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Prevalence of subclinical mastitis and associated risk factors at cow and herd level in dairy farms in North-West Ethiopia

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ABSTRACT

Knowledge of mastitis pathogens and their predominance as well as understanding of risk factors are prerequisites to improve udder health in a herd, region or country. In Ethiopia, such information is scarce, despite the fact that mastitis is an important cattle disease in the country. A cross-sectional study that describes prevalence and causative agents of subclinical mastitis (SCM) as well as risk factors at cow and herd level was conducted on 167 dairy farms in North-West Ethiopia. On average, 33% of the quarters and 62% of the cows were California Mastitis Test (CMT) positive, but the within herd quarter level prevalence ranged between 0 and 100%. A total of 1543 milk samples, being 27 quarters that showed signs of CM, 606 CMT positive quarters and 910 CMT negative quarters were cultured, respectively 40%, 67% and 47% was positive on bacteriological culture. Coagulase negative staphylococci (CNS) (31%) followed by *Staphylococcus aureus* (9%) were the pathogens most frequently isolated.

Based on face-to-face questionnaire data, 35 herd level and 13 cow level factors were evaluated for their association with SCM (based on CMT) and with a positive culture for any bacteria, CNS or *S. aureus*. Cows with a history of CM, of higher parity, >150 days in milk (DIM) and herds with owners that have >10th grade level of education had higher odds of SCM. The odds of being culture positive for any bacteria was higher in cows with ≥25% Holstein Friesian blood level (HBL), >150 DIM, housed on cemented floors, and milked by squeezing rather than stripping. Similarly, the odds of culturing CNS was higher in cows with 25–50% HBL, >150 DIM, and milked by squeezing. *Staphylococcus aureus* was more often found in cows with a history of CM and in larger herds. Checking the udder for mastitis, feeding cows according to their requirements and allowing calves to suckle the cows were negatively associated with SCM, with culturing any bacteria and with culturing CNS, respectively. Higher odds of SCM and of culturing CNS were found in herds owned by members of a dairy cooperative.

In summary, we identified a high prevalence of SCM and intramammary infections with substantial variation between farms, and we found a number of risk factors explaining this variation. The risk factors for mastitis that were identified in this study can form the basis of an udder health control program specific for the dairy industry in North-West Ethiopia.

1. Introduction

Dairy farming is expanding in Ethiopia (Mekonnen et al., 2006). The Ethiopian dairy industry can be divided into rural, urban and peri-urban dairy farms. In rural dairy farms, indigenous breeds are used to produce milk only for household consumption. Urban and peri-urban farms, which we are focussing on in this paper, mainly keep Holstein-Friesian and Zebu cross-breed cows to produce milk

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that is sold on the market (Redda, 2001). Cross-breed cows have a much higher milk production level than indigenous breeds. However, mastitis is also a larger problem in these cross-breed cows (Lema et al., 2001). Dego and Tareke (2003) and Almaw et al. (2008) reported a significantly higher prevalence of mastitis in cross-breed cows than in the indigenous breeds. Almaw et al. (2008) found no clinical mastitis (CM) in indigenous breeds. In southern Ethiopia, subclinical mastitis (SCM) measured by the California mastitis test (CMT) was reported to have a prevalence as high as 62% at cow level (Tolosa et al., 2013).

Clinical mastitis is recognized as an important disease by Ethiopian dairy farmers. But SCM although much more prevalent than CM, receives little attention by most dairy farmers (Almaw et al., 2008; Tolosa et al., 2013). Subclinical mastitis affects milk yield and leads to economic losses which have been estimated for Ethiopia to vary from US$38 (Mungube et al., 2005) to US$79 (Tesfaye et al., 2010) per cow per lactation. Additionally, SCM may lead to CM (Ma et al., 2000; Reksen et al., 2006), and may be a source of new intramammary infections (IMI) in healthy cows (Oliver et al., 2004). Moreover, SCM has an impact on milk quality such as shelf life of fresh milk (Busato et al., 2000).

Mastitis is caused by many different bacterial species associated with different dynamics of IMI and production losses (Gröhn et al., 2004). In Ethiopia, variation in distribution of dominant mastitis causing bacteria has been reported. Dego and Tareke (2003) and Abera et al. (2012) from Southern, Getahun et al. (2008) from Central, and Haftu et al. (2012) from Northern parts of Ethiopia reported that *S. aureus* was the dominant pathogen while other authors found CNS dominating (Almaw et al. (2008) from North-West and Mekonnen and Tesfaye (2010) from Central Ethiopia). Each species may have distinct risk factors (Tolosa et al., 2015), so in order to establish a targeted mastitis control system, the predominant types of mastitis pathogens as well as species specific risk factors need to be identified.

In the present study, our objectives were (1) to estimate the prevalence of SCM based on CMT scores, (2) to estimate the prevalence of positive culture for mastitis pathogens, and (3) to identify risk factors associated with SCM and species specific IMI at cow and herd level in the urban and peri-urban cross-breed dairy herds of North-West Ethiopia.

2. Materials and methods

2.1. Study area

The study was carried out at two sites, Bahir Dar and Gondar (both in North-West Ethiopia). The altitude in Bahir Dar and Gondar varies from 1805 to 1,966 meters above the sea level and average annual rain fall varies from 1077 to 1,460 mm.

2.2. Study herds and cows

A total of 510 milking cows from 167 herds kept in urban and peri-urban dairy herds were included in the study. All sampled cows in the study were cross-breeds. The studied herds had an average herd size of 5 (median 4) head of cattle (lactating cows, dry cows, heifers and weaned calves) and the median number of lactating cows was 3. Thirty eight herds had only one lactating cow. The studied cows had an average milk yield of approximately 10 L per cow per day. Cows were milked by hand twice a day by stripping (milking by thumb and index finger), squeezing (milking by five fingers) or both, depending on the habit of the farmer and the size of the teats. Less than half (40%) of the herds use a cloth or a towel for drying the udder. Breeding practice was either by artificial insemination or bull mating depending on availability and the farmers' choice. The milk produced was sold either house to house, to families, to restaurants or hotels, or through a farmers' cooperative.

2.3. Selection of herds and cows

Because there is no systematic registration of dairy farmers in Ethiopia, we first made list of urban and peri-urban dairy farms in Bahir Dar and Gondar aiming to be exhaustive and to represent the population as good as possible. Names and addresses of dairy farmers were collected from private and governmental artificial insemination records, from two dairy cooperatives, and from veterinary clinics. In this way, a longlist of 1209 dairy farmers from Gondar and of 272 from Bahir Dar was made. Of note, the list for Gondar was expected to contain many records of farms that were not-existing anymore, whereas the list from Bahir Dar was constructed more recently based on more reliable records. Computer generated random numbers were assigned to each farm on the longlist, although stratified for Gondar and Bahir Dar. As we aimed for approximately equal sampling probabilities (about 25% of all currently existing farms) in both regions, and as we expected the actual number of farms in Gondar to be much smaller than the number of farms on the list because we knew many farmers had stopped in this region since the list was constructed, we started out with the goal to enrol approximately 100 farms from Gondar and 70 farms from Bahir Dar. The number of included herds, the number of cows, and the number of milk samples collected was based on a balance of local logistic possibilities, processing costs (time and money) and enrolling representative numbers of samples of the population of herds with cross-breed dairy cows. Farmers were enrolled based on the order of the assigned random numbers. If a farmer was not willing to participate in the study (n = 7), had stopped dairy farming (n = 32), if the farm could not be found (n = 18), or if a farm only had cows of indigenous breeds (n = 178), that farm was skipped and the farm with the next number was approached. For these reasons, 230 farms in Gondar and 5 farms in Bahir Dar were skipped. Finally, we managed to include a total of 167 farms, 102 form Gondar and 65 from Bahir Dar.

2.4. Design of the questionnaire and data collection

Based on the literature and our own expertise with regard to the Ethiopian dairy circumstances, two questionnaires on risk factors for SCM were prepared. The first questionnaire addressed personal characteristics of the farmer, herd characteristics, farm management, milking practices and prevention practices of diseases other than mastitis. The second questionnaire addressed cow characteristics. For reasons of data handling, both questionnaires contained mainly closed questions. The order of the questions was carefully chosen in order to avoid leading responses. The original English questionnaire was translated to the farmers' local language (Amharic), and subsequently translated back into English by an external translator to validate the translation. Corrections were subsequently made to the translation based on the comparison of the different versions. The questionnaire was validated by performing five pilot interviews in a convenience sample of farmers. The pilot interviews were also used to train two final year veterinary students who were participating in the data collection in order to prevent differences between interviewers. Based on the pilot interviews, some questions were modified. Finally, a total of 60 questions remained and were used for data collection. The second questionnaire contained questions regarding each cow enrolled in the study. The English version of the two questionnaires are available (Supplementary files 1 and 2).

All 510 lactating cross-breed cows in the 167 participating herds were examined for CM by visual inspection of the udder and the

Table 1
Cow level variables potentially related to subclinical mastitis in North-West Ethiopia as included in the questionnaire.

Variable	Levels
CM history[a]	No/Yes
Treatment history for clinical mastitis	No/Yes
Presence of blind quarter(s)	No/Yes
Holstein Friesian blood level	<25%, 25–50% or >50%
Experience of common dairy health problems[b]	No/Yes
Parity	Parity: 1, 2, 3 or ≥4
Days in milk	≤60, >60 to 150, >150 to 250 or >250
Pregnancy	No/Yes
History of lameness	Absent or Present
Average daily milk yield (ADMY)	<8 L, 8–15 L or >15 L
Posture	Normal or aberrated
Body Condition Score	Poor, good or very good
Udder symmetry	Symmetric or asymmetric

[a] Cow had clinical mastitis at least once in her life.
[b] Common Dairy Health Problems are dystocia, retained placenta, hypocalcaemia and abortion.

milk and for SCM by performing the CMT. Milk samples for bacteriological culture were collected from all lactating quarters of a cow with CM or with one or more CMT positive quarters. Additionally, from 50% of the cows that had 4 CMT negative quarters, milk samples were collected from all lactating quarters. The cows that had 4 CMT negative quarters were selected randomly after numbers were assigned for each cow that had 4 CMT negative quarters in a farm. However, if a herd had one cow that had 4 CMT negative quarters, samples were collected from that cow. When milk sample collection from a selected cow was impossible because of the cows' behaviour, the next cow was sampled.

Of the 167 farms enrolled, in 16 farms the questionnaire data could not be used because milk samples from these farms were lost to follow up. Out of the remaining 151 questionnaires, nine were not completed because the farmers cancelled their appointment more than twice. In addition, in six farms, the data on parity and days in milk (DIM) was incomplete because the farmers did not remember this information or because the cows had been purchased without information on parity. Therefore, data from only 136 herds and 345 cows were used for the risk factor analysis.

Questionnaires were administered by face-to-face interviews. Information on general farm hygiene and management, and on body condition score (BCS) was collected directly by the first author. Body condition was scored in three levels; poor, good and very good. The information on the level of Holstein Friesian blood (HBL) was estimated based on the cow's exterior by the first author together with the farmer. Potential cow and herd level risk factors are described in more detail below.

2.4.1. Cow level variables

Thirteen cow level variables deemed to be potential risk factors for SCM and positive culture, and were included in the questionnaire (Table 1). Average daily milk yield (ADMY) and DIM were grouped into three classes. Experience of dystocia, retained placenta, hypocalcaemia, ketosis and abortion at least once in a cows' life time were recorded as common dairy health problems (CDHP), and were analysed as one variable.

2.4.2. Herd level variables

Thirty five herd level variables referring to dairy farmers' personal characteristics, herd characteristics, farm management practices applied, milking practices and other factors potentially related to mastitis were included, and are presented in Table 2. The level of feeding was recorded in three levels: below the requirement, at the requirement and above the requirement according to the farmers' opinion.

2.5. Diagnosis of mastitis and milk sample collection

All herds were visited once for sampling. During the visit, all quarters of all lactating cows were examined clinically and by CMT. Clinical mastitis was defined as an abnormal udder and/or changes in the appearance (colour) and consistency of the milk (clots, flakes or blood). The CMT was performed and interpreted as negative (0), trace (T), weakly positive (1), distinctively positive (2), and strongly positive (3) as described by the NMC (1999). A quarter was considered to have SCM if the CMT was positive (defined as CMT score T, +1, +2, or +3). A cow was considered to have SCM if one or more quarters were CMT positive.

Milk samples were collected as described by the NMC (1999). Briefly, loose dirt, bedding, and hair from the gland and teats was brushed off. Grossly dirty teats and udders were washed. After cleaning each of the quarters thoroughly, quarters were dried by using towels. Then, teat ends were scrubbed vigorously (10–15 s) with cotton balls moistened (not dripping wet) in 70% alcohol until no more dirt was visible on the cotton ball or on the teat end. First, teats on the far side of the udder were disinfected, followed by the two nearest teats. The first two squirts of milk were discarded, before individual quarter milk samples were collected from each quarter, beginning with the nearest teats, followed by the two on the far side. Sampling tubes were maintained at a 45° angle while stripping, trying to avoid the rim of the tube to touch the teat end. Approximately 3–4 ml of milk was collected, filling approximately ¾ of the sampling tubes. After collection, samples were kept in a cooler jar containing melting ice, and were shipped to the laboratory within 5 h where they were kept at −20 °C until further processing.

2.6. Culturing and identification of bacteria

Bacteriological culturing and identification was done at the University of Gondar Veterinary Medicine microbiology laboratory. Frozen samples were warmed to room temperature (22–25 °C) for about an hour and then homogenized using a Vortex mixer. Approximately 0.01 ml of milk was spread on a 5% sheep blood agar plate and was incubated aerobically at 37 °C. Plates were examined after 24 h. A quarter was considered culture-positive if any bacteria were cultured, and was considered culture-negative if no growth occurred after 24 h of incubation. If two different types of colonies were noticed, the quarter was considered to have a mixed infection. Milk samples that were culture-positive for more than two types of colonies were considered contaminated and were excluded from the analyses. A cow of which at least one quarter was culture-positive was considered bacteriologically positive.

Morphology, haemolysis, Gram staining, catalase production, growth on manitol salt agar, mannitol fermentation, slide and tube coagulase testing, hydrolyzation of esculin and the CAMP reaction were used to identify bacterial species (NMC, 1999).

2.7. Statistical analysis

After removing farms that were ineligible (i.e. because they had only indigenous breeds) or did not exist anymore, sampling weights were created for Gondar and Bahir Dar herds, separately, by accounting the probabilities of selection of herds. Additionally, sampling weights were created for each cow from which milk samples were collected by accounting for the probabilities of selection of a cow within herds. The probabilities of selection were based on the effective sampling rate. Probabilities of selection of participated herds were given after removing herds that were not willing for

Table 2
Herd level variables potentially related to subclinical mastitis in North-West Ethiopia as included in the questionnaire.

Group of variable	Variable	Levels
Farmers' personal characteristics	Farmers' level of education	≤6 grade, grade 7–10, grade >10
	Dairy farming experience	≤5years, >5–15 years, >15 years
	Membership of a dairy cooperative	No/Yes
	Followed dairy management training	No/Yes
	Receiving expert advice	No/Yes
	Knowledge whether mastitis exists	No/Yes
	Believes mastitis can be prevented	No/Yes
Herd characteristics	Stall type	Tie stall or free stall
	Floor type	Soil, stone or cement
	Presence of stall drainage	No/Yes
	Occurrence of mastitis last year	No/Yes
	Herd size	≤5, 6–10 or >10
	Level of feeding	Below requirement, meet requirement or above requirement
Farm management practice	Calves suckling with cow	No/Yes
	Checking the udder for clinical mastitis	No/Yes
	Use of bedding	No/Yes
	Washing the stall	No/Yes
	Removing the dung between the regular cleaning times	No/Yes
	Treatment of cows with clinical mastitis	No/Yes
	Use of pasture grazing	No/Yes
	Deworming	No/Yes
	Vaccinate cows	No/Yes
	Antibiotic treatment of clinical mastitis cows by farmer himself	No/Yes
	Consistency of feeding concentrates	No/Yes
Milking practice	Restraining before milking	No/Yes
	Foremilk stripping	No/Yes
	Cleaning udder before milking	No/Yes
	Use of towel for drying udder	No/Yes
	Milking method	Stripping, squeezing or both
	Use of separate milking equipment for each cow's milking	No/Yes
	Milking mastitis quarters	No/Yes
Other variables	Tick infestation present	No/Yes
	Source of water for cleaning	Tap, tap and river, well
	Lameness is a problem	No/Yes
	Interviewers' evaluation of general hygiene	Poor, good, very good

interview and herds that were inaccessible in each of the regions. Probabilities of selection of cows within herds were given taking into account CM, CMT score and cows from which milk samples were not collected because of their behaviour. Subsequently, the survey adjusted overall prevalence of SCM, at cow and quarter level, and of mastitis causing pathogens, was calculated by using proportions in survey data analysis.

For the risk factor analyses, 4 dependent variables were used: SCM, culture of any bacteria (including minor pathogens), culture of CNS, and culture of *S. aureus*. When *S. aureus* as well as CNS were cultured (mixed culture), data were analysed as positive in both. Potential herd and cow level risk factors were tested. Univariable screening was done using mixed logistic regression models with a random herd effect and a cow effect nested within herd, taking the stratified sampling design and sampling weights into account using the survey data analysis procedure in Stata release 14 (StataCorp LLC, USA). Both herd-level and cow-level risk factors that were statistically significant at $P < 0.15$ in the univariable analyses were tested starting from the most significant variable by adding one variable at a time in the same multivariable multi-level survey logistic regression models using forward selection. Correlations between pairs of independent variables were evaluated using the Spearman Rank correlations. If two variables had a correlation coefficient of ≥0.7, only one of the variables was included in the further multivariable analysis. Variables in multivariable models with $P < 0.05$ from the Wald test were retained. All two-way interactions between variables in the final multivariable models were tested, but no significant interactions were found. Confounding was checked during the model building process by evaluating the change in the beta estimate of other variables when a variable was added to the models. If this change in beta estimate was >30%, the variable was considered a confounder. Herd-size and region, being likely confounders, were kept in the model throughout the analyses.

As our logistic regression models had random herd and cow effects, population-averaged odds ratios of the fixed effects were calculated according to Dohoo et al. (2009). First, regression coefficients were averaged across the population of herds and cows (1), and then population-averaged odds ratios (2) were calculated.

$$\beta^{PA} \frac{\beta^{HS}}{\sqrt{1 + 0.346 * \sigma 2}} \quad (1)$$

$$OR^{PA} = \exp(\beta^{PA}) \quad (2)$$

Where β^{PA} is the population-averaged regression coefficient, β^{HS} is the herd and cow-specific regression coefficient, σ^2 is the sum of the variances of the herd and cow random effects, and OR^{PA} is the population-averaged odds ratio.

3. Results

3.1. Descriptive statistics

In total, 2040 quarters from 510 cows in 167 herds (38 were one cow herds) were enrolled in the study. Of these, 58 quarters were blind and 27 quarters had CM. Therefore, a total of 1955 quarters was examined by CMT. In 16 farms milk samples were lost to follow up. In 28 CMT positive cows, after the CMT testing, collection of milk samples was not possible due to the cows' behaviour or because the farmer didn't want to cooperate. Finally, a total of 1543 milk samples of 400 cows in 151 herds were cultured, 96 (6%) from quarters of cows with CM (n = 25), 1050 (68%) from quarters of CMT positive cows (n = 273) and 397 (26%) from quarters of a subset (n = 102) of CMT negative cows (n = 184).

Table 3
California mastitis test scores in 1955 quarters and bacteria isolated from 1543 quarter milk samples from 400 dairy cows in 151 herds, and the estimated overall prevalence in 167 dairy farms in North-West Ethiopia.

Isolated bacteria	CMT score					CM (%)	Total (%)
	N (%)	T (%)	1 (%)	2 (%)	3 (%)		
CNS	230 (25)	67 (46)	95 (46)	65 (42)	34 (36)	5 (19)	496 (32)
S. aureus	44 (5)	10 (7)	32 (15)	29 (19)	16 (17)	4 (15)	135 (9)
S. agalactiae	10 (1)	4 (3)	2 (1)	4 (3)	2 (2)	2 (7)	24 (2)
C. bovis	12 (1)		4 (2)	2 (1)	1 (1)	1 (4)	20 (1)
S. dysgalactiae	3 (0)		3 (1)	7 (5)	4 (4)		17 (1)
Enterococcus spp.	5 (1)	4 (3)	1 (0)	4 (3)	1 (1)		15 (1)
S. uberis	5 (1)		1	2 (1)	1 (1)		9 (1)
Micrococcus spp.	2 (0)	1 (1)	2 (1)	3 (2)	1 (1)		9 (1)
Coliforms	2 (0)		1 (0)				3 (0)
Clostridium spp.			1 (0)				1 (0)
Bacillus spp.	3 (0)						3 (0)
Culture negative	588 (66)	61 (41)	69 (33)	40 (26)	35 (37)	15 (56)	808 (53)
Total	910 (100)	147 (100)	208 (100)	156 (100)	95 (100)	27 (100)	1543 (100)

CMT = California Mastitis Test; N = negative; T = trace; 1–3 = degree of CMT score; CM = Clinical mastitis; CNS = Coagulase negative staphylococci.

The sampling probability was 27% for herds located in Gondar and 25% for herds located in Bahir Dar. The overall prevalence of SCM, based on CMT, corrected for the sampling design was 62% (95% CI: 58%–67%), and 33% (95% CI: 30%–36%), at cow and quarter level, respectively. Bacteria were cultured from 49% (95% CI: 43%–4%), of the milk samples. Bacterial growth occurred in 67% of CMT positive, 47% of CMT negative, and 40% of CM quarters. A total of 81% (95% CI: 75%–87%), of the cows from which milk was cultured had at least one culture-positive quarter. The culture results for the different types of quarters are presented in Table 3. Coagulase-negative staphylococci were the most frequently isolated bacteria in all types of quarters, followed by *S. aureus*. Cows with CM or SCM in one or more of the quarters were more often positive in culture than the CMT negative cows. The overall cow level prevalences of mastitis causing pathogens were 81% and 87% in cows with CM and SCM respectively, and 62% in cows with 4 CMT negative quarters. The overall quarter level prevalences were 48% and 55% in cows with CM and SCM quarters and 30% in quarters from cows with 4 CMT negative quarters.

Most farms were small in herd size (Fig. 1A) and in number of lactating cows (Fig. 1B), and were positive for SCM, CNS and culturing any bacteria with substantial variation in prevalence between herds (Fig. 1C–E). The prevalence of *S. aureus* was generally low (Fig. 1F).

3.2. Cow level and herd level risk factors

Treatment history for mastitis was strongly correlated with CM history (r = 0.96), therefore CM history was excluded from the analyses. In the univariable analyses, 6 cow-level factors were found to be associated with SCM, 3 were associated with culturing any bacteria, 5 were associated with culturing *S. aureus*, and 2 were associated with culturing CNS. Based on the approach used, in the model with SCM as dependent variable, membership of dairy cooperative was confounded with farmers' level of education, and herd size was confounded with floor type. Of the potential herd level risk factors tested by the univariable analyses, 10 were associated with SCM, 13 were associated with culturing any bacteria, 6 were associated with *S. aureus* and 7 were associated with CNS. Variables that remained significant in the multivariable models are presented in Table 4.

4. Discussion

The objectives of this study were to estimate the prevalence of SCM and of specific mastitis pathogens, and to identify associated risk factors at cow and herd level, specific to the situation of urban and peri-urban farmers using cross-breed dairy cattle in North-West Ethiopia.

The prevalence of SCM estimated in our study (62% at cow level and 33% at quarter level) was substantially higher than the earlier reported 22.3% at cow and 10.1% at quarter level in central Ethiopia (Getahun et al., 2008) and 34.4% at cow and 17.9% at quarter level in North-West Ethiopia (Almaw et al., 2008). These studies, however, used CMT score ≥1 as cut-off for SCM, which is higher than the cut-off value (≥trace) we used. The prevalence in our study would, however, still be higher (49% at cow and 24% at quarter level) if a cut-off value of ≥1 would have been used.

From 87% of the cows and 48% of the quarters, in our study at least one species of bacteria was identified. This is almost the same as reported by Tolosa et al. (2015) in Southern Ethiopia who found 85% at cow level and 51% at quarter level. These estimates are much higher than what was reported by Abera et al. (2012) who identified 30.3% at cow level and 10.3% at quarter level in Southern Ethiopia. These findings indicate the prevalence of SCM has increased over the years in different parts of Ethiopia. This might be due to chance, but possibly also to changes in infection pressure, to an increase in the number of cows with higher HBL or to other factors, which needs further study. Unlike some previous studies that reported *S. aureus* to be dominating (Getahun et al., 2008; Abera et al., 2012), CNS was the most prevalent bacteria in our study, followed by *S. aureus* (Table 3), as was described by Almaw et al. (2008).

Not surprisingly, the prevalence of mastitis pathogens in quarters of cows with CM or SCM quarters was almost twice as high as in quarters of cows with four CMT negative quarters. Additionally, substantial variation in prevalence of SCM and positive culture between farms was found. This between herd variation could to a certain degree be explained by differences in cow and herd level risk factor status. The HBL, the level of feeding and checking the udder for CM are the most important risk factors in our models. Five of the risk factors associated with culture of any bacteria were also associated with culture of CNS, which can be explained by the fact that the vast majority of positive culture results consisted of CNS. There were less risk factors that were associated with culturing *S. aureus* than with CNS, due to the fact that *S. aureus* was less prevalent than CNS, yielding a lower power to detect significant associations. Except one, no more significantly associated risk factors were shared between CNS and *S. aureus*. This suggests that factors that affect the prevalence of *S. aureus* are different from those driving the prevalence of CNS. The odds of *S. aureus* was higher in herds with more than 10 cows. The strong effect of herd size on *S. aureus*, which was not found for other dependent variables, suggests that contagious transmission plays a more important role for *S. aureus* than for other pathogens which is in line with earlier reports (e.g., Lam et al.,

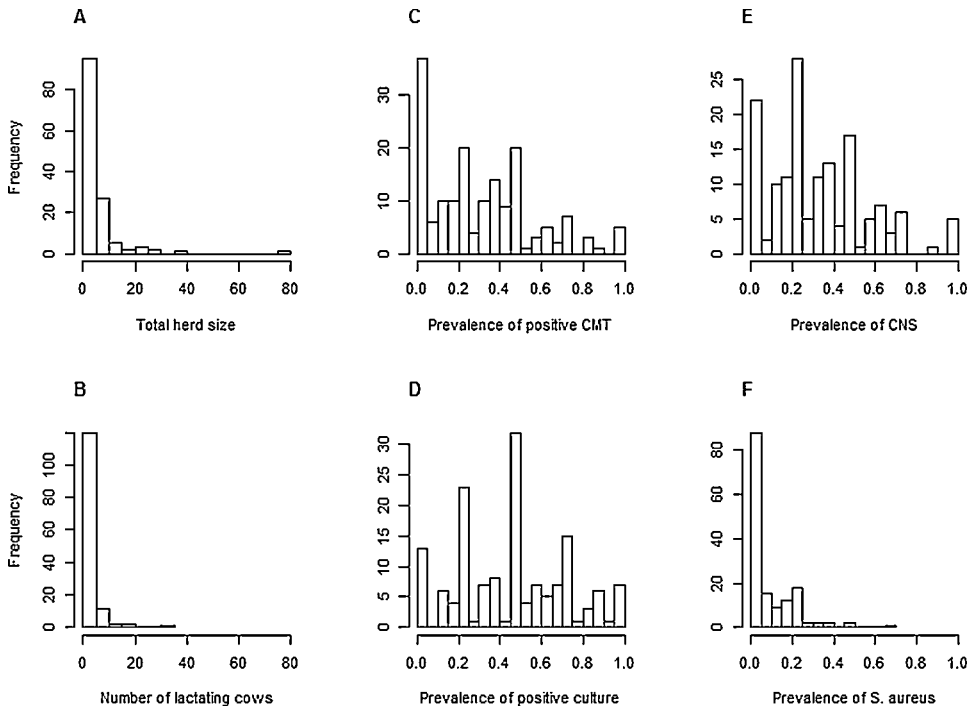

Fig. 1. Histograms showing the distribution of the total number of cattle per herd (A) and the number of lactating cattle per herd (B), based on questionnaire data of 136 herds. Herd-level prevalence of quarters with subclinical mastitis by CMT score (C), based on 167 herds, and herd-level prevalence of quarters from which any bacteria (D), coagulase-negative staphylococci (CNS) (E), and *Staphylococcus aureus* (F) were cultured in 151 herds in Gondar and Bahir-Dar, Ethiopia.

1996). These findings indicate that management measures should be tailored to the specific herd situation, including information on the pathogen causing problems in a particular herd. We identified a large number of risk factors for SCM in general, culturing any bacteria and for CNS, of which DIM was found important in all of them. For *S. aureus*, however, we only found that cows in larger herds are at greater risk. This indicates that if *S. aureus* is found in herds with SCM problems, on top of the measures mentioned above, specific attention for reducing transmission of mastitis pathogens is needed. To decrease pathogen transmission, previously management measures such as increasing hand hygiene and post milking teat disinfection (PMTD) were found to be helpful (Workineh et al., 2002; Mungube et al., 2004; Barkema et al., 2006).

History of CM is the only risk factor that was found for both, SCM and *S. aureus*. Sarker et al. (2013) has reported higher odds of SCM in cows with history of CM in Bangladesh. The likely explanation is that CM cases did not fully cure due to ineffective treatment which leads to chronic SCM. There are many factors that may influence the bacteriological cure after treatment of CM (Sol et al., 1997). Barkema et al. (2006) have reported that in *S. aureus*, the probability of cure depends on cow, pathogen, and treatment factors. Low cure rates may, for instance, be due to suboptimal treatment or detection of CM cases at a chronic stage of infection. We found that checking udders for CM reduced the odds of SCM, probably due to the fact that these farmers find new cases at an earlier stage, allowing them to take appropriate measures such as contacting a veterinarian for treatment. In that way, they may also reduce the spread of infection in their herds.

Higher HBL was associated with significantly higher odds of culturing *S. aureus*. This may be related to feeding practices; in our data, cows with higher HBL produced significantly more milk, with correlation coefficient between HBL and milk yield 0.41, 95% CI = 0.32–0.49, P-value < 0.05, therefore such cows need more feed. However, farmers in North-West Ethiopia may not take the blood level into account when feeding their cows. Overall, we found lower odds of CNS and of any bacteria when cows were fed more, but the interaction between HBL and feeding level was not statistically significant. Nowadays, many farmers in Ethiopia are using Holstein semen to improve the milk yield of their indigenous breeds. This shift to higher HBL may be necessary to be accompanied by an improved feeding and stall and milking hygiene, because cows with higher HBL seem to be more susceptible to intramammary infections.

Cows >150 DIM are at higher risk of SCM, of culturing any bacteria, and of having a positive culture for CNS. This is consistent with previous reports from Ethiopia (Getahun et al., 2008; Mekonnen and Tesfaye, 2010; Tolosa et al., 2015) and from other countries (Busato et al., 2000; Unnerstad et al., 2009). The fact that we did not find a significant effect of lactation stage on culture of *S. aureus* may again have resulted from a lack of power to detect such an effect. Cows that have been milked longer have had more exposure to mastitis pathogens than cows in early lactation. Coagulase-negative staphylococci are frequently isolated from quarters with SCM, and are abundantly present in extramammary habitats such as teat apices (De Visscher et al., 2016). This type of infection can be prevented by optimal hygiene of the udder and through PMTD (Pyörälä and Taponen, 2009) a measure hardly ever practiced in Ethiopia (Workineh et al., 2002; Mungube et al., 2004).

The higher odds of SCM in cows owned by farmers who have an education level >10th grade likely is the result of confounding. Most dairy farmers who have had >10th grade level of education have an additional source of income, and their dairy farm often is not their main source of income. The dairy activities in these farms are done by hired workers, who are often poorly educated and may perform suboptimal mastitis management, leading to transmission of mastitis pathogens.

Soil floor reduced the odds of SCM, as measured by CMT score, and culturing of any bacteria as compared to cows on a cemented floor. Even though direct comparison is not possible, Abera et al. (2012) in Ethiopia found lower prevalence of mastitis in cows housed in concrete-floored than in cows housed in soil-floored while Kivaria et al. (2004) from Tanzania didn't find an association

Table 4
Summary of the final multivariable mixed models describing the associations (P < 0.05) between risk factors and subclinical mastitis or culture of any bacteria, S. aureus or CNS based on data on 1291 quarters of 345 dairy cows on 136 dairy farms in North-West Ethiopia, taking the sampling design into account and modeling herd and cow random effects.

Variable level	Variable	Quarter level SCM[a] OR[d] (95% CI)	Any bacteria[b] OR (95% CI)	S. aureus OR (95% CI)	CNS[c] OR (95% CI)
	Region				
	Bahir Dar			Ref.[e]	
	Gondar			0.34 (0.22–0.52)	
Cow level	CM history[f]				
	No	Ref.		Ref.	
	Yes	1.86(1.35–2.57)		1.96 (1.36–2.80)	
	Days in milk				
	≤60	Ref.	Ref.		Ref.
	>60 to 150	1.50 (0.94–2.38)	1.02 (0.73–1.42)		1.28 (0.89–1.85)
	>150 to 250	1.93(1.20–3.11)	1.57 (1.08–2.29)		1.45 (0.97–2.16)
	>250	2.63 (1.57–4.39)	2.61 (1.64–4.15)		2.52 (1.68–3.78)
	Parity				
	Parity1	Ref.			
	Parity 2	0.94 (0.62–1.41)			
	Parity 3	2.12 (1.39–3.23)			
	Parity≥4	1.36 (0.91–2.04)			
	HBL[g](%)				
	<25		Ref.	Ref.	Ref.
	25–50		1.77 (1.16–2.70)	1.17 (0.62–2.22)	1.69 (1.04–2.75)
	>50		1.28 (0.79–2.08)	1.92 (1.03–3.60)	0.89 (0.52–1.52)
Herd level	Farmers' level of education				
	≤6 grade	Ref.			
	7 to 10 grade	1.07 (0.69–1.68)			
	>10 grade	1.89 (1.18–3.02)			
	Membership of a dairy cooperative				
	No	Ref.			
	Yes	1.36 (0.92–2.00)			
	Checking the udder for clinical mastitis				
	No	Ref.			
	Yes	0.47 (0.27–0.82)			
	Floor type				
	Cement	Ref.	Ref.		
	Stone	0.95 (0.58–1.57)	0.63 (0.42–0.95)		
	Soil	0.43 (0.24–0.76)	0.51 (0.27–0.95)		
	Herd size				
	≤5	Ref.		Ref.	Ref.
	6 to 10	1.22 (0.79–1.89)		1.66 (1.03–2.67)	1.48 (1.04–2.10)
	>10	1.05 (0.60–1.84)		1.74 (1.11–2.73)	0.77 (0.52–1.14)
	Milking method				
	Stripping		Ref.		Ref.
	Squeezing		1.41 (0.83–2.38)		1.76 (1.05–2.93)
	Both		3.00 (1.25–7.22)		4.03 (1.60–10.15)
	Calves suckling with cow				
	No		Ref.		Ref.
	Yes		0.66 (0.46–0.95)		0.54 (0.37–0.78)
	Level of feeding				
	Below requirement		Ref.		Ref.
	Meet requirement		0.76 (0.54–1.07)		0.60 (0.42–0.86)
	Above requirement		0.40 (0.22–0.73)		0.44 (0.29–0.68)
	Cleaning udder before milking				
	No				Ref.
	Yes				1.88 (1.03–3.44)
	Interviewers' evaluation of general hygiene				
	Poor		Ref.		
	Good		0.70 (0.49–0.99)		
	Very good		0.47 (0.26–0.86)		

[a] Subclinical mastitis based on the California Mastitis Test.
[b] Quarters from which any one or two species of bacteria were isolated.
[c] Coagulase-negative staphylococci.
[d] OR = population-averaged odds ratio.
[e] Reference category.
[f] Cow had clinical mastitis at least once in her life.
[g] Holstein-Friesian blood level.

between floor type and CMT positive quarters. Unlike a soil floor, infected milk is not absorbed by cement and thus can be a source of new IMI. Also, cemented floors may damage the teats, which increases the chance of new infections. Our finding of a lower CNS prevalence and lower odds of culturing of any bacteria in cows with suckling calves supports the report of Karimuribo et al. (2006). They studied the association between residual calf suckling and culturing mastitis pathogens and found that residual calf suckling was protective for a bacteriologically positive quarter. A possible explanation may be that calves remove residual milk from the udder, leading to less new IMI.

Milking by stripping was associated with lower odds of culturing CNS and any bacteria, in line with Karimuribo et al. (2008) who also found significantly less CMT positive quarters in Tanzanian farms practising stripping compared to farms practising squeezing. In contrast, Tolosa et al. (2013) reported a higher likelihood of SCM in cows milked by stripping. As we do not know the underlying biological mechanisms, more work is needed to come to evidence-based advice on the optimal milking technique in tropical circumstances.

If cows were, according to the farmers, fed above their requirement, a lower odds of CNS and of any bacteria was found. This might be due to the fact that well-fed animals are less susceptible to disease. It is reported that deficiencies of energy, protein, minerals, or vitamins have been associated with increased disease susceptibility (Valde et al., 2007). Nutrition affects udder health by affecting immune response (Hogan et al., 1993). As insufficient feeding may have an important influence on the risk of mastitis, dairy farmers who already think that they are feeding their cows insufficiently, probably should improve the feeding management, but this is not always possible.

Not surprisingly, better general hygiene was associated with lower odds of culturing of any bacteria. However, in contrast with our expectation, practising udder cleaning increased the odds of culturing CNS. This may be related to how udder cleaning is performed. Of farmers that use a cloth or towel for drying of the udders, 88% (53/66) use the same cloth/towel for drying of the udders of all lactating cows in the herd. This udder drying practice may contribute for the spread of udder pathogens abundantly present on teat apices and udder. Coagulase-negative staphylococci are known to be among the contagious types of pathogens (De Visscher et al., 2016).

In this observational field study, we used both CMT and bacteriological culture to determine udder health status of the cows and quarters. Both methods are imperfect tests to identify the true mastitis status (Dohoo et al., 2011), with both imperfect sensitivity and specificity. It seems reasonable to assume that these test characteristics are independent of risk factor status, resulting in non-differential misclassification. Therefore the strength of the associations between independent and dependent variables has probably been underestimated suggesting that some risk factors could not be identified.

5. Conclusions

The prevalence of SCM in urban and semi-urban dairy farms in Ethiopia varied substantially between herds, but generally was high compared to previous reports, with CNS and *S. aureus* being the dominant causative agents. Few risk factors were associated with culture of *S. aureus*, indicating that a better understanding of the drivers of transmission of this important pathogen is needed. Although further study is needed at some points, a number of herd and cow level factors were identified, that can be helpful in tailoring udder health improvement programs in urban and peri-urban dairy farms of North-West Ethiopia.

Conflict of interest statement

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2017.06.009.

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