milking machine maintenance all represent significant risk factors of pathogen presence and bacterial increment counts in BTM (Elmoslemany and others 2009, 2010).

Segregation practices (Wilson and others 1995), or milking at the end, were well implemented in Azorean herds as well as for cows presenting high individual somatic cell count levels in order to minimise somatic cells presence in BTM, mainly due to economic and legal constraints.

The objectives of the present study were (1) to evaluate the influence of using mobile or fixed milking machines in Azorean herds on the apparent prevalence of several milk pathogens from BTM and (2) to determine whether separated subclinical mastitic cows can serve, in real time, as predictors of milk pathogen prevalence for the remaining animals at the herd level.

MATERIALS AND METHODS

Samples

From all regions of San Miguel-Azores, 92 dairy herds for a total of 5520 adult cows were used (total population=1833 herds and 51,684 females; SDASM 2014). From each one of the eight regions (Table 1), at least 25 per cent of the herds in each region, on dependence of the local Young Farmers' Association (http://www.ajamcja.com/), were preselected for a previous study (Azevedo and others 2016) by a researcher under local veterinarian assessment. From 100 preselected herds, 92 also presented segregated subclinical mastitic cows and were used for the present study.

The authors considered subclinical mastitis in agreement with the selection of segregated cows by farmers due to persistent high

TABLE 1: Number of herds selected in each region of San Miguel Island (25° 30′ west longitude and 37° 50′ north latitude) and on dependence of São Miguel Young Farmers' Association

Region	Herds	
	Total number	Selected
Nordeste	35	9
Povoação	30	8
Vila Franca do Campo	20	6
Lagoa	26	8
Ribeira Grande/Lomba da Maia	39	10
Ponta Delgada	79	17
Arrifes	86	22
Sete Cidades	30	10

individual somatic cell count levels, normally more than one week, without apparent clinical signs, other than the chronic inflammatory consequences, such as alterations of quarter conformation.

Pooled milk samples were collected from all quarters of each one of the segregated cows (average=3) in each farm. Simultaneously, after milking all cows, a sample was collected from the BTM.

Finally, the criteria for inclusion were large herd size (from 20 to 260 lactating cows), farmer permission, the presence of segregated milking cows with subclinical mastitis and use of mobile (n=47; Fig 1) or fixed (n=45) milking machines. A lower mean number of lactating cows was observed in herds using mobile (39.4±4.8; ±SEM) than fixed (70.0±4.7; P≤0.001) milking machines.

All final samples were collected using the Startcheck sampling kit (HIPRA, Spain) to test the presence of coagulase-negative staphylococci (CNS), *Escherichia coli*, *Staphylococcus aureus*, *Corynebacterium bovis*, *Enterococcus* species, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Actinomyces pyogenes/Peptostreptococcus indolicus*, *Klebsiella* species, *Streptococcus agalactiae* and *Serratia marcescens*. A total of 250 µl of milk were taken from each tank or pool samples, immediately after milking, and transferred on to a fast technology for analysis (FTA) card that was dried (GE Healthcare, Barcelona, Spain), according to the instructions of the manufacturer, and posted to Diagnos Laboratory (HIPRA).

DNA extraction and (qPCR) amplification

DNA was extracted and amplified using the PathoProof Mastitis Complete-12 Kit (Thermo Fisher Scientific, Massachusetts, USA) as described by Koskinen and others (2009). All procedures concerning the collection and preparation of samples, DNA extraction, and PCR and qPCR amplification for this study were reported by Azevedo and others (2016).

For each milk pathogen, a positive qPCR result, that is, the presence of bacteria in the milk samples, was recorded when the cycle threshold value was ≤37.

Statistical analysis

Descriptive statistics were used for the apparent prevalence of milk bacteria and 95 per cent CI determination.

The effect of herds using fixed or mobile milk machines on the presence or absence of milk bacteria was tested using univariate logistic regression models. For likelihood ratio tests at the 0.05 level, the respective ORs were also calculated.

Considering all herds, a Spearman correlation considering the apparent prevalence of each milk pathogen between milk from segregated cows and BTM was used.

Fig 1: Picture of milking practice in herds using mobile milking machines and mobile tank milk at San Miguel- Azores

The κ coefficient value and the McNemar-Bowker test were used to determine the intra-herd agreement of the apparent prevalence of milk pathogens between BTM and pooled samples from segregated mastitic cows.

All statistical analyses were performed using SAS (SAS Institute Inc, 2007. JMP User's Guide. Version 7.0. SAS Institute Inc: Cary, NC, USA).

RESULTS

Prevalence of milk pathogens

CNS were observed in 100 per cent of samples and only the prevalence of *S aureus* or *Klebsiella* species in milk sampled from bulk tanks was influenced (P≤0.05) by milking machines types in Azorean herds. In fact, *S aureus* (95 per cent CI OR 1.1 to 6.0) and *Klebsiella* species (95 per cent CI OR 1.0 to 5.8) were 2.5 times more likely (P<0.05) to increase in the BTM of herds using mobile milking machines. The prevalence of all other milk pathogens remaining similar between herds is described in Table 2.

Agreement of milk pathogens between bulk tank and mastitic segregated cows

Overall, a high Spearman correlation of apparent prevalence from each pathogen across herds was observed between milk from segregated cows and BTM (r=0.90; n=11; P≤0.005).

The agreement between intra-herd apparent prevalences (Table 3) was low (κ coefficient <0.70) or not significant.

DISCUSSION

Although probably the milk dilution of pooled and milk samples and their potential contamination during collect can affect the sensibility and specificity of the PCR technique, there are several studies indicating their advantages regarding the classical bacterial cultures methods (Taponen and others 2009, Katholm and others 2012, Mahmmod and others 2013a,b, Zanardi and others 2014), notwithstanding different Ct-value cut-offs can change the mastitis infection definition (Cederlöf and others 2012). Reyher and Dohoo (2011) estimate the sensitivity and specificity of bacterial cultures techniques using composite milk samples, that is, from cow's four mammary quarters, for several milk pathogens or group pathogens (CNS, *S aureus*, *E coli*, *S dysgalactiae*, *S uberis*, *Enterococcus* species and *Corynebacterium* species). In general, these researchers observed that the composite samples have very high specificity (≥86.7) but not high sensitivity, which ranged from 24.5 (*Corynebacterium* species) to 77.1 (*S aureus*). Probably an agreement in the same direction can also observed using BTM samples at level herd. On the other hand, the sampling technique effect on PCR-based

TABLE 2: Apparent prevalence (95% CI) of milk pathogens in Azorean herds using a mobile or fixed milking machine

	Herds with mobile v fixed milking machine		
	Prevalence (95% CI)		
Bacteria species	**BTM – mobile milking machine**	**BTM – fixed milking machine**	**P value***
Coagulase-negative staphylococci	100.0%; n=47	100.0%; n=45	–
Escherichia coli	72.3%; n=34 (58.2–83.1)	77.8%; n=35 (63.7–87.5)	NS
Staphylococcus aureus	**72.3%; n=34 (58.2–83.1)**	**51.1%; n=23 (37.0–65.0)**	**0.04**
Corynebacterium bovis	59.6%; n=28 (45.3–72.4)	55.6%; n=25 (41.2–69.1)	NS
Enterococcus species	48.9%; n=23 (35.3–62.8)	62.8%; n=28 (47.6–74.9)	NS
Streptococcus dysgalactiae	57.5%; n=27 (43.3–70.5)	44.4%; n=20 (30.9–58.8)	NS
Streptococcus uberis	38.3%; n=18 (25.8–52.6)	44.4%; n=20 (30.9–58.8)	NS
Actinomyces pyogenes/Peptostreptococcus indolicus	36.2%; n=17 (24.0–50.5)	46.7%; n=21 (32.9–60.9)	NS
Klebsiella species	**46.8%; n=22 (33.3–60.8)**	**26.7%; n=12 (16.0–41.0)**	**0.05**
Streptococcus agalactiae	36.2%; n=17 (24.0–50.5)	28.9%; n=13 (17.7–43.4)	NS
Serratia marcescens	6.4%; n=3	n = 0	–

Diferences between prevalence values in bold are statistically significant for the same line.
Coagulase-negative staphylococci: *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*, *Staphylococcus simulans*, *Staphylococcus warneri* and *Staphylococcus xylosus*
Klebsiella species: *Klebsiella oxytoca* and *Klebsiella pneumoniae*
*Likelihood ratio test (1 d.f.) for logistic regression
BTM, bulk tank milk; NS, not significant

TABLE 3: Agreement (κ coefficient) between the bulk tank milk and pooled samples from mastitic cows

	BTM v milk pools from segregated cows of all herds			
	Prevalence (95% CI)			McNemar-Bowker
Bacteria species	BTM	Milk pools	κ coefficient	test
Coagulase-negative staphylococci	100.0%; n=92	100.0%; n=92	–	–
Escherichia coli	75.0%; n=69 (65.3–82.7)	73.9%; n=66 (64.1–81.8)	0.51 ± 0.10	0.81
Staphylococcus aureus	**62.0%; n=57 (51.8–71.2)**	**45.7%; n=42 (35.9–55.8)**	**0.51 ± 0.08**	**0.002**
Corynebacterium bovis	57.6%; n=53 (47.4–67.2)	50.0%; n=46 (40.0–60.0)	0.24 ± 0.10	0.24
Enterococcus species	**55.4%; n=51 (45.3–65.2)**	**42.4%; n=39 (32.8–52.6)**	**0.19 ± 0.10**	**0.05**
Streptococcus dysgalactiae	**51.1%; n=47 (41.0–61.1)**	**27.2%; n=25 (19.1–37.0)**	**0.27 ± 0.09**	**<0.001**
Streptococcus uberis	41.3%; n=38 (31.8–51.5)	48.9%; n=45 (39.0–59.0)	0.30 ± 0.10	0.22
Actinomyces pyogenes/Peptostreptococcus indolicus	**41.3%;n=38 (31.8–51.5)**	**29.3%; n=27 (21.0–39.3)**	**0.27 ± 0.10**	**0.05**
Klebsiella species	**37.0%; n=34 (27.8–47.2)**	**21.7%; n=20 (14.5–31.2)**	**0.29 ± 0.10**	**0.008**
Streptococcus agalactiae	**32.6%; n=30 (23.9–42.7)**	**20.7%; n=19 (13.6–30.0)**	**0.26 ± 0.11**	**0.03**
Serratia marcescens	3.3%; n=3 (1.1–9.6)	n = 0	–	–

K coeficiente values in bold are statistically significant
Coagulase-negative staphylococci: *Staphylococcus chromogenes, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus saprophyticus, Staphylococcus simulans, Staphylococcus warneri* and *Staphylococcus xylosus*
Klebsiella species: *Klebsiella oxytoca* and *Klebsiella pneumoniae*
BTM, bulk tank milk

bacteriological results was well evidenced regarding the bacterial contamination in teat or extra-mammary sites (Hiitiö and others 2016) mainly for non-obligate intramammary pathogens resulting in false-positive intramammary infections. Consequently, the interpretation of the PCR results tests alone from BTM and composite milk should be done with caution and other records such as somatic cell counts and clinical observations are important tools for intramammary infection diagnosis.

CNS were observed in all samples of this study, which is in agreement with Danish dairy herds (Katholm and others 2012). In the last decade, these minor pathogens have been considered by some as emerging pathogens (Pyörälä and Taponem 2009).

S aureus is a major agent of contagious bovine mastitis associated with high economic losses (Hogeveen and others 2011), and the study's findings (up to 72.3 per cent) are in agreement with the prevalence estimates ranging from 31 per cent to 100 per cent in Europe and North America (Richard and others 2006), the cumulative prevalence of 74 per cent in Prince Edward Island, Canada (Riekerink and others 2006), and the prevalence of 57 per cent in New Zealand (Howard 2006). The transmission of this milk-contagious pathogen between cows is very well studied and many tasks related with milking process represent significant risk factors. In fact, *S aureus* can be isolated from several utensils used during milking as well as from animal body surfaces and the hands of the milking operator (Benić and others 2012). In a recent study (Azevedo and others 2016), the authors observed, using a six-point scale, that the poor hygiene during milking is an important risk factor for Azorean herds. Unfortunately, the milking machine maintenance and their vacuum levels and even the teat's condition of dairy cows were not assessed, and further studies were needed in order to evaluate the impact of these factors in the incidence of *S aureus*.

The prevalence of *Klebsiella* species (up to 46.8 per cent) observed in this study was higher than the 13 per cent observed by Katholm and others (2012), who also used a PCR technique. However, milk contamination with *Klebsiella* species occurs from numerous sources, including sampling techniques (Zadoks and others 2011), and care must be taken in comparing the prevalence values of different studies. These environmental bacteria are normally shed only for a short time by cows with clinical mastitis, and their presence in BTM is suggestive of poor stall management, udder hygiene and milking practices, resulting in the contamination of milk (Hogan and Smith 2012).

The higher likelihood of *S aureus* or *Klebsiella* species observed in BTM from herds with mobile than fixed machines can be probably attributed to some poor milking practices. In another study, the authors observed

that the absence of practices concerning the gloves used during milking procedures and the cleaning of the milking machine with hot water were significant risk factors for coliform presence, including *Klebsiella* species in milk from BTM (Azevedo and others 2016). These practices should be improved, especially in Azorean herds using mobile machines.

Concerning the other major contagious mastitis pathogen, the authors found that the prevalence of *S agalactiae* in the present study is higher than the prevalence found in Denmark, which ranged between 3 per cent and 7 per cent (Rynasek and others 2009, Katholm and others 2012), and the 7.7 per cent prevalence found in Québec (Francoz and others 2012), but this result is similar to the 26 per cent of positive BTM samples found in Australian herds (Phuektes and others 2003). Being an obligate intramammary pathogen, the bovine udder is recognised as the only reasonable source of *S agalactiae*, and consequently, the specificity in BTM approaches 100 per cent (Keefe 2012, De Carvalho and others 2015).

In countries that have had long-term control plans for the control and eradication of *S agalactiae*, prevalence has declined, but in emerging dairy industries without effective milk control plans prevalence remains high (Francoz and others 2012, Keefe 2012). Efforts should be undertaken to achieve the goal of control and eradication.

The prevalence of *S dysgalactiae* observed in the Azorean BTM samples is a much lower result compared with the 86 per cent apparent prevalence found by Katholm and others (2012), but this result is very similar to the 55 per cent found by Phuektes and others (2003) in the BTM of 42 herds in Australia. The higher prevalence of this environmental bacteria might be related to the limited use of dry cow therapy in Danish herds due to restrictive legislation concerning the use of antimicrobials without microbiological diagnosis (Danish Veterinary and Food Administration 2010). The prevalence found in both these studies for *S uberis* (95 per cent and 83 per cent, respectively) was also much higher than the apparent prevalence found in this study for this mastitis pathogen.

In the same way, the apparent *Enterococcus* species prevalence reported in this study is much lower than the prevalence (78 per cent) reported by Katholm and others (2012).

The presence of environmental *Streptococcus* species has been exacerbated due to the increasing implementation of control strategies against contagious pathogens such as *S aureus*. These programmes have reduced the incidence of contagious mastitis, but they have had low impacts on mastitis caused by *Streptococcus* species (Ruegg 2012). Moreover, the dairy environment is a determinant factor for mastitis development due to *Streptococcus* species, and stabled dairies are at greater risk than those held in open pastures (NMC 1999).

Minor pathogens such as *C bovis* and *A pyogenes* or *P indolicus* were found in the BTM samples were much lower values than the 90 per cent and 63 per cent positive samples found in Danish farms (Katholm and others 2012). In fact, in herds using dry off therapy, a common practice in countries without antibiotic use restrictions, such as Portugal, levels of infection with minor pathogens such as *Corynebacterium* species are low (Honkanen-Buzalski and others 1984).

The high Spearman correlation of apparent prevalence from each pathogen across herds observed between milk from segregated cows and BTM suggested that mastitic cows in herds are in fact the reservoir of bacteria in BTM. However, the low κ coefficients observed in the present study indicated that separated mastitis cows cannot serve as predictors of milk pathogen prevalence for the remaining animals at the herd level.

The authors concluded that the likelihood of finding *S aureus* or *Klebsiella* species in BTM was higher in herds using mobile milking machines. The milk pathogens of segregated cows with subclinical mastitis at milking time are not good predictors for BTM at the herd level.

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Contributors Substantial contributions to the conception or design of the work: CA, DP and LS. Acquisition of data and milk samples: LS and MM. Laboratory milk samples analysis (responsibility): JM and RG. Statistical analysis: JS. Drafting and interpretation of data for the work: CA, JS, DP, MMand LS. Final approval of the version to be published: all authors.

Competing interests CA (Technical and Marketing Manager Ruminants for Portugal), RG (Corporate Marketing Manager Mastitis and Small Ruminants Vaccines) and JM (DIAGNOS Manager) are employed by HIPRA SA Laboratorios - Amer (Girona), Spain. These authors declare that this professional relationship did not affect the design, sample collection and analysis, results, discussion or conclusions of the present study.

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Data sharing statement Blaz gene was also evaluated using the PathoProof Mastitis Complete-12 Kit (Thermo Fisher Scientific, Massachusetts, USA) and may be available at HIPRA for researchers who wish to deeply confirm the genotypic characterization of antimicrobial resistance in milk pathogens from Azorean dairy herds.

REFERENCES

Azevedo C., Pacheco D., Soares L., Romão R., Moitoso M., Maldonado J., Guix R., Simões J. (2016). Prevalence of contagious and environmental mastitis-causing bacteria in bulk tank milk and its relationships with milking practices of dairy cattle herds in São Miguel Island (Azores). *Tropical Animal Health and Production* 48, 451–459

Bava L., Zucali M., Sandrucci A., Brasca M., Vanoni L., Zanini L., Tamburini A. (2011) Effect of cleaning procedure and hygienic condition of milking equipment on bacterial count of bulk tank milk. *Journal of Dairy Research* 78, 211–219

Benić M., Habrun B., Kompes G. (2012) Clinical and epidemiological aspects of cow mastitis caused by *Staphylococcus aureus* and its methicillin-resistant strains. *Rad 511- Medical Sciences* 37, 113–122.

Berry D. P., O'brien B., O'callaghan E. J., Sullivan K. O., Meaney W. J. (2006) Temporal trends in bulk tank somatic cell count and total bacterial count in Irish dairy herds during the past decade. *Journal of Dairy Science* 89, 4083–4093

Cederlöf S. E., Toft N., Aalbaek B., Klaas I. C. (2012) Latent class analysis of the diagnostic characteristics of PCR and conventional bacteriological culture in diagnosing intramammary infections caused by *Staphylococcus aureus* in dairy cows at dry off. *Acta Veterinaria Scandinavica* 54, 65

DANISH VETERINARY and FOOD ADMINISTRATION (2010) [Bek. nr 785 of 25/06/2010 Notice of the veterinary use and dispensing and prescribing of drugs for animals]. https://www.retsinformation.dk/Forms/R0710.aspx?id=132613. Accessed August 26, 2015

De Carvalho N. L., Gonçalves J. L., Botaro B. G., Silva L. F., Dos Santos M. V. (2015) Detection and enumeration of *Streptococcus agalactiae* from bovine milk samples by real-time polymerase chain reaction. *Current Microbiology* 71, 363–372

Elmoslemany A. M., Keefe G. P., Dohoo I. R., Jayarao B. M. (2009) Risk factors for bacteriological quality of bulk tank milk in Prince Edward Island dairy herds. Part 2: bacteria count-specific risk factors. *Journal of Dairy Science* 92, 2644–2652

Elmoslemany A. M., Keefe G. P., Dohoo I. R., Wichtel J. J., Stryhn H., Dingwell R. T. (2010) The association between bulk tank milk analysis for raw milk quality and on-farm management practices. *Preventive Veterinary Medicine* 95, 32–40

Francoz D., Bergeron L., Nadeau M., Beauchamp G. (2012) Prevalence of contagious mastitis pathogens in bulk tank milk in Québec. *Canadian Veterinary Journal* 53, 1071–1078

Hiitiö H., Simojoki H., Kalmus P., Holopainen J., Pyörälä S., Taponen S. (2016) The effect of sampling technique on PCR-based bacteriological results of bovine milk samples. *Journal of Dairy Science* 99, 6532–6541

Hogan J., Smith K. L. (2012) Managing environmental mastitis. *Veterinary Clinics of North America: Food Animal Practice* 28, 217–224

Hogeveen H., Huijps K., Lam T. J. (2011) Economic aspects of mastitis: new developments. *New Zealand Veterinary Journal* 59, 16–23

Honkanen-Buzalski T., Griffin T. K., Dodd F. H. (1984) Observations on *Corynebacterium bovis* infection of the bovine mammary gland. I. Natural infection. *Journal of Dairy Research* 51, 371–378

Howard P. (2006) Mastitis pathogens present in bulk tank milk from seven dairy herds in the Waikato region, New Zealand. *New Zealand Veterinary Journal* 54, 41–43

Katholm J., Bennedsgaard T. W., Koskinen M. T., Rattenborg E. (2012) Quality of bulk tank milk samples from Danish dairy herds based on real-time polymerase chain reaction identification of mastitis pathogens. *Journal of Dairy Science* 95, 5702–5708

Keefe G. (2012) Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. *Veterinary Clinics of North America: Food Animal Practice* 28, 203–216

Koskinen M. T., Holopainen J., Pyörälä S., Bredbacka P., Pitkälä A., Barkema H. W., Bexiga R., Roberson J., Sølverød L., Piccinini R., Kelton D., Lehmusto H., Niskala S., Salmikivi L. (2009) Analytical specificity and sensitivity of a real-time polymerase chain reaction assay for identification of bovine mastitis pathogens. *Journal of Dairy Science* 92, 952–959

Mahmmod Y. S., Toft N., Katholm J., Grønbæk C., Klaas I. C. (2013a) Bayesian estimation of test characteristics of real-time PCR, bacteriological culture and California mastitis test for diagnosis of intramammary infections with *Staphylococcus aureus* in dairy cattle at routine milk recordings. *Preventive Veterinary Medicine* 112, 309–317

Mahmmod Y. S., Toft N., Katholm J., Grønbæk C., Klaas I. C. (2013b) Estimation of test characteristics of real-time PCR and bacterial culture for diagnosis of subclinical intramammary infections with *Streptococcus agalactiae* in Danish dairy cattle in 2012 using latent class analysis. *Preventive Veterinary Medicine* 109, 264–270

NMC (1999) *Laboratory Handbook on Bovine Mastitis*. Madison, WI: National Mastitis Council

Phuektes P., Browning G. F., Anderson G., Mansell P. D. (2003) Multiplex polymerase chain reaction as a mastitis screening test for *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* in bulk milk samples. *Journal of Dairy Research* 70, 149–155

Piepers S. (2014) Vaccination against mastitis: an overview. *Proceedings of the National Mastitis Council Regional Meeting*. Ghent, Belgium

Pyörälä S., Taponen S. (2009) Coagulase-negative staphylococci-emerging mastitis pathogens. *Veterinary Microbiology* 134, 3–8

Reyher K. K., Dohoo I. R. (2011) Diagnosing intramammary infections: evaluation of composite milk samples to detect intramammary infections. *Journal of Dairy Science* 94, 3387–3396

Richard G. M., Riekerink O., Barkema H., Veenstra S., Poole D., Dingwell R. T., Keefe G. P. (2006) Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island. *Canadian Veterinary Journal* 47, 567–572

Riekerink R. O., Barkema H., Kelton D. F., Keefe G. P., Scholl D. T. (2006) Risk factors for herd-level infection of contagious mastitis pathogens on Canadian dairy farms. *Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, Cairns, Australia,* p. 598

Ruegg P. L. (2012) New perspectives in udder health management. *Veterinary Clinics of North America: Food Animal Practice* 28, 149–163

Rysanek D., Zouharova M., Babak V. (2009) Monitoring major mastitis pathogens at the population level based on examination of bulk tank milk samples. *Journal of Dairy Research* 76, 117–123

SDASM (2014) [São Miguel Agricultural Improvement Services. Azores Regional Administration]. http://www.azores.gov.pt. Acessed August 25, 2015

Taponen S., Salmikivi L., Simojoki H., Koskinen M. T., Pyörälä S. (2009) Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. *Journal of Dairy Science* 92, 2610–2617

Wilson D. J., Gonzalez R. N., Sears P. M. (1995) Segregation or use of separate milking units for cows infected with *Staphylococcus aureus*: effects on prevalence of infection and bulk tank somatic cell count. *Journal of Dairy Science* 78, 2083–2085

Zadoks R. N., Middleton J. R., Mcdougall S., Katholm J., Schukken Y. H. J. (2011) Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. *Journal of Mammary Gland Biology and Neoplasia* 16, 357–372

Zanardi G., Caminiti A., Delle Donne G., Moroni P., Santi A., Galletti G., Tamba M., Bolzoni G., Bertocchi L. (2014) Short communication: comparing real-time PCR and bacteriological cultures for *Streptococcus agalactiae* and *Staphylococcus aureus* in bulk-tank milk samples. *Journal of Dairy Science* 97, 5592–5598

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