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Research article

Risk factors associated with environmental mastitis in clinical cases at small dairies in western Thailand

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Abstract

The risk factors were identified associated with the environmental and non-environmental pathogens that cause bovine clinical mastitis. A cross-sectional study using a questionnaire was conducted from September 2013 to July 2014 to identify the risk factors associated with different types of bacteria. The study area consisted of the regions served by the Kasetsart University Veterinary Teaching Hospital. A questionnaire was given to farmers, and milk was collected from 110 cows in 37 small dairies in western Thailand. The results showed that environmental pathogens were the most frequently isolated type of pathogen (76.36%). The analysis of potential risk factors indicated that unpracticed hand stripping after machine milking (odds ratio (OR) = 0.26; $p < 0.05$) and cleaning the udders of several cows using the same towel (OR = 0.18; $p < 0.05$) were significantly associated with the occurrence of environmental bacterial mastitis. Furthermore, environmental pathogens presented risk factors associated with this milking practice. Farmers and the relevant authorities should apply this information to control the incidence of clinical mastitis.

Introduction

Bovine clinical mastitis mainly refers to inflammation of the mammary glands in dairy cattle and is mostly caused by bacterial infections, with bacterial clinical mastitis resulting in considerable economic loss worldwide and the maximum attributable loss in the USA was USD 403 per cow (Bar et al., 2008). The cost of clinical mastitis during the early lactation period has been reported as USD 444 per case (Rollin et al., 2015). According to the literature, there are no current studies that have reported the estimated economic loss attributable to bovine mastitis in Thailand; however, the development of a mastitis prevention program for small dairies in Thailand can increase farm profits by more than would result from reproductive prevention programs (Hall et al., 2004).

There have been reports of bovine mastitis in several provinces in Thailand. In Nakhon Pathom, a province in western Thailand, the farm prevalence of bacterial clinical mastitis was in the range 0–14.3% (Ajariyakhajorn et al., 2003). Leelahapongsathon et al. (2014) reported that the cow-level prevalence of clinical mastitis in Chiang Mai province in northern Thailand was 5.35%, which included infections by a variety of pathogens. In general, environmental pathogens, such as coliform bacteria, are the most important pathogens causing clinical mastitis (Fairbrother et al., 2015; Morin, 2009; Kampa et al., 2010). In particular, factors such as parity, body condition score, days in milk, the vacuum pressure of the milking machine, use of the teat dipping method, towel usage, dry cow therapy and the season of year all considerably influence the occurrence of mastitis (Green et al., 2007; Jarassaeng et al., 2012; Oliveira et al., 2015). A study in Thailand showed that use of a cloth rag was a risk factor

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for infections by *Streptococcus [Str.] agalactiae* and *Staphylococcus [S.] aureus*, and the infusion of an intramammary drug into a dirty teat was significantly associated with mammary gland infection by environmental pathogens in small dairy herds (Kampa et al., 2010).

Western Thailand is home to an important milk industry and contains nearly 30% of the total milking cow population, with most of these farms belonging to smallholders, with an average of approximately 20 cows per farm (Department of Livestock Development, 2013). Although the farmers are well educated regarding the hygiene practices to be followed while milking and have considerable experience in raising cows, coliform mastitis has still been reported in this area. Making information available to farmers regarding the risk factors that predispose an animal to clinical mastitis and about the groups of bacteria that cause mastitis would enhance the efficiency of veterinary services. Consequently, the aim of this study was to identify the risk factors for clinical mastitis that are specific to the environmental pathogens in the area of the Kasetsart University Veterinary Teaching Hospital (KUVTH) in Kamphaeng Saen and Nong Pho, which serves the dairy farms located in Nakhon Pathom, Ratchaburi and Kanchanaburi provinces in western Thailand.

Materials and Methods

Study design

A cross-sectional study using a questionnaire was conducted from September 2013 to July 2014 to identify the risk factors for clinical mastitis. The study was conducted in regions covered by the KUVTH in Kamphaeng Saen and Nong Pho, both in Nakhon Pathom province (Fig. 1). The sample size was calculated using the one-proportion formula in EpiTools (Ausvetplan, 2013) and based on the following inputs: 5% of the estimated true proportion, 95% confidence interval, 5% for the desired precision,and 11,525 for the population size in the study area (Department of Livestock Development, 2013). At least 73 cows were required for this study. However, by the end of the study, 110 questionnaires and milk samples were collected for data analysis.

The questionnaire used in this study was reviewed by two epidemiologists with expertise in the field of dairy diseases. Ten questionnaires were tested on farmers in the study area but who did not take part in the final survey. The approved questionnaire was then discussed and explained to the veterinarians from the KUVTH in Kamphaeng Saen and Nong Pho before starting data collection. The questionnaire comprised both open-ended and closed-ended questions that covered topics regarding farm management, milking practices and possible factors that associated with clinical mastitis.

Data and sample collection

Data were collected when a farmer had a cow or cows with clinical mastitis and called the KUVTH in Kamphaeng Saen or Nong Pho for veterinary services. At the farm, the veterinarian who responded to the call collected the health and medical history of the mastitis-infected cow. Only cows with a new mastitis infection (whose owners had consented to participate in the study) were given a physical examination; milk samples were collected from these cows aseptically. The definition of a new clinical mastitis case was any cow that displayed abnormal milk (inflammation of mammary gland or the presence of watery, flakey milk or clotted milk) with or without systemic signs (Morin, 2009). In addition, cows with a history of mastitis had to have been infection-free for at least 2 wk. Individual cow records from the owner were used for case confirmation. If a cow presented signs of clinical mastitis in more than one quarter, then each quarter with suspected clinical mastitis was sampled for the bacterial culture and identification. After the samples were collected, they were stored in an icebox at 4° C and delivered to the laboratory within 24 hr.

Fig. 1 Sampling locations of small dairies in three provinces (Nakhon Pathom, Ratchaburi, and Kanchanaburi) in Thailand

Bacteriological culture

Bacteriological identification was performed according to the clinical veterinary microbiology and laboratory procedures outlined in Quinn et al. (1994). In short, 10 µL of each milk sample were streaked on 5% bovine blood agar and MacConkey agar (Oxoid Limited; Basingstoke, UK) for the bacterial cultures. The samples were then incubated at 37° C for 24–48 hr. A sample was identified as positive if at least one colony was visible on the culture plate. If no microorganisms were isolated, it was reported that the result showed no growth was detected. For positive samples, the bacteria were identified using colony morphology, Gram staining and biochemical tests (Quinn et al., 1994).

Environmental pathogen infections were recorded based on cultures positive for environmental streptococci (e.g., *Str. uberis, Str. dysgalactiae, and Str. bovis*), enterococci (e.g., *Enterococcus [En.] faecalis* and *En. faecium*), coliform bacteria (such as *Escherichia [E.] coli, Enterobacter* spp., and *Klebsiella* spp.), and other microorganisms that had been previously reported as having been isolated from the environment in clinical mastitis cases, such as *Pseudomonas* spp. and yeast (Osborne et al., 1981; Parkinson et al., 1999; Morin, 2009). Non-environmental pathogens were sampled that tested positive for bacteria such as *S. aureus*, *S. epidermidis*, *S. haemolyticus, S. intermedius, Staphylococcus spp., Str. Agalactiae, and Pasteurella [P.] haemolytica.*

Statistical analyses

Data were managed using Microsoft Excel® 2016 (Microsoft Corp.; Redmond, WA, USA). Samples with missing values and incomplete data (cases where the farmer did not answer a question or some data were not recorded for some herds) from the questionnaires were excluded from the analysis (n = 14). For cows with multiple infected quarters, the primary quarter of the infection was selected. If the primary quarter of the infection could not be recorded or the bacterial identification indicated multiple species of pathogen, the corresponding data were removed from analysis (n = 22). In the end, 110 questionnaires and milk samples from 37 dairies were used in the data analysis. Finalizing data sets were imported and analyzed in R release 3.5.1 (R Core Team, 2018) and Stata release 15.1 (StataCorp LLC; College Station, TX, USA).

The definitions and descriptions for all variables are shown in Table 1. Potential risk factors were used as independent variables. The dependent variables were categorized into two levels with binary outcomes: 1) clinical mastitis from environmental mastitis pathogens and 2) clinical mastitis from other pathogens. The clinical mastitis with environmental mastitis pathogens category included cows who displayed clinical mastitis signs and cultured positive for environmental pathogen infections. Clinical mastitis with other pathogens referred to cows who displayed clinical mastitis signs and cultured positive for non-environmental pathogens. Univariate analysis was performed using a permutation for Pearson's chi-square test with R to determine the associations between the dependent and independent variables (Table 1). This analysis was performed with 10,000 iterations. In this way, to increase the chance of maximizing the number of independent variables in the final model, the associations that were statistically significant at $p < 0.15$ were selected for a mixed-effects logistic regression analysis with the farm used as a random effect using Stata. Goodness-of-fit was reported using log-likelihood ratios in the final model. The mapping of the sampling locations was presented using ArcGIS 10.2.1 (Esri, Inc.; Redlands, CA, USA).

Results

Descriptive statistics and univariate analyses

Almost all the farms (34/37, 91.89%) used milking machines; only three farms (3/37, 0.08%) performed milking manually. The average number of cows per farm was 22 (SD = 14.94). The average number of lactating cows per farm was 15 (SD = 10.34) and the average milk yield per farm was 163.85 (SD = 123.79) kg/d. The average milk yield per cow was 11.38 (SD = 4.33) kg/d and the average number of years of farmer experience was 10.76 (SD= 8.52). The median number of cows with clinical mastitis by parity was 2 (interquartile range [IQR] = 1–3) and the average number of days in milk was 122 (IQR = 40.25–238.75) as shown in Table 2.

The results of the bacteriological identification are illustrated in Table 3. In the cows with clinical mastitis, the most commonly isolated pathogens were in the environmental pathogen group (84/110, 76.36%). There were six samples (6/110, 5.45%) with no pathogen growth. *E. coli* (14/110, 12.73%) and *Str. dysgalactiae* (14/110, 12.73%) were the most frequently isolated pathogens, followed by *Streptococcus* spp. (13/110, 11.82%), *En. faecalis* (10/110, 9.09%), *Str. faecalis* (8/110, 7.27%), *Str. uberis* (7/110, 6.36%), *Klebsiella* spp. (4/110, 3.64%), *Candida* spp. (4/110, 3.64%), *Pseudomonas aeruginosa* (3/110, 2.73%), *Str. bovis* (3/110, 2.73%), *Str. pyogenes* (1/110, 0.91%), *Pseudomonas* spp. (1/110, 0.91%), *Enterobacter* spp. (1/110, 0.91%) and *Bacillus cereus* (1/110, 0.91%). For the non-environmental pathogens, the most commonly isolated pathogens found were coagulase-negative *Staphylococcus* spp. (10/110, 9.09%), followed by *S. aureus* (2/110, 1.82%), *S. intermedius* (2/110, 1.82%), *Str. agalactiae* (2/110, 1.82%), *P. haemolytica* (2/110, 1.82%), *S. epidermidis* (1/110, 0.91%) and *S. haemolyticus* (1/110, 0.91%).

The univariate analyses showed that at 15% of α-error, the occurrence of environmental or non-environmental pathogens causing clinical mastitis was significantly associated with five independent variables: the practice of using the same towel to clean the udders of multiple cows before milking (p = 0.03); the treatment of cleaning water before using it on cows (p = 0.02); not practicing hand stripping after machine milking (p = 0.13); cow appearance before milking (p = 0.07); and milking machine vacuum noise during milking (p = 0.03).

Table 1 Definitions of independent and dependent variables.

Variable type	Definition and coding
Dependent variable	
Clinical mastitis infected by environmental mastitis pathogens	1 = clinical mastitis with environmental mastitis pathogens 2 = clinical mastitis with other pathogens (non-environmental pathogen)
Independent variable	
Farm husbandry type	1 = tied stall, 2 = pasture and/or free stall
Floor type	1 = ground (concrete or rubber), 2 = other
Floor crack	1 = yes, 2 = no
Bedding	1 = yes, 2 = no
Feeding proportion by milk yield	1 = yes, 2 = no
Water source for cow bathing	1 = pipe water, 2 = other
Water undergoes treatment process before cow bathing	1 = yes, 2 = no
Parts cleaned before milking	1 = only udder, 2 = whole body
Cow appearance before milking	1 = wet, 2 = dry
Usage of towel for udder cleaning	1 = one towel for one cow, 2 = same towel for multiple cows
Disinfection of towel before cleaning udder	1 = yes, 2 = no
Teat looks clean before milking	1 = yes, 2 = no
Teat dipping	1 = yes, 2 = no
Separate milking parlor section	1 = yes, 2 = no
Milking parlor cleaned before use	1 = yes, 2 = no
Hand milking after using machine	1 = yes, 2 = no
Milking machine vacuum noise during milking	1 = yes, 2 = no
Milking machine routine cleaning	1 = yes, 2 = no
Milking machine deep cleaning	1 = yes, 2 = no
Milking machine pipe cleaning	1 = yes, 2 = no
Vacuum pipe bent	1 = yes, 2 = no
Dry cow therapy	1 = yes, 2 = no
Method of dry cow therapy	1 = only selected cow, 2 = all cows
Cow stands for 30 min after dry cow therapy	1 = yes, 2 = no
Culling program for mastitis cows	1 = yes, 2 = no
Separate pregnant cows before parturition	1 = yes, 2 = no
Clean floor after parturition	1 = yes, 2 = no
Time to collect foremilk after parturition	1 = within 6 h, 2 = after 6 h
Calves suckling other cows and contracting clinical mastitis	1 = yes, 2 = no
Feces cleaned during the day	1 = yes, 2 = no
Cow standing ≥ 30 min after milking	1 = yes, 2 = no
Days in milk	1 = < 90 days, 2 = 91–180 days, 3 = > 180 days,
Parity	1 = parity 1–3, 2 = parity 4–6, 3 = parity > 6
Hoof problems	1 = yes, 2 = no
Milk leaking from teat	1 = yes, 2 = no
History of previous chronic mastitis	1 = yes, 2 = no
Number of quarters that show clinical sign of mastitis	1 = one quarter, 2 = more than one quarter

Table 2 Descriptive statistics from general information about farms

General information	Mean (SD)	Range (minimum–maximum)	Median (IQR)
Number of total cows (cows/farm)	22 (14.94)	73 (3–76)	19 (11–26.5)
Number of lactating cows (cows/farm)	15 (10.34)	44 (2–46)	12 (6.5–19)
Total milk yield per farm (kg/farm/d)	163.85 (123.79)	510.6 (13.4–524)	130 (67.5–239.5)
Total milk yield per cow (kg/cow/d)	11.38 (4.33)	28.13 (1.87–30)	11.39 (9.36–13.21)
Number of years of experience (years)	10.76 (8.52)	34 (1–35)	10 (3–15.5)
Parity (cow)	2.5 (1.32)	6 (1–7)	2 (1–3)
Days in milk (d)	151.85 (127.87)	649 (1–650)	122 (40.25–238.75)

IQR = inter-quartile range.

Table 3 Bacteriological identification from milk samples.

Bacteriological result	Number of cows	Percentage
Environmental pathogen	84	76.36
Bacillus cereus	1	0.91
Candida spp.	4	3.64
Escherichia coli	14	12.73
Enterobacter spp.	1	0.91
Enterococcus faecalis	10	9.09
*Klebsiell*a spp.	4	3.64
*Pseudomonas aeruginos*a	3	2.73
Pseudomonas spp.	1	0.91
*Streptococcus bovi*s	3	2.73
*Streptococcus dysgalactia*e	14	12.73
*Streptococcus faecali*s	8	7.27
*Streptococcus pyogene*s	1	0.91
Streptococcus spp.	13	11.82
Streptococcus uberis	7	6.36
Non-environmental pathogen	26	23.64
*Staphylococcus aure*us	2	1.82
*Staphylococcus epidermidi*s	1	0.91
*Staphylococcus haemolyticu*s	1	0.91
*Staphylococcus intermediu*s	2	1.82
Staphylococcus spp.	10	9.09
*Streptococcus agalactia*e	2	1.82
*Pasteurella haemolytic*a	2	1.82
No growth	6	5.45
Total	110	100

Mixed-effects logistic regression

Since the univariate analysis identified five independent variables that were significantly associated with the dependent variables, all five of these independent variables were selected for mixed-effects logistic regression analysis. The final model showed that only two factors (the practice of hand stripping after machine milking and the practice of using the same towel to clean the udders of multiple cows before milking) were significantly associated with environmental mastitis on these particular farms ($p < 0.05$) (Table 4). To provide more detail, the odds ratio (OR) for non-environmental bacteria versus environmental infection was significantly ($p < 0.05$) less than 1 for the influence of unpracticed hand stripping after machine milking (OR = 0.26; 95% confidence interval [CI] = 0.07–0.91). On the other hand, farms where efficient hand stripping was practiced after cows were removed from a milking machine were at risk of being infected with non-environmental bacteria. The OR of non-environment bacterial-infected quarters compared with environmental infection were also significantly ($p < 0.05$) less than 1 for a farmer using the same towel for udder cleaning on multiple cows (OR = 0.18, 95% CI = 0.05 – 0.65). On the other hand, on farms where the same towel was used for multiple cows, there was the risk of infection with environmental bacteria.

Discussion

This study identified risk factors for bovine clinical mastitis associated with the bacterial cultures found in cows of small dairies in western Thailand. The study found that unpracticed hand stripping after machine milking was significantly associated with environmental bacterial mastitis.

All farms in this study were small dairies. The largest farm had 46 lactating cows during the study period. The average milk yield per cow was 11.38 kg/d. Therefore, the presence of clinical mastitis on these farms had a considerable negative financial impact. Environmental pathogens were the most frequently isolated mastitis pathogens (76.36%), which agreed with the results of a study conducted in Chiang Mai in northern Thailand where environmental pathogens were the most prevalent form identified in intramammary infections (Leelahapongsathon et al., 2016). Moreover, in the current study, *E. coli* and *Str. dysgalactiae* were equally the most prevalent among the environmental pathogens which was consistent with these two pathogens being the most common causing bovine clinical mastitis in other areas (Lundberg et al., 2014; Fairbrother et al., 2015).

The results of the mixed-effects logistic regression analysis showed that only one of the risk factors was associated with clinical mastitis caused by either environmental or non-environmental pathogens. The practice of hand stripping after machine milking increased the risk of non-environmental bacterial infection. This indicates that hand stripping is more likely to cause infections by non-environmental

Table 4 Final model of mixed-effects logistic regression analysis.

Dependent variable	Independent Variable	β[a]	SE	Model log likelihood	Odds ratio	95% CI of odds ratio	*p*–value
En[c]		Ref.[b]					
Non En	Intercept	0.57	0.62	-54.77	-	-	0.36
	Hand stripped after machine milking						
	Yes	Ref.					
	No	-1.34	0.64	-	0.26	0.07–0.91	0.04*
	Usage of towel for udder cleaning						
	One towel for one cow	Ref.					
	Same towel for multiple cows	-1.69	0.65	-	0.18	0.05–0.65	0.01*

CI = 95% confidence interval

[a] = Regression coefficient.

[b] = Reference.

[c] = Clinical mastitis with environmental pathogens infection.

* = Significantly different at $p < 0.05$.

pathogens than by environmental bacteria. Based on their study conducted in Tanzania, Karimuribo et al. (2008) reported that hand stripping is a protective factor for California mastitis test (CMT)-positive quarters in cows with subclinical mastitis. Previous studies have indicated that residual milk or incomplete milking can increase the somatic cell count and encourage bacterial growth in the teat canal (González-Sedano et al., 2010; Williamson and Lacy-Hulbert, 2013). Thus, hand stripping after machine milking and post-dipping by well-trained farmers using good sanitary practices might help empty the milk in the teat canal, possibly decreasing the occurrence of clinical mastitis and reducing the risk of new intramammary infections. Hence, competent hand striping is appropriate on farms with a high incidence of environmental bacterial infection to help reduce bacterial growth in the teat canal and the risk of environmental mastitis. However, insufficiently hygienic hand striping may increase the risk of mastitis infection. Farmers must carefully practice hand stripping using aseptic technics. Moreover, the use of a single towel to clean the udders of multiple cows before milking presented a significant association with environmental mastitis. This indicated that farms with problems from environmental mastitis should be concerned about this practice. Similar findings were reported in earlier studies conducted in Ethiopia (Tolosa et al., 2015) and Thailand (Jarassaeng et al., 2012). Based the experience of the authors, Thai farmers tend to use the same towel to clean the udders of multiple cows, usually cleaning the towel after each use by hand-washing it and soaking it in a bucket of chlorinated water. Before using the towel on the next cow, the farmers twist the towel to squeeze out the water. This practice leads to the accumulation of organic matter in the bucket, possibly reducing the potency of the chlorinated water and increasing the risk of mastitis via environmental pathogen infection. Therefore, farmers must follow the practice of using a different towel to clean the udder of each cow, because this is currently the most effective method for controlling environmental mastitis.

This study was limited by the low number of samples. Nevertheless, the cited significance factors and presence for biological plausibility were met. For the sample selection, a purposive sampling method was used to collect the clinical mastitis milk samples. This method uses non-probability sampling that selected the sample based on the cow owner calling the animal hospital. This limited the representativeness of the sample, but an advantage for this study was that it was possible integrate the research activities into routine work. All farmers welcomed the study team and allowed the study to be undertaken on their farms. Therefore, it is suggested that our analyses can improve the measures used to control clinical mastitis in western Thailand because the results are scientifically valid and will help farmers limit their economic losses due to mastitis.

In conclusion, the most frequently isolated pathogens in the area were the environmental pathogens associated with hand stripping practices and using a towel for udder cleaning. These results should be used to establish control measures that help farmers reduce their economic losses due to coliform mastitis. In other geographical areas, the results of this study might help guide the establishment of control measures, especially for application in small dairies.

Conflicts of Interests

The authors declare that there are no conflicts of interest.

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References

Ajariyakhajorn, K., Samngamnim, S., Boonserm, T., Inchaisri, C., Thiraoatsakun T, Farnsworth, R.J. 2003. Mastitis in small dairy holders of Nakhonpathom province, Thailand. In: Proceedings of The 11th International Symposium of the World Association of Veterinary Laboratory Diagnosticians and OIE Seminar on Biotechnology. 9–13 November 2003. Bangkok, Thailand, pp. 122–123.

Ausvetplan. 2013. EpiTools. Ausvet. Canberra, Australia. https://epitools.ausvet.com.au/samplesize, 27 November 2018.

Bar, D., Tauer, L.W., Bennett, G., et al. 2008. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. J. Dairy Sci. 91: 2205–2214.

Department of Livestock Development. 2013. Farmer and Livestock Population Statistics Annual Report in 2013. http://ict.dld.go.th/th2/images/stories/stat_web/yearly/2556/summaryreport/3.report56 _milk.pdf, 6 June 2018

Fairbrother, J.H., Dufour, S., Fairbrother, J.M., Francoz, D., Nadeau, E., Messier, S. 2015. Characterization of persistent and transient *Escherichia coli* isolates recovered from clinical mastitis episodes in dairy cows. Vet. Microbiol. 176: 126–133.

González-Sedano, M., Marin-Mejia, B., Maranto, M.I., Leme de Magalhaes-Labarthe, A.C., Alonso-Diaz, M.A. 2010. Effect of residual calf suckling on clinical and sub-clinical infections of mastitis in dual-purpose cows: Epidemiological measurements. Res. Vet. Sci. 89: 362–366.

Green, M.J., Bradley, A.J., Medley, G.F., Browne, W.J. 2007. Cow, farm, and management factors during the dry period that determine the rate of clinical mastitis after calving. J. Dairy Sci. 90: 3764–3776.

Hall, D., Ehui, S., Shapiro, B. 2004. Economic analysis of the impact of adopting herd health control programs on smallholder dairy farms in Central Thailand. Agric. Econ. 31: 335–342.

Jarassaeng, C., Aiumlamai, S., Wachirapakorn, C., Techakumphu, M., Noordhuizen, J.P.T.M., Beynen, A.C., Suadsong, S., 2012. Risk factors of subclinical mastitis in small holder dairy cows in Khon Kaen Province. Thai J. Vet. Med. 42: 143–151.

Kampa, J., Sukolapong, V., Chaiyotwittakun, A., Rerk-u-suke, S., Polpakdee, A. 2010. Chronic mastitis in small dairy cattle herds in Muang Khon Kaen. Thai J. Vet. Med. 40: 265–272.

Karimuribo, E.D., Fitzpatrick, J.L., Swai, E.S., Bell, C., Bryant, M.J., Ogden, N.H., Kambarage, D.M., French, N.P. 2008. Prevalence of subclinical mastitis and associated risk factors in smallholder dairy cows in Tanzania. Vet. Rec. 163: 16–21.

Leelahapongsathon, K., Piroon, T., Chaisri, W., Suriyasathaporn, W. 2016. Factors in dry period associated with intramammary infection and subsequent clinical mastitis in early postpartum cows. Asian-Australas. J. Anim. Sci. 29: 580–585.

Leelahapongsathon, K., Schukken, Y.H., Suriyasathaporn, W. 2014. Quarter, cow, and farm risk factors for intramammary infections with major pathogens relative to minor pathogens in Thai dairy cows. Trop. Anim. Health Prod. 46: 1067–1078.

Lundberg, A., Nyman, A., Unnerstad, H.E., Waller, K.P. 2014. Prevalence of bacterial genotypes and outcome of bovine clinical mastitis due to *Streptococcus dysgalactiae* and *Streptococcus uberis*. Acta Vet. Scand. 56: 80. doi.org/10.1186/s13028-014-0080-0

Morin, D.E. 2009. Mammary gland health and disorder. In: Smith, B.P. (Ed.). Large Animal Internal Medicine, 4th ed. Elsevier. St. Louis, MO, USA, pp. 1112–1143.

Oliveira, C.S., Hogeveen, H., Botelho, A.M., Maia, P.V., Coelho, S.G., Haddad, J.P. 2015. Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. Prev. Vet. Med. 121: 297–305.

Osborne, A.D., Armstrong, K., Catrysse, N.H., Butler, G., Versavel, L. 1981. An outbreak of *Pseudomonas* mastitis in dairy cows. Can. Vet. J. 22: 215–216.

Parkinson, T.J., Merrall, M., Fenwick, S.G. 1999. A case of bovine mastitis caused by *Bacillus cereus*. N. Z. Vet. J. 47: 151–152.

Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R. 1994. Clinical Veterinary Microbiology, 1st ed. Mosby. London, UK.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/, 25 November 2018.

Rollin, E., Dhuyvetter, K.C., Overton, M.W. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Prev. Vet. Med. 122: 257–264.

Tolosa, T., Verbeke, J., Ayana, Z., Piepers, S., Supre, K., De Vliegher, S. 2015. Pathogen group specific risk factors for clinical mastitis, intramammary infection and blind quarters at the herd, cow and quarter level in smallholder dairy farms in Jimma, Ethiopia. Prev. Vet. Med. 120: 306–312.

Williamson, J.H., Lacy-Hulbert, S.J. 2013. Effect of disinfecting teats post-milking or pre- and post-milking on intramammary infection and somatic cell count. N. Z. Vet. J. 61: 262–268.