Mastitis management in urban and peri-urban dairy herds of North-Western Ethiopia

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Mastitis management in stedelijke en peri-urbane melkveestapels van Noordwest-Ethiopië
(met een samenvatting in het Nederlands)

Proefschrift

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Contents

Chapter 1

General introduction

Introduction

With 7.2 million dairy cows, Ethiopia has the largest cattle population of all African countries (CSA, 2017). It has, for instance, twice the number of dairy cows in France (Eurostat, 2016). Dairy cows are mainly kept in the Ethiopian highlands, which occupy the central part of the country, cover over 40% (approximately 490.000 km^2) of the country, and have a temperature ranging between 13-24 °C (Fazzini et al., 2015). In most parts of the country the climate makes crop and pasture production possible (SNV, 2008). Currently, Ethiopia already has a shortage of dairy products and imports these products from other countries (Tegegne et al., 2013), while it has an increasing demand for these products. This provides potential for further growth of the dairy industry. Besides increasing the number of animals, an increase in production per animal, therefore, is a more likely way to go, also because the current milk production per cow often is relatively low with quite some potential for improvement. This asks for improving the efficiency of production, for instance by improving animal health and specifically udder health.

Mastitis is one of the major production diseases in the dairy sector worldwide. With the introduction of Holstein-Friesian blood in Ethiopian cattle, mastitis hampering the dairy production has become a bigger problem (Lema et al., 2001; Mungube et al., 2005; Tesfaye et al., 2010). A lack of knowledge about the consequences of mastitis in the Ethiopian context hinders quantification of the potential of improving the mastitis situation. In order to be able to judge the potential gains associated with working on udder health in the country, assessing the current mastitis situation is a first step. Subsequently, knowledge of the important mastitis determinants, such as the dominant pathogens and their transmission routes, and risk factors for poor udder health, is essential. Once the mastitis situation is known and the key management factors are identified, in theory, one only has to implement this knowledge. It is well known, however, that technical knowledge alone is not enough to improve udder health (Kuiper et al., 2005; Jansen et al., 2009; Jansen et al., 2010). Hence, understanding what motivates dairy farmers to actually apply available knowledge and to control mastitis is also crucial to successfully improve the mastitis situation.

For these reasons, this thesis deals with the epidemiology of mastitis as well as with the motivation of dairy farmers to control mastitis. In this general introduction, first the dairy production sector in North-Western Ethiopia will be described, followed by the mastitis situation (occurrence, costs and control) and the current knowledge on the mindset of dairy farmers with respect to udder health. Finally, the outline of the thesis will be given.

Dairy production in Ethiopia

Until the second half of the 20th century, dairying in Ethiopia was done in the same traditional way it had been done during many ages. The system was not market oriented and most of the milk, which was produced by indigenous Zebu cows, was used as fresh milk for home consumption. Surplus milk produced was often processed manually to milk products such as butter, *ayib* (a local product resembling cottage cheese), and sour milk, again primarily for consumption by the household, although some of these products were marketed (Redda, 2001). Modern dairying started in the early 1950s, when the country received the first Holstein Friesian (HF) cows from the United Nations Relief and Rehabilitation Administration. With the introduction of these cattle in Holleta and the former Shola dairy farms in Addis Ababa, commercial liquid milk production started in

Ethiopia (Ketema, 1998). The government encouraged dairy production through the introduction of these high-yielding dairy cattle and by promoting cross-breeding HF cows with indigenous Zebu cattle. This crossbreeding was introduced in 1967 and is nowadays widely practiced (Gebremedhin et al., 2009).

Dairy production is of great economic importance for Ethiopia. The sector contributes 45% of the Agricultural Production Value, and 12-16% of the total national Gross Domestic Product (Tegegne et al., 2013), with a large number of people being employed in the sector. For individual farmers, dairy production leads to the availability of food, as well as to a regular cash flow (Tangka et al., 2002). For rural people, livestock often is an important part of their assets. Even though the total milk production in Ethiopia is currently growing rapidly, there still is a shortage of dairy products. Figure 1, which is drawn based on data obtained from the FAOSTAT database, depicts the development of the number of milking cows in Ethiopia between 1961 and 2014 (FAO, 2017). The milk production is expected to continue to grow over the forthcoming years. Based on an increasing human population and a growing income of the majority of these people, a higher demand and therefore increased commercial potential for dairy products is to be expected.

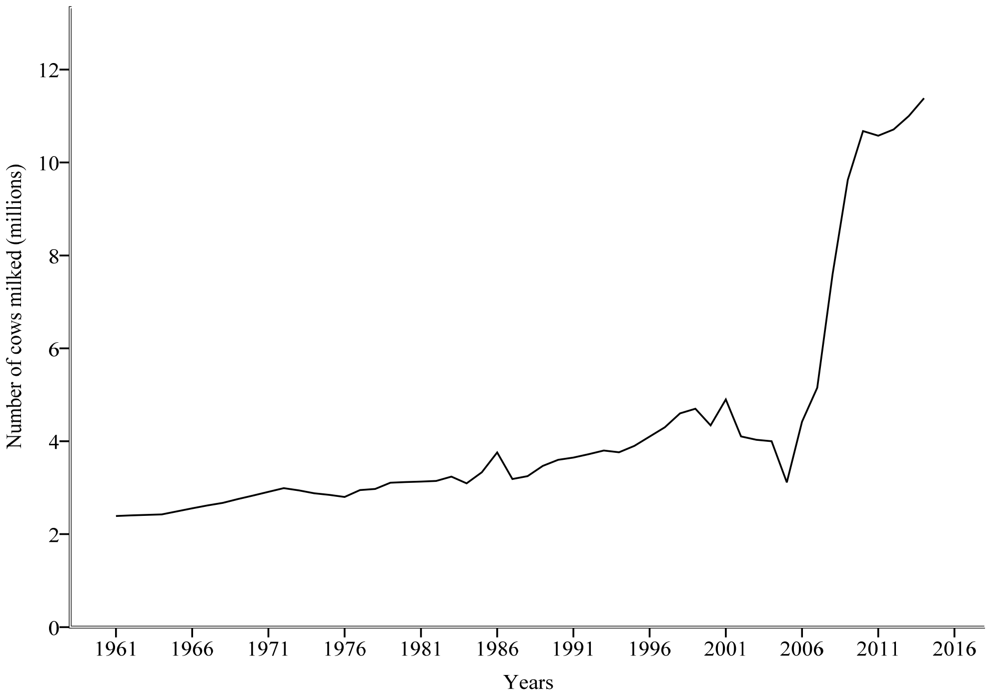

Figure 1. Development in population of milking cows during the years 1961-2014 in Ethiopia based on the FAOSTAT database (FAO, 2017).

Types of dairy farms in Ethiopia

There are three different types of dairy farms in Ethiopia that are primarily classified based on location of the farms and further on connection with crop farming and orientation of the milk production to the market (Gebre-Wold et al., 1998; Redda, 2001; Yilma et al., 2011). Although some of the smaller holdings might be considered as households with one or two cows, we consider them as dairy farms in this thesis. The types of dairy farms are: urban, peri-urban and rural. The urban and peri-urban dairy production is market-oriented and includes smallholder as well as specialized commercial dairy farms, mainly concentrated in and around Addis Ababa and other regional towns

(Yilma et al., 2011). The rural dairy production system predominantly uses indigenous breeds and includes pastoralist and mixed crop-livestock producers (Ahmed et al., 2004). Unlike the urban and peri-urban dairy production, the rural dairy production system is not market-oriented and most of the milk produced is used for home consumption. The characteristics of these three types of dairy farms are summarized in Table 1.

Table 1. Characteristics of urban, peri-urban and rural dairy farms of Ethiopia.

Characteristics	Urban	Peri-urban	Rural
Location	In cities and/or towns	Close to cities and/or towns	In the rural areas of the country
Scale based on number of cows[1]	Small and medium	Small, medium and large	Small
Market orientation	Market-oriented	Market-oriented	Not market-oriented
Milk and milk product selling	Formal and informal	Formal and informal	None
Use of inputs (e.g. feed) and services (e.g. AI[2])	High	Medium	Low
Breed of cows	Mainly HF×Z[3]	Mainly HF×Z	Indigenous, mainly Zebu
Main purposes	Milk production	Milk production	Draught power is the main purpose; milk is a by product
Breeding	AI and bull mating	AI and bull mating	Mostly by bull mating
Husbandry	Intensive	Semi-intensive	Extensive
Feeding	Stall feeding	Stall feeding	Grazing
Development	Expanding	Expanding	Slowly substituted by market oriented production system
Major constraints	SFL[4]; lack of extension services; seasonality in demand for milk	SFL; shortage of AI and extension services; seasonality in demand for milk	SFL; poor access to inputs and services

[1]small = 1-5, medium = 6-10, large = > 10 cows in a herd; [2]Artificial insemination; [3]Holstein Friesian × Zebu; [4]Shortage of feed and land.

Sources: Gebre-Wold et al. (1998), Holloway et al. (2000), Redda (2001), Tangka et al. (2002), Ahmed et al. (2004), Ayenew et al. (2009), Yilma et al. (2011), Tegegne et al. (2013).

Dairy management in Ethiopia

In this thesis, dairy farm management is defined as the use of human, natural and material resources for the purpose of dairy production and directing them in order to maximize profit while optimizing the use of the available input. The way dairy farm management is executed is the result of a combination of human, physical and financial resources, and of cultural values. Although the dairy farmer does not always perform all of the work on the farm by himself, the farmer generally is the person who decides on management issues. In order to be able to improve the management of dairy farmers, it is important to fill gaps of knowledge in farm management. Because dairy farming in Ethiopia differs in many aspects from dairy farming in developed countries, the optimal management, including udder health management, also differs. Milking is, for example, performed manually twice daily by stripping (milking by thumb and index finger), squeezing (milking by five fingers) or a mixture of both. In some farms calves suckle the cow before milking (Tolosa et al., 2013). Most dairy farmers use a towel/cloth to dry multiple udders. Furthermore, although teat disinfection and dry cow therapy are considered important in controlling mastitis in western countries (Barkema et al., 2006), these mastitis control measures are not practiced at all by Ethiopian dairy farmers (Workineh et al., 2002; Mungube et al., 2004).

Mastitis in Ethiopia

Occurrence of mastitis pathogens in Ethiopia

Mastitis, an inflammation of the mammary gland, is generally caused by bacterial intramammary infections (**IMI**) and can either present itself as clinical mastitis (**CM**) or have no clinical symptoms, which is then described as subclinical mastitis (**SCM**). Subclinical mastitis is often diagnosed by means of the somatic cell count (**SCC**). Although no absolute SCC minimum can be used for detection of an IMI, the probability that an IMI is present increases as the SCC increases. Somatic cell counts that are greater than 200,000 cells/mL generally indicate an abnormality, mostly due to microorganisms infecting the gland (NMC, 1999). Mastitis is economically one of the most important diseases in dairy cows worldwide (Seegers et al., 2003; Halasa et al., 2007). Subclinical mastitis, as compared to CM, is considered to account for a considerable proportion of the economic losses due to production losses and increased risk of CM on dairy farms (Petrovski et al., 2006; Abrahmsén et al., 2014). Subclinical mastitis can also result in decreased milk quality such as reduced shelf life of fresh milk (Busato et al., 2000; Seegers et al., 2003). Additionally, SCM may lead to clinical flare-ups (Ma et al., 2000; Reksen et al., 2006), and may be a source of new IMI in healthy cows (Oliver et al., 2004). Also in Ethiopia SCM is reported to be highly prevalent, with over 60% of the cows being infected (Dego and Tareke, 2003; Tolosa et al., 2013) and to cause significant economic losses (Mungube et al., 2005; Tesfaye et al., 2010). Dairy farmers in Ethiopia, however, generally do not seem to recognize SCM as an important problem (Almaw et al., 2008; Tolosa et al., 2013).

Mastitis can be caused by many different bacterial species, subspecies and serovars (Radostits et al., 2007). In Ethiopia, over the years, several studies have been carried out in which mastitis pathogens were identified from different types of milk samples. The results of these studies are summarized in Table 2. Staphylococci are worldwide described as an important cause of mastitis (Pyörälä and Taponen, 2009) and have been found to be the most common mastitis pathogen in Ethiopia (Mekonnen et al., 2005; Almaw et al., 2008; Lakew et al., 2009; Haftu et al., 2012). *Staphylococcus aureus* is cultured from milk samples from both CM and SCM cases, whereas CNS is predominantly cultured from SCM cases (Mekonnen et al., 2005; Lakew et al., 2009; Haftu et al., 2012).

Table 1. List of pathogens identified from milk samples from clinical and subclinical mastitis and from healthy quarters, and the proportion of identified pathogens in earlier Ethiopian studies.

Bacteria	Study							
	Dego and Tareke (2003)	Mekonnen et al. (2005)	Almaw et al. (2008)	Getahun et al. (2008)	Lakew et al. (2009)	Mekonnen and Tesfaye (2010)	Haftu et al. (2012)	Abera et al. (2012)
S. aureus	36.7	36.7	17.8	41.5	24.1	14.7	36	48.6
CNS[1]		15.5	49.6		17.3	21.2	8.6	15.7
S. epidermidis	1.5			22.5				
S. hyicus	1.0							
S. agalactiae	13.1	14.1	8.2	13.7	12.9	11.6	7.0	11.4
S. uberis	5.1	6.4	1.5	9.8	3.8	3.3		
S. dysgalactiae	5.6	5.7	6.7	2.9	6.8	6.4	3.1	
S. intermedius			5.2	2.0				
E. coli	10.1	3.9		0.5	7.5	7.5	27.3	2.9
A. pyogenes	6.1	1.8	0.7	2.0	7.5	3.3		
B. cereus	0.005		1.5	2.0		2.2		
C. bovis	1.0		3.7	0.5	3.8			
E. faecalis					1.5	10.6		
K. pneumoniea	4.1	0.7					8.6	
S. marcescens						4.2		
P. aeruginosa							2.3	
Micrococcus spp.	8.1	10.2	5.2	2.0	6.0	15.9		
Corynebacterium spp.		2.5					1.6	
Klebsiella spp.								1.4
Bacillus spp.	7.6	2.5			9.0			2.9
Source of milk samples[1]	CM+SCM	CM+SCM	CM+SCM+H	CM+SC+H	CM+SCM	CM+SCM	CM+SCM	CM+SCM

[1] CM = clinical mastitis quarters, SCM = quarters positive by California mastitis test, H = quarters negative by California mastitis test.

Risk factors for mastitis in Ethiopia

The identification of risk factors associated with the occurrence of mastitis and IMI can help to improve programs for the prevention and control of mastitis in dairy herds. Worldwide, many studies have been conducted on mastitis management and many risk factors were found to be associated with the occurrence of mastitis. In tropical dairy producing countries, for example, the following risk factors for SCM were described: water scarcity, barn size, residual suckling, use of a single udder towel for milking more than one cow (Kivaria et al., 2004), rainy season, history of CM cases, parity, breed (Oliveira et al., 2015), pendulous type of udder, body condition score (Sarker et al., 2013), age, breed, milking hygiene (Shittu et al., 2012), teat-end damage, cow dirtiness and breed (Iraguha et al., 2015). These studies have shown that risk factors of mastitis vary between studies, stressing the fact that risk factors may vary, depending on farm circumstances, region or country. As described above, the dairy industry in Ethiopia differs from that in the western world and there is only a very limited amount of studies that reported on one or more risk factors of mastitis in Ethiopia (Tolosa et al., 2013; Getaneh and Gebremedhin, 2017). Almaw et al. (2008) and Tolosa et al. (2013) have shown that Holstein Friesian × Zebu cross bred cows to be more susceptible to diseases such as mastitis than indigenous Zebu cows. Therefore, specific attention for risk factors for mastitis in this country is valuable.

Control of mastitis

The traditional 5-point plan of mastitis control consists of 1) effective post-milking teat dipping, 2) use of antibiotic dry cow therapy in every quarter at the end of each lactation, 3) appropriate treatment of clinical cases, 4) culling of chronically affected cows, and 5) optimal milking methods and use of a functionally adequate milking machine (Neave et al., 1969). This approach has provided an effective basis to dairy producers to manage mastitis for almost 5 decades (Nickerson and Oliver, 2014). Nowadays, the program has been extended and often a 10-pointplan is used (Ruegg, 2017). Applicability of these programs, however, depend on the local situation and the motivation of farmers, which both differ between countries and need specific attention if improvement of udder health is pursued.

In Ethiopia treatment of CM seems to be generally considered as an important way to control mastitis. In other parts of the world, antibiotic treatment of SCM is also an established component of mastitis control programs (Barlow, 2011) and is responsible for the largest proportion of use of antimicrobial products on dairy farms (Erskine et al., 2003). It is generally recommended that treatment of mastitis should be based on bacteriological diagnosis and treatment guidelines (Pyörälä and Taponen, 2009). In Ethiopia, for the treatment of CM only intramammary antimicrobials are applied, which can be purchased without veterinary prescription. Farmers treat their cows without having had adequate training and whilst being unaware of possible side-effects such as development of antimicrobial resistance. In previous Ethiopian studies on antimicrobial susceptibility of mastitis pathogens isolated from milk samples, resistance of *S. aureus* to commonly used antimicrobials was described (Getahun et al., 2008; Haftu et al., 2012). The level of antimicrobial resistance, however, is unknown. The level of antimicrobial resistance is relevant given the policy on the use of antimicrobials in animal husbandry, and the actual use in dairy cows in Ethiopia.

Motivation to control mastitis

There are several factors that influence motivation of farmers to change their behaviour with respect to mastitis management (Jansen et al., 2009; Jansen et al., 2010). It is important to have knowledge on these factors, in order to facilitate implementation of the available technical knowledge in the field. The motivation of dairy farmers to work on mastitis, which includes economic aspects of mastitis, therefore asks for specific attention.

Dairy farmers are responsible for their cows' health and production and make their own decisions with respect to implement or not implement mastitis control measures. To be able to decide on that, they first need to have access to the available knowledge on mastitis management, which is provided in Ethiopia by the livestock extension services. In Ethiopia, as in other countries, actual mastitis management will deviate from the advices given for reasons such as costs, attitude and mindset (Jansen et al., 2009; van den Borne et al., 2014). Understanding drivers of dairy farmers' motivation is important to tailor intervention programs aiming to improve udder health, through increasing the internal motivation of Ethiopian dairy farmers to implement mastitis control measures.

Worldwide, only recently, motivation of farmers to improve cattle health has received attention (e.g., Valeeva et al., 2007; Garforth, 2011; Bruijnis et al., 2013; van den Borne et al., 2014). In Ethiopia, only one study in this field was done, and that was related to foot and mouth disease (Jemberu et al. 2015). In that study, it was found that farmers were willing to change their behaviour and to apply foot and mouth disease control measures, but market oriented and rural farmers differed in motivation to control foot and mouth disease. This indicates that different types of farmers may need a different approach in order to change their behaviour with regard to animal health.

Costs of mastitis

The costs of mastitis consist of failure costs and of preventive costs (Hogeveen and Van der Voort, 2017). Visualizing failure costs of mastitis at the herd level is one of the factors that may motivate dairy farmers to improve mastitis control (Valeeva et al., 2007; Huijps et al., 2008). The failure costs of mastitis contain several types of costs, such as discarded milk, drugs for treatment, and veterinary service, but also less visible costs such as losses due to decreased milk production, increased labour for managing sick cows and culling (Wolfová et al., 2006; Gunay and Gunay, 2008; Boujenane et al., 2014). In many countries, dairy farmers underestimate the costs of mastitis, probably due to the invisibility of big parts of the these costs and the chronic nature of the disease (Hogeveen et al., 2011). Quantifying the costs of mastitis may therefore help to create awareness about the importance of the mastitis problem. Ideally, it is combined with a quantification of preventive costs to be able to compare advantages and costs of practicing mastitis control measures.

Outline of the thesis

The aim of this thesis is to contribute to the knowledge on mastitis which can help to improve udder health in Holstein Friesian × Zebu breed market-oriented dairy farms in North-Western Ethiopia. Factors of importance in this respect are the prevalence of mastitis, mastitis management, factors associated with occurrence of mastitis, and motivational aspects including economic factors. These subjects will be described in five separate chapters.

Chapter 2: In this chapter, prevalence of mastitis and mastitis causing pathogens are described. Risk factors associated with SCM and **IMI** are identified.

Chapter 3: Because *S. aureus* was found to be an important mastitis pathogen, the diversity of *S. aureus*, the prevalence of some virulence genes and of the antimicrobial susceptibility of *S. aureus* isolates are described.

Chapter 4: In this chapter, the costs of mastitis are quantified normatively using a bio-economic stochastic simulation model. Losses of milk yield related to mastitis as well as costs associated to milk yield losses are estimated based on Ethiopian data or on literature, data from other countries or expert opinions.

Chapter 5: In chapter 5, the mastitis costs were estimated using an economic model based on empirical data with the goal to validate the results from chapter 4, based on a different approach.

Chapter 6: This chapter describes how dairy farmers' attitude, subjective norms and perceived behavioural control are related to their intentions to control mastitis and to apply different measures of prevention.

Chapter 7: In this final chapter, the implications of the main findings are summarized and discussed in relation to the practical application of the findings to improve udder health in dairy farms in Ethiopia. The gaps that need further research are highlighted and main conclusions are drawn.

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Chapter 2

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 1,543 milk samples, being 27 quarters that showed signs of clinical mastitis (**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.

Keywords
Intramammary infection, dairy, Ethiopia, risk factor, mastitis.

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 focusing on in this paper, mainly keep Holstein-Friesian and Zebu cross-breed cows to produce milk 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 coagulase negative staphylococcus (**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.

Materials and methods

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 1,805 to 1,966 meters above the sea level and average annual rain fall varies from 1,077 to 1,460 mm.

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 liters 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.

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 1,209 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 enroll 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.

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 (Annex 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 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 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.

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.

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 litres, 8-15 litres or >15 litres

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

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.

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 to 15 seconds) 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 45º 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 hours where they were kept at -20°C until further processing.

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 24h. A quarter was considered culture-positive if any bacteria were cultured, and was considered culture-negative if no growth occurred after 24h 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).

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 to 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

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 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.

Results

Descriptive statistics

In total, 2,040 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 1,955 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 1,543 milk samples of 400 cows in 151 herds were cultured, 96 (6%) from quarters of cows with CM (n=25), 1,050 (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).
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% to 67%), and 33% (95% CI: 30% to 36%), at cow and quarter level, respectively. Bacteria were cultured from 49% (95% CI: 43%-54%), 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.

Table 3. California mastitis test scores in 1,955 quarters and bacteria isolated from 1,543 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)	1,543 (100)

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

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

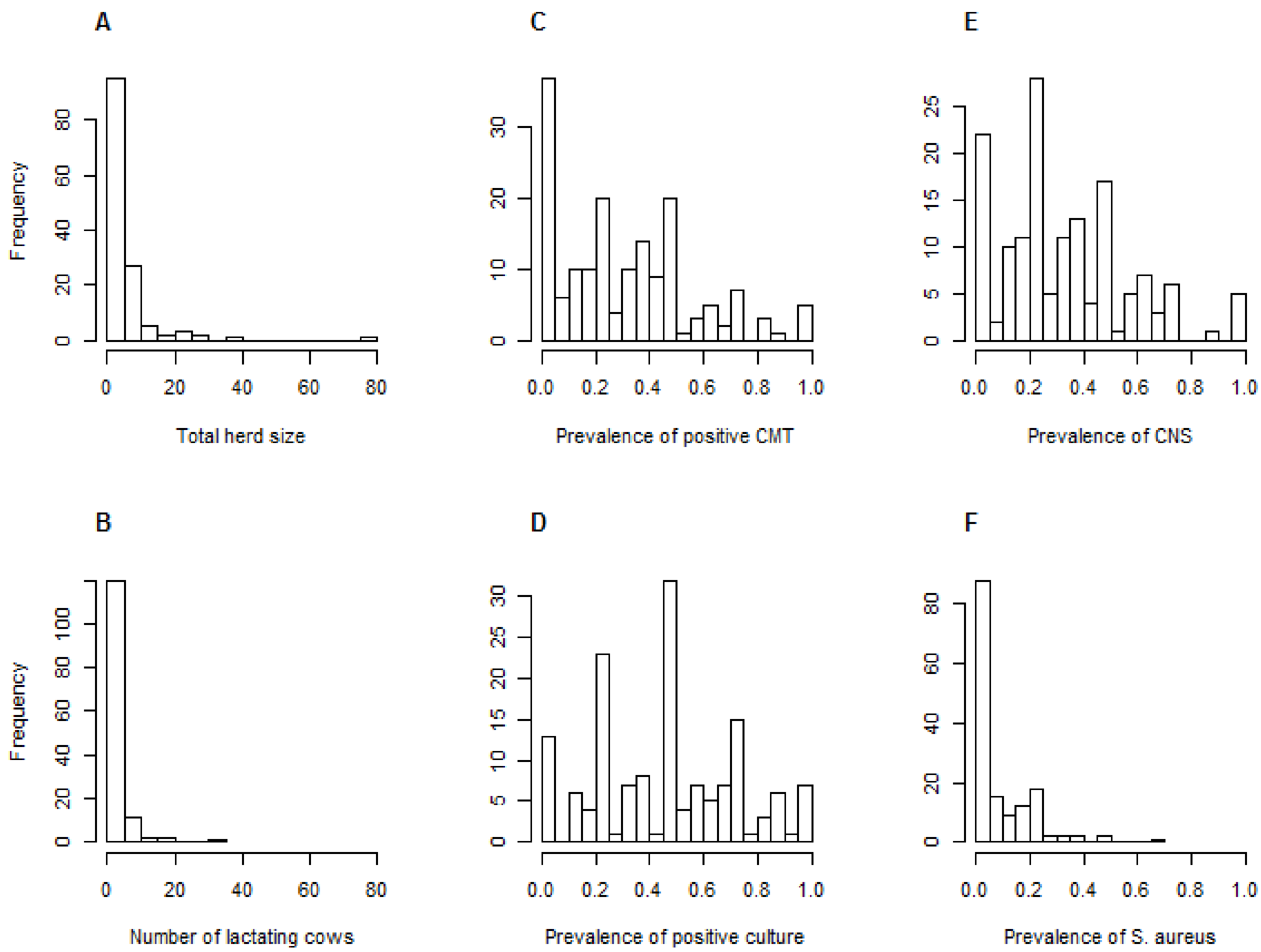

Figure. 1. Histograms showing the distribution of the total number of cattle per herd (A) and the number of lactating cattle per herd (B), based on questionnaire data of 136 herds. Herd-level prevalence of quarters with subclinical mastitis by CMT score (C), based on 167 herds, and herd-level prevalence 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.

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.

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 (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).

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 1,291 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]	Any bacteria[b]	*S. aureus*	CNS[c]
		OR[d] (95% CI)	OR (95% CI)	OR (95% CI)	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.

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., 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 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 practicing stripping compared to farms practicing 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, practicing 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.

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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Annex 1: The final English version of the herd level questionnaire on risk factors for mastitis used in this study.

Date____
Time ____
Dairy farm number: ___________
Name of the dairy farm ____
Zone ____
District/Woreda ____
Kebele ____
Village ____
Owners' demographic profile:

- Gender: O Male O Female
- Age _________________________________
- Level of education______________________________________

General questions

1. When did you start dairy farming? ______________________________
2. Total herd size _______________
   Heifers: Lactating __________________ Non lactating _____________________
   Cows : Lactating __________________ Non lactating ______________________
3. Number of animals in Holstein blood level: < 25% _______, 25-50% _______, >50% _______
4. Stall size? ________________________ (in m^2)
5. Are you member of a dairy farmers association? O Yes O No
6. Did you have any training about udder health? O Yes O No
   If yes, how many times you participated training?____
7. Do you get advice about mastitis control? O Yes O No
   If yes, a) who give you the advice?
   O Livestock extension workers
   O Veterinarian who has regular visit to my farm
   O Public employed veterinarians when I visit public veterinary clinic
   b) how often do you get advice? ____________________________________

Monitoring for mastitis

8. Do you have a veterinarian who supervise/visit your farm for mastitis and other health issues?
   O Yes O No
   If yes, how often is the visit of your veterinarian? ____________________________________
9. Do you inspect the udder of cows for mastitis? O Yes O No
   If yes, how often do you inspect? O Always before milking O Sometimes
   Other___________________
10. Do you check teats for mastitis before milking?
    If yes, how often? O Always O sometimes Other ___________________
11. Do you milk away the first streams of milk while milking? O Yes O No
    If yes what is the reason?
    O To open the teats O To check mastitis Other ____________________________

12. Do you check the udder for mastitis when you purchase new cows?
    O Yes O No
    If yes, how do you check? ____

Type of stall and stall hygiene

13. Type of stall: O Tie-stall O Free-stall
14. Floor material of the stall: O Soil O Stone O Concrete
15. Does the stall floor has drainage?
    O Yes, it drains urine and other liquid wastes.
    O No, urine and other liquid wastes accumulate on the floor.
16. Roof material of the stall: O n O Grass O Plastic
17. Do you use bedding in the stall? O Yes O No
    If yes, how often do you add new bedding? ______________________________
18. How often do you clean the stall? ____
19. Do you remove the dung? O Yes O No
    If yes, how often ____

Mastitis and mastitis treatment

20. Do you know whether mastitis exists? O yes O No
21. Do you know that different types of mastitis exist? O Yes O No
    If yes, describe the different types of mastitis____
    ____
    ____
22. How do you recognize clinical mastitis?
    ____
23. Do you believe that mastitis is a preventable disease?
    O Yes O Partly O No
24. Did any of the cows in your farm experience clinical mastitis during the last one year?
    O Yes O No If yes, how many of them? _________________________
25. Do you isolate cows that get sick due to udder infection or another disease?
    O Yes O No
26. Do you treat cows when affected by clinical mastitis? O Yes O No
27. How many of the cows affected by clinical mastitis in the last one year: were treated? _______ Not treated? ________
28. Do you treat by yourself? O Yes O No
    - If yes, how many of them treated? by you_________ by an expert_______________
    - If you treat clinical mastitis cases yourself, how do you treat them?
      ____
      ____
29. How many of treated cases had got repeated infection? Total ____; 2x _____, 3x _____; 4X ______
30. In those cows affected by clinical mastitis, how many quarters were affected in each cow?

Number of cows	No. of quarters per cow affected at the same time
	1
	2
	3
	4

31. Do you have any cow affected with clinical mastitis at the moment?
O Yes O No

Milking hygiene and milking

32. Do you restrain cows during milking? O Yes O No
If yes, how often the restraining material cleaned?
O After every milking O Once a day O Only when dirty O Not washed
33. Do you clean/wash the udder before milking? O Yes O No
If yes, when do you clean/wash the udder ? O at every milking O when the udder/quarter looks dirty; Other
____
How often do you clean the udder? O before milking only O after milking only
O both before and after milking,
Other____
34. Part of the udder you are washing? O whole udder O teats only
35. Do you use soap while cleaning/washing the udder? O Yes O No
If yes, how often do you use soap? O before milking only, O both before and after milking O after milking only Other_____________.
36. Do you use cloth/towel to clean/dry the udder? O Yes O No
If yes, how often do you use the cloth/towel ?
O before milking only, O after milking only, O both before and after milking.
37. How often do you clean the cloth/towel used to dry the udder?
O after every milking -------times/week, ----------times/month;
Other __________
38. Do you use one cloth/towel for more than one cow? O Yes O No
If yes, for how many cows do you use one cloth/towel? ____________cows
39. Do you wash your hands before milking? O Yes O No
If yes, how often do you wash your hands?
O Once only before milking all cows O before milking every cow
40. Do you use disinfectant for teats? O Yes O No
If yes, when ____
41. Do you use calves to stimulate milk letdown? O Yes O No
If yes, how do you avoid the calf from suckling to start milking?
____
42. Do you allow the calves to suckle after milking? O Yes O No
If no, how do you feed milk to the calves?
____
43. Do you milk mastitis and healthy cows separately? O Yes O No
If yes, when do you milk mastitic cows? O First O Last
44. Do you collect mastitic cows' milk into a separate milking equipment?
O Yes O No
45. Do you clean milking equipment between milking one cow and the other?
O Yes O No
If no, how often do you clean milking equipment?
O Twice, before all morning milking and before all afternoon milking
O Four times, before and after all morning milking and before and after all afternoon milkings

O Other ______________________________________

46. How is your milking method?
O Five fingers squeezing O Using two fingers/stripping

Feed and feeding practice

47. Do your cows graze on pasture? O Yes O No
If yes, for how many hours do they graze on pasture/day? minimum ________ maximum ________
48. Do you use grazing throughout the year? O Yes O No
If no, mention the months you use grazing on pasture? ______________________________
49. What is the source of water you are using for cows to drink and for cleaning milking utensils?
For cows to drink: OTap water O Spring O River O Pond/Well
For cleaning: O ap water O Spring O River O pond/Well
50. How often you supply water? O Two times a day Other (specify) ________________________________
51. Do you supply concentrate? O Yes O No
If yes, how often? O Consistently O infrequently
How much do you estimate the amount of concentrate you are supplying per cow per day? ________Kg
52. What do you think about the level of feeding of your cows?
O below the requirement O they get the requirement O above the requirement

Other important points

53. Did you encounter tick infestation on the udder?
O Never O Frequently O rarely
54. Do you use deworming to your cows? O Yes O No
If yes, how often? ________________
55. Do you vaccinate your cows? O Yes O No
If yes, for which vaccines? O When vaccines are free of charge O only vaccines with charge O vaccine both with and without charge
56. Is lameness a problem in your cows? O Never O frequently O rarely
57. What do you do with the milk immediately after milking? O Stored O Selling immediately
If stored, do you use refrigerator? O Yes O No
58. Who are your milk customers?
O People in the neighbourhood O Cooperative
O Shops, kiosks, and supermarkets O Restaurants, coffee and tea sales and hotels
59. Are there any other mastitis preventive measures that you practice? Mention them.
____
____
60. What is the general hygienic and management condition of the farm?

Factors	Poor	Good	Very good
General hygienic condition			
General management condition			

Annex 2: The final English version of the cow-level questionnaire used in this study.

Date______________________

Time ____________________

Dairy farm number: __________

Name of the dairy farm ____________________

Cow Id number/specific name_______________________________

1. Holstein Friesian blood level O < 25% O 25-50% O >50%
2. Presence of blind quarter(s) O Yes O No
3. History of Clinical mastitis O Yes O No
4. Experience of common dairy health problems (dystocia, retained placenta, hypocalcaemia and abortion) O Yes O No
5. Average daily milk yield ________________ liters
6. Parity ________________
7. Days in milk/Calving date ________________
8. Pregnancy status O Pregnant O Not preganant
9. Posture O Normal O Aberrated
10. History of lameness/fracture O Yes O No
11. Body condition score O poor O good O very good
12. Udder shape and symmetry O Symmetrical O Not symmetrical
13. Supernumerary teats O Present O Absent

Chapter 3

Genetic diversity and antimicrobial resistance of *Staphylococcus aureus* isolated from milk samples in dairy farms of North-Western Ethiopia

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Abstract

Staphylococcus aureus causes disease in many species and is an important cause of mastitis in dairy cattle. In Ethiopian dairy farms, *S. aureus* is often found as a cause of clinical or subclinical mastitis, but the genetic background and antimicrobial susceptibility of these isolates has not been studied. The aim of this study therefore was to characterize *S. aureus* from Ethiopian dairy cows, which was done by *spa* and MLST typing, testing for several virulence factors and antimicrobial susceptibility testing. A total of 79 *S. aureus* isolates from intramammary infections was used. A PCR was used to detect *lukMF'* and *pvl* genes encoding the bovine and human associated bi-component leukocidins, and the toxic shock syndrome toxin gene-1 (*tst*). Antimicrobial susceptibility was determined using the drug microdilution method. Twenty different *spa* types were identified, of which most were of *spa*-types t042 (58%), and the closely related t15786 (11%). The proportion of isolates positive for *lukMF'*, *tst* and *pvl* was low at 0.04, 0.10 and 0.09 respectively, with *lukMF'* often co-occurring with *tst*, but not with *pvl*. All isolates were susceptible to methicillin, but resistance to penicillin/ampicillin (86%) and tetracycline (54%) was common.
We found a high degree of similarity between bovine *S. aureus* isolates in North-Western Ethiopia, suggesting contagious within and between farm transmission of strains that are often resistant to commonly used antimicrobials. This highlights the need for effective preventative measures aiming to limit transmission of bacteria rather than using antimicrobials to control mastitis in Ethiopia.

Keywords
Staphylococcus aureus, antimicrobial resistance, dairy cow, mastitis.

Introduction

Staphylococcus aureus is a contagious pathogen that causes mastitis in dairy cattle (Cremonesi et al., 2015; Boss et al., 2016), and is an opportunistic pathogen in humans and many other animal species (Nübel et al., 2011; Zadoks et al., 2011; Sangvik et al., 2011). Also in Ethiopia, *S. aureus* is frequently identified from cows with mastitis (Getahun et al., 2008; Haftu et al., 2012; Mekonnen et al., 2017), and from milk or milk products (Tarekgne et al., 2016).

In dairy cows, *S. aureus* can be isolated from milk as well as different body sites (Joo et al., 2001; Middleton et al., 2002). Still, transmission of *S. aureus* intramammary infections (IMI) is believed to mainly occur during the milking process (Vanderhaeghen et al., 2015). As in Ethiopia cows are mainly milked by hand, the transmission routes of *S. aureus* may differ from countries in which machine milking is common. Additionally, hand milking may introduce possibilities for transmission between farmers and their cattle. Typing of *S. aureus* isolates may give insight in their likely origin, because MLST and spa-types are often host-associated and virulence factors can also be host specific (Herron-Olson et al., 2007; Smith et al., 2014). Furthermore, the genetic similarity of isolates within and between farms gives an indication of the importance of contagious rather than environmental transmission in the spread of *S. aureus* (Zadoks et al., 2011). Bovine *S. aureus* isolates often belong to clonal complexes (CC) 97, 133, 151 or 479 (Zadoks et al., 2011) and, depending on their clonal lineage, may carry the phage-encoded leukocidin *lukMF'* (Hata et al. 2010; Schlotter et al., 2012). This virulence factor is mainly present in *S. aureus* of ruminant origin (Vrieling et al., 2015) is a potent *S. aureus* toxin specifically killing bovine neutrophils (Vrieling et al., 2016), and likely contributes to the clinical severity of mastitis. The proportion of bovine *S. aureus* isolates encoding this toxin varies largely between countries (Koop et al., 2017), but to our knowledge this proportion has not been estimated in isolated for IMI in African cattle.

Because antimicrobial use, both in humans and animals is high and poorly controlled in Ethiopia, multidrug resistant *S. aureus* are frequently isolated from animals (Getahun et al., 2008; Haftu et al., 2012) and humans (Lemma et al., 2015; Eshetie et al., 2016; Dilnessa and Bitew, 2016). In *S. aureus* isolated from humans, there is a trend of increasing antimicrobial resistance (AMR) rates to commonly prescribed antimicrobials (Moges et al., 2014). Describing AMR patterns in both humans and animals may contribute to the knowledge on the importance of the issue in Ethiopia and to a more prudent and effective antimicrobial use.

In small holder dairy farming, effective control of *S. aureus* mastitis is important because mastitis can have a substantial effect on family income and because of the zoonotic potential resulting from the close interaction between animals and their owners. Molecular typing of isolates may help to identify what control strategies are most effective from an animal health as well as public health perspective. There is, however, only a limited number of studies that characterized bovine *S. aureus* isolated from Ethiopia (Seyoum et al., 2015; Tarekgne et al., 2016). The aim of this study, therefore, was to characterize *S. aureus* isolates from bovine milk samples in dairy farms of North-Western Ethiopia by *spa* typing, testing for virulence genes and quantifying antimicrobial susceptibility.

Materials and Methods

Isolate collection

For this study, a total of 79 *S. aureus* isolates was available (two from clinical mastitis (CM), 53 from California mastitis test (CMT) positive and 24 from CMT negative quarters) from an earlier described study in which milk samples were collected in a cross-sectional study in 167 urban and peri-urban small holder dairy farms in the regions Gondar and Bahir Dar, in the North-West of Ethiopia (Mekonnen et al., 2017). The 79 isolates were derived from 60 dairy cows in 42 dairy herds.

DNA extraction

Single colonies from an overnight culture were suspended in 1 mL of Milli-Q, homogenized using Vortex and centrifuged for 1 minute at 13,000 rpm. Supernatant was removed and the bacteria were re-suspended in 200 µL of Milli-Q, boiled for 10 minutes at 100 °C and centrifuged at 13,000 rpm for 1 minute. The extracted DNA was diluted 1:10 in Milli-Q and stored at -20 °C until further analysis.

Virulence genes detection

Polymerase chain reactions were performed on all 79 isolates using 10 µL of the DNA and different primers. Table 1 summarizes primer sets with the corresponding PCR protocol used. Amplifications were carried out in a final volume of 25µL using a T100™ Thermal Cycler (Bio-Rad, USA). The presence of the genes was examined after running 10 µL of the PCR-products on 1.5% agarose gel at 100 V for 40 min. PCR products were visualized with a molecular Imager Gel Doc XR+ Imaging system (Bio-Rad, USA).

Table 1. Sequences of primer sets with their corresponding PCR protocols and product sizes

Target gene	Primer sequence (5′-3′)	PCR-protocol	Amplicon size	Reference
FemA	F: TGCCTTTACAGATAGCATGCCA R: AGTAAGTAAGCAAGCTGCAATGACC	1	142 bp	(Francois et al., 2003)
mecA	F: GGCTATCGTGTCACAATCGTT R: TCACCTTGTCCGTAACCTGA	2	689 bp	(Melo et al., 2014)
Spa	F: AGACGATCCTTCGGTGAGC R: GCTTTTGCAATGTCATTTACTG	3	Variable	(Harmsen et al., 2003)
lukM	F: TGAGTGGGTATGGCATGAAAGA R: TGGACATTTTGTGTTACACCCC	4	572 bp	This study
lukF'	F: ACTCAGGCTATACCAACCCA R: CGAGCTACTCTGTCTGCCAC	1	425 bp	This study
Pvl	F: GCTGGACAAAACTTCTTGGAATAT R: GATAGGACACCAATAAATTCTGGATTG	5	85 bp	(Pajić et al., 2014)
Tst	F: CAACATACTAGCGAAGGAACT R: GATATGTGGATCCGTCATTCA	6	277 bp	(Cosandey et al., 2016)

[1] 95 °C for 2 min, 35x (95 °C for 30 s, 59.5 °C for 30 s, 72 °C for 35 s), 72 °C for 5 min and 12 °C for ∞
[2] 95 °C for 1.5 min, 30x (95 °C for 45 s, 55 °C for 30 s, 72 °C for 45 s), 72 °C for 5 min and 12 °C for ∞
[3] 95 °C for 2 min, 30x (95 °C for 45 s, 60 °C for 45 s, 72 °C for 1:30 min), 72 °C for 5 min and 12 °C for ∞
[4] 95 °C for 2 min, 33x (95 °C for 30 s, 58.2 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min and 12 °C for ∞
[5] 95 °C for 2 min, 35x (95 °C for 30 s, 55.5 °C for 30 s, 72 °C for 35 s), 72 °C for 5 min and 12 °C for ∞
[6] 95 °C for 2 min, 35x (95 °C for 30 s, 59 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min and 12 °C for ∞

Spa and MLST typing

The PCR-product of the *spa* gene was cleaned up from unbound primers and nucleotides (ExoSAP-IT, Affymetrix Inc., USA) and submitted for Sanger sequencing (Baseclear BV, The Netherlands). The *spa* types were assigned using BioNumerics version 7 (Applied Maths, Belgium), using the *spa*-typing plugin and Ridom SpaServer (http://www.spaserver.ridom.de). Multilocus sequence typing (MLST) was performed as described by Enright et al. (2000) on a subset of 10 selected isolates: 6 of most frequently identified spa types and 4 isolates of other *spa* types. Isolates were selected equally from the two regions (Gondar and Bahir Dar) and from 10 different farms. Sequence types were assigned using BioNumerics version 7 (Applied Maths, Belgium).

Antimicrobial susceptibility testing

Antimicrobial susceptibility (AMS) testing was performed by determining minimum inhibitory concentrations (MICs) using the MICRONAUT system (Merlin Diagnostika, Germany) in customized prepared 96-wells microtiter plates containing dilution series of 17 antimicrobials. Inoculum preparation, broth composition and incubation conditions were performed as recommended by the manufacturer. Reading of the plates after incubation was done with a photometer (Skan, Merlin Diagnostika, Germany). *Staphylococcus aureus* ATCC 29213 was used as quality control strain. Clinical breakpoints were used according to the Clinical and Laboratory Standards Institute (CLSI) standards (CLSI, 2015; CLSI, 2017). Veterinary breakpoints were used for ceftiofur (cattle), cephalotin (dogs) and enrofloxacin (dogs). For all other antimicrobials, human breakpoints were used in the absence of veterinary breakpoints. For fusidic acid, EUCAST-criteria were used (www.eucast.org), because no breakpoints were available in the CLSI standards.

Results

Genetic typing

In total, twenty different *spa* types were found, the majority belonging to t042 (58%) and the closely related t15786 (11%). The proportion of *lukMF'* positive *S. aureus* in our sample was low (3/79 = 4%). Seven isolates carried *pvl* (7/79 = 9%) and *tst* was detected in eight isolates (8/79 = 10%). All t14061 isolates were positive for *tst*, and *pvl* was present in all t355 and t1376 isolates. Table 2 summarizes the spa typing results and presents the isolates positive for the three toxin genes.

Table 2. Molecular characterization of 79 *Staphylococcus aureus* isolates from quarter milk samples from 42 dairy farms in North-Western Ethiopia.

spa type	Total (%)	*N isolates positive for*			Repeat sequence											
		lukMF'	*tst*	*pvl*												
t042	46 (58)	0	1	0	26	23	12					34	34	33		34
t15786	9 (11)	0	0	0	26	23	12				34	34	34	33		34
t355	3 (4)	0	0	3	07	56	12	17	16	16	33	31	57	12		
t14061	3 (4)	2	3	0	04	31	17									
t1376	2 (3)	0	0	2	07	12	21	17	13	13	34	34	33	13		
t488	2 (3)	0	0	0	07	12	21	17	13	13			34	33		34
t10018	1 (1)	1	1	0	04	31	17	25	17	25	17					
t306	1 (1)	0	1	0	26	23	17	34	17	20	17	12	17	17		16
t17835	1 (1)	0	1	1	14	22	75	16	24	24	24	24	24			
t223	1 (1)	0	1	0	26	23	13	23	05	17	25	17	25	16		28
t273	1 (1)	0	0	1	07	23	21	17	13		34	16	34	33		13
t2085	1 (1)	0	0	0	26									33		34
t4206	1 (1)	0	0	0	26	23						34	34	33		34
t9300	1 (1)	0	0	0	26	23	21									34
t4701	1 (1)	0	0	0	26	12	21	17			34	34	34	33		34
t17184	1 (1)	0	0	0	26	23	13			17	25	17				28
t2801	1 (1)	0	0	0	07	23	34	12	12	23	02	12	23			
t17834	1 (1)	0	0	0	07	23	02	12	23	02	02	02			34	
t17185	1 (1)	0	0	0	07	500	22	31	17							
t409	1 (1)	0	0	0	60	61	34	22	34	17						

All six t042 isolates subjected to MLST typing belonged to a new ST, ST4550 which is a double locus variant of ST97. The other isolates subjected to MLST were ST1 (t273), ST22 (t17184), ST88 (t488) and ST848 (t409).

Antimicrobial resistance

All 79 isolates were classified as methicillin-susceptible *S. aureus* as they were susceptible to cefoxitin and lacked the *mecA* gene. A high proportion AMR was found for penicillin/ampicillin (86%) and tetracycline (54%), but against other antimicrobials, resistance was limited. The MIC values and number of isolates resistant to the tested antimicrobials are presented in Table 3. No obvious associations between spa-type and AMR patterns were observed (Annex).

Table 3. Minimum inhibitory concentration (MIC) values of 79 *S. aureus* isolates cultured from quarter milk samples in 42 dairy farms in North-Western Ethiopia tested for susceptibility to 17 antimicrobials[1].

Antimicrobial	MIC (µg/mL)										Resistant n (%)
	0.0625	0.25	0.5	1	2	4	8	16	32	64	
PEN	11			2	10	27	18	8	3		68 (86)
AMP		11		2	30	22	7	4	1	2	68 (86)
TET			36				4	34	5		43 (54)
CLI			76			3					3 (4)
ERY		70	6					3			3 (4)
T/S			76	1	1		1				1 (1)
RAM	78			1							1 (1)
AMC		11	31	35		2					0
CTN					79						0
CET					79						0
COX						79					0
CMP						42	37				0
FUS				79							0
ENR		79									0
GEN					78	1					0
KAN								79			0
NEO							79				0

[1]PEN=penicillin, AMP=ampicillin, TET=tetracycline, CLI=clindamycin, ERY=erythromycin, T/S=Trimethoprim sulfamethoxazole, RAM=rifampicin, AMC=amoxicillin/clavulanic acid, CTN=cephalothin (1st generation cephalosporin), CET=ceftiofur (3rd generation cephalosporin), COX=cefoxitin, CMP=chloramphenicol, FUS=fusidic acid, ENR=enrofloxacin, GEN=gentamicin, KAN=kanamycin, NEO=neomycin.
Vertical lines indicate clinical breakpoints, with values to the left of the line being susceptible or intermediate and those to the right being resistant.

Discussion

The aim of this study was to characterize *S. aureus* isolates cultured from milk samples in North-Western Ethiopia based on *spa* typing, virulence genes and AMR. We identified t042 and the related t15786 as the dominant *spa* types in our sample, found in 69% of all isolates. Such a high degree of relatedness of isolates cultured suggests that contagious transmission takes place (Herron-Olson et al., 2007; Smith et al., 2014). The fact that many of our isolates were derived from different farms in two regions suggests that contagious transmission is not only taking place within farms, but also between farms, possibly through cattle movements between farms, and from the government breeding and heifer distribution centers. Four of the *spa* types (t042, t223, t306 and t355) identified in the current study were previously found by Tarekgne et al. (2016) in cattle in the Tigray region, Northern Ethiopia. Interestingly, in that study the dominant spa type was t314, which has a repeat sequence entirely different from the dominant types in our study. This suggests that, although contagious transmission seems to occur at a local scale, exchange of strains between regions does not always happen, resulting in dominance of different clones in different regions. Different regions have their own cross-breeding and breeding distribution centers, which make exchange of strains from the same source between regions less likely. The six t042 isolates subjected to MLST belonged to the new ST4550, which is a double locus variant of ST97, that is commonly found in bovine mastitis worldwide (Smith et al., 2005; Delgado et al., 2011; Rabello et al., 2007).

The proportion of isolates positive for *lukMF'*genes was low. As the majority of isolates in our study belonged to a ST related to ST97, this low *lukMF'* prevalence is in line with previous reports (Hata et al. 2010; Schlotter et al., 2012). The isolates that were positive for *lukMF'* belonged to t14061 and t10018, the latter of which has previously been found in nasal swabs from Nigerian abattoir workers (Odetokun et al., 2018). The bi-component leukocidin *lukMF'* is predominantly found in strains isolated from bovine *S. aureus* mastitis (Barrio et al., 2006) and specifically kills bovine neutrophils, whereas human neutrophils are not affected (Vrieling et al., 2015). Based on an experimental study, Vrieling et al. (2016) reported that LukMF' is produced during the course of infection and its level in milk was associated with severity of mastitis. The low proportion of *lukMF'* positive isolates in our samples may be partly due to the fact that they came from a cross-sectional study that included only two cases of clinical mastitis. In our collection the proportion of isolates positive for *pvl* was higher than that of *lukMF'*, despite the fact that bovine neutrophils are insensitive to the effect of this human-associated leukocidin (Koop et al., 2017). Although several authors have reported *pvl*-positive cattle *S. aureus* before (Zecconi et al., 2006; Hata et al. 2010), *pvl* is mainly detected in *S. aureus* of human origin (Dinges et al., 2000; Rainard et al., 2003; Vrieling and et al., 2016) while cattle isolates are often negative for *pvl* (Yamada et al., 2005; Monecke et al., 2007). The finding of *pvl* in isolates cultured from cows may suggest human to cow transmission of *S. aureus*, possibly during unhygienic hand milking. Further characterization of the *pvl*-positive *S. aureus* in this study and of *S. aureus* isolates cultured from farmers and their families may help quantifying the importance of human to cattle transmission of *S. aureus*. Likewise, similar studies on bovine isolates that may be found in humans may also show the importance of zoonotic transmission between cattle and their farmers, which likely occurs along the same transmission routes. The *tst* gene encodes the toxic shock syndrome toxin, which *S. aureus* uses to facilitate colonization (Omoe *et al* 2003). Although the *tst* gene is documented to be more prevalent in bovine than in human *S. aureus* strains (van Leeuwen et al., 2005), we only found it in a low number of isolates.

Comparable to the report of Tigabu et al. (2015) from the Central highlands of Ethiopia, we did not find *mec*A positive *S. aureus* in our samples. The majority of our isolates were, however, resistant to penicillin and ampicillin, which are the commonly used antimicrobials to treat mastitis and tetracycline mostly used to treat other bacterial infections, in Ethiopia. The high level of resistance to these antimicrobials is in line with previous Ethiopian reports on isolates from bovine mastitis (Mekonnen et al., 2005; Getahun et al., 2008; Haftu et al., 2012). This consistent picture of widespread AMR in livestock pathogens is alarming likely results from the high usage of antibiotics, probably due to the fact that farmers can obtain antimicrobials over the counter without a prescription. Additionally, farmers are often not instructed on a proper treatment regimen, because guidance by veterinarians is lacking. A final reason may be that Ethiopian farmers are not inclined to cull animals, but rather continue treating their cows for an extended period of time. The high level of resistance against commonly used antimicrobials makes the current standard treatment regimens virtually useless. Farmers therefore should be instructed to use other antimicrobials such as amoxicillin for treating mastitis cases and, more importantly, should be motivated to prevent transmission of *S. aureus*, rather than to use antimicrobials for mastitis control.

Conclusions

We identified a high degree of similarity between the bovine *S. aureus* isolates in North-Western Ethiopia, suggesting contagious transmission within and between farms. Moreover, there was a high level of resistance to antimicrobials that are commonly used to treat mastitis in Ethiopia. Therefore, implementing effective preventative measures aiming to limit transmission, rather than using antimicrobials to control mastitis, is important to decrease AMR.

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Annex. Percentage resistance to antimicrobials by *spa*-type for 79 *S. aureus* isolates identified from milk samples in dairy farms of North-Western Ethiopia. Percentages of 0 are not shown.

spa type	N isolates	Percentage of isolates resistant to antimicrobial							
		PEN[1]/AMP	CLI	FUS	RAM	ERY	TET	T/S	Other [2]
t042	46	78					50	2	
t15786	9	100	11			11	44		
t14061	3	100	33			33	66		
t355	3	100							
t488	2	100			50		50		
t1376	2	100					50		
t10018	1	100							
t2085	1								
t223	1	100	100			100	100		
t273	1	100							
t4701	1	100							
t17184	1	100		100			100		
Other[3]	8	100					100		

[1] PEN=penicillin, AMP=ampicillin, CLI=clindamycin, FUS=fusidic acid, RAM=rifampicin, ERY=erythromycin, TET=tetracycline, T/S=Trimethoprim sulfamethoxazole.

[2] Other antimicrobials: amoxicillin/clavulanic acid, cephalotin (1st generation cephalosporin), ceftiofur (3st generation cephalosporin), cefoxitin, chloramphenicol, enrofloxacin, gentamicin, kanamycin, neomycin were with proportions of 0.

[3] Other spa types: *spa* types t2801, t9300, t17185, t306, t409, t4206, t17384 and t17385.

Chapter 4

Stochastic bio-economic modeling of mastitis in Ethiopian dairy farms

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Abstract

Mastitis is an inflammation of the mammary gland that is considered to be one of the most frequent and costly diseases in the dairy industry. Also in Ethiopia, bovine mastitis is one of the most frequently encountered diseases of dairy cows. However, there was no study, so far, regarding the costs of clinical mastitis and only two studies were reported on costs of subclinical mastitis. Presenting an appropriate and complete study of the costs of mastitis will help farmers in making management decisions for mastitis control. The objective of this study was to estimate the economic effects of mastitis on Ethiopian market-oriented dairy farms. Market-oriented dairy farming is driven by making profits through selling milk in the market on a regular basis. A dynamic stochastic Monte-Carlo simulation model (bio-economic model) was developed taking into account both clinical and subclinical mastitis. Production losses, culling, veterinarian costs, treatment, discarded milk, and labour were the main cost factors which were modeled in this study. The annual incidence of clinical mastitis varied from 0 to 50% with a mean annual incidence of 21.6%, whereas the mean annual incidence of subclinical mastitis was 36.2% which varied between 0and 75%. The total costs due to mastitis for a default farm size of 8 lactating cows were 6,709 Ethiopian Birr (**ETB**) per year (838 ETB per cow per year). The costs varied considerably, with 5th and 95th percentiles of 109ETB and 22,009 ETB, respectively. The factor most contributing to the total annual cost of mastitis was culling. On average a clinical case costs 3,631 ETB, varying from 0 to 12,401, whereas a sub clinical case costs 147 ETB, varying from 0 to 412. The sensitivity analysis showed that the total costs at the farm level were most sensitive for variation in the probability of occurrence of clinical mastitis and the probability of culling. This study helps farmers to raise awareness about the actual costs of mastitis and motivate them to timely treat and/or take preventive measures. As a result, the dairy industry will improve.

Keywords

Dairy cow, market-oriented dairy farms, mastitis, bio-economic modeling, dynamic Monte-Carlo simulation.

Introduction

The rapidly increasing urban population size in Ethiopia creates a growth of demand for milk and dairy products. Following this, in the recent decade, milk production in Ethiopia has been evolving from a byproduct of draft power by indigenous breeds to market-oriented milk production. Generally, the dairy farming sys-tem in Ethiopia classified as pastoralists, agro-pastoralists, mixed crop–livestock producers, the peri-urban and urban dairy systems (Azage and Alemu, 1998; Ketema, 2000; Zegeye, 2003; Dereje et al., 2005). In the first three farming systems, most of the milk produced is retained for home consumption. However, in the peri- urban and urban dairy system, that constitute 14.3% of the dairy production system in Ethiopia (Tolosa et al., 2013), most of the milk produced is sold in the market. Market-oriented dairy farming is driven by making profits through selling milk in the market on a regular basis. In the market-oriented production system, cross-breeds (cross between local and exotic-breeds) are mainly used so that the milk production level has increased. A cross- breed cow in these dairy farms produces an average of 8.5 kg of milk per day (Yoseph et al., 2003). Typically, farms in the market-oriented dairy system are small (8 lactating cows on average) but relatively intensive. However, this production system is subjected to diseases of intensification including mastitis (Lemma et al., 2001). Mastitis is an inflammation of the mammary gland that is considered to be one of the most frequent and costly diseases in the dairy industry (Halasa et al., 2007). The economic effects of mastitis are due to production losses, treatment, veterinarian costs, discarded milk, culling and changes in product quality; and using the basic cost elements around mastitis and mastitis management, costs can be calculated (Hogeveen, 2005). The economic effects of mastitis and mastitis control have been estimated in several ways ranging from budget calculations (Swinkels et al., 2005), dynamic stochastic models (Huijps and Hogeveen, 2007) to dynamic optimization models (e.g. Cha et al., 2011). In Ethiopia, the available information indicated that bovine mastitis is one of the most frequently encountered diseases of dairy cows (Lemma et al., 2001). However, to our knowledge there was no study, so far, regarding the total costs of mastitis. Two studies were reported on costs of subclinical mastitis (Mungube et al., 2005; Tesfaye et al., 2010). In those two studies the authors used the average milk production per year to estimate the costs associated with milk production losses due to subclinical mastitis. However, those studies were quite rough and did not contain much detail, such as differences in milk production throughout the lactation with regard to the occurrence of mastitis. This is important because milk loss will vary with both stage of lactation and time after the occurrence of the disease (Grohn et al., 2004). Relevant estimations about disease costs can be used in evaluating the benefits of applying preventive measurements to decrease disease incidence, disease losses, or both, and in the process allow the evaluation of individual cow disease treatment options (Baret al., 2008). Similarly, presenting the full costs of mastitis will help farmers make management decisions on mastitis control. According to Seegers et al. (2003) to estimate the costs of a disease, the use of dynamic models that permit modeling the herd dynamics is more appropriate than the use of static models. Therefore, the objective of this study was to estimate the economic effects of clinical and subclinical mastitis in Ethiopian market oriented dairy farms by representing the best available knowledge in a dynamic stochastic simulation model.

Material and methods

Model description

A dynamic stochastic discrete event bio-economic model was developed using @risk 6.2 (Palisade Corporation, Ithaca, NY, USA) software to calculate the costs of mastitis at the farm level. Monte-Carlo simulation is a computer technique used to simulate the reaction of a model under repeated iterations. By taking different values from appropriate distributions of a parameter, the model becomes stochastic and thus can take the variation into account (Huijps et al., 2009). The basis of our model (biological part) was the simulation of the dynamics of mastitis. Results of these simulations were then used to calculate the economic effects of mastitis. The basic stochastic process was carried out at the cow level using 26 time steps of 2 weeks to simulate one complete year.

Herd dynamics

A specific parity, lactation stage and calving interval were assigned to each cow, based on different distributions. The parity and the lactation stage of a cow changed when either the cow gave birth or was culled. When the cow gave birth, the parity increased by one whereas it becames one if the cow was culled in the previous time step. In both events the lactation stage went to 1. Before calving, a cow was assumed to have a dry period of 8 weeks. Calving interval (**CI**) of each cow (i) was determined with a pert distributions with minimum, most likely and maximum values.

$$CI_i = Pert\ (CI_{min}, CI_{ml}, CI_{max})` \quad [1]$$

where, CI_{min} is the minimum, CI_{ml} is the most likely, and CI_{max} is the maximum values of calving interval. Parity was classified into six distinct categories and it was determined based on a discrete distribution as follows:

$$Par_{it} = \begin{cases} t = 1 & \rightarrow Discerete([1,2,3,4,5,6],[P_{Par1}, P_{Par2}, P_{Par3}, P_{Par4}, P_{Par5}, P_{Par \geq 6}]) \\ Cull_{it-1} > 0 & \rightarrow 1 \\ LS_{it-1} > (CI) - 14 & \rightarrow Par_{it-1} + 1 \\ Par_{it-1} \end{cases} \quad [2]$$

where, $P_{Par1}, P_{Par2}, P_{Par3}, P_{Par4}, P_{Par5}, P_{Par \geq 6}$ are the probabilities of the distribution of parity 1, 2, 3, 4, 5, 6 and above in a farm, respectively. Par_{it} indicates the parity of each cow (i) at a time period (t). $Cull_{it-1}$ and LS_{it-1} denote the status of culling (0 = not culled and > 0 = culled) and lactation stage for each cow (i) in the previous time period (t-1), respectively. Lactation stage (days after calving) was determined using uniform distribution according to Eq.(3).

$$LS_{it} = \begin{cases} t = 1 \rightarrow Round(Uniform\ (1,26)\ ; 0) \\ Cull_{it-1} > 0 \rightarrow 1 \\ LS_{it-1} > (CI) - 14 \rightarrow 1 \\ LS_{it-1} + 1 \end{cases} \quad [3]$$

where LS_{it} is the lactation stage (days after calving) of each cow (i) at a time period (t). $Cull_{it-1}$ and LS_{it-1} were explained previously. The lactational milk yield (LMY_i) which is 305 days of milk yield, was assigned to each cow using normal distribution with average annual milk production and standard deviation, and adjusted to parity (k) and calving seasons (s) as follows:

$$LMY_{iks} = Normal\left(MeanLMY, \ Sd\right) \times Adj\ Par_k \times Adj\ Cas_s \qquad [4]$$

where $Adj\ Par_k$ and $Adj\ Cas_s$ are the adjustment factor for each parity and calving season, respectively. The calving seasons were categorized into three groups as dry season (October to February), short rainy season (March to May) and long rainy season (June to September). The basic milk production of each cow during each time period (BMP_{ikst}) was modeled using Wood's curve (Wood, 1976) based on the following equation:

$$BMP_{ikst} = \frac{LMY_{iks} \times a \times \left(LS_{it} \times 2 - 0.5\right)^b \times e^{-c \times \left(LS_{it} \times 2 - 0.5\right)}}{14 \times \sum_{LS=1}^{22} \left(a \times \left(LS_{it} \times 2 - 0.5\right)^b \times e^{-c \times \left(LS_{it} \times 2 - 0.5\right)}\right)} \qquad [5]$$

Where; a, b and c or Wood's constants represent the slope of the lactation curve during the different lactation stages of each cow (i). The rest of the acronyms were explained previously.

Mastitis dynamics

Modeling the dynamics of mastitis was based on the schematic representation given in Figure 1. Depending on the status of the previous time period, each cow had different transition probabilities for each time period (t). Cows can remain longer than one time period in a status except for the clinical status. Cows in the clinical mastitis status were assumed to be treated with antibiotics and either changed status within the same time period to recovery, or became subclinical in the next time period.

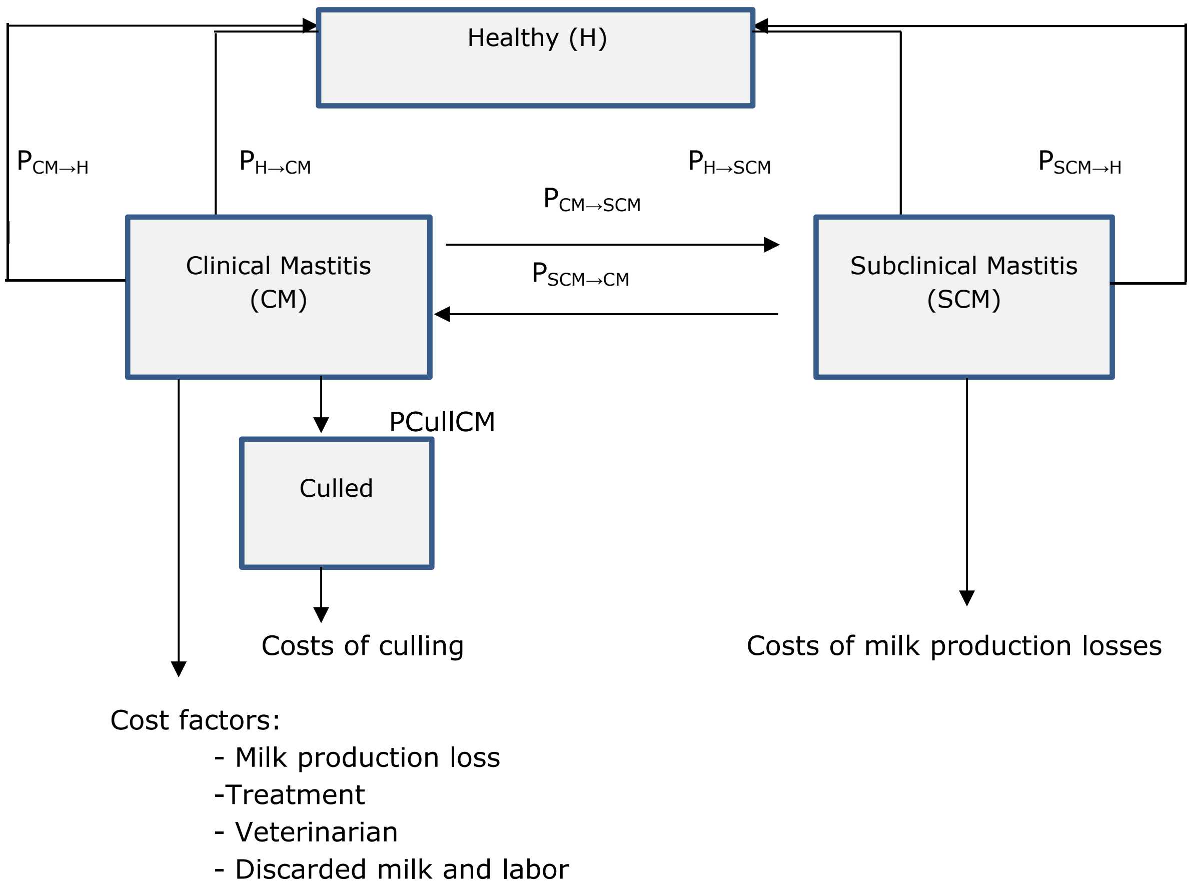

Figure 1.The dynamics of mastitis and its economic consequences as modeled. $P_{H \rightarrow CM}$ = probability of change from healthy status to clinical mastitis status; $P_{H \rightarrow SCM}$ = probability of change from healthy status to subclinical mastitis status; $P_{CM \rightarrow SCM}$ = probability of change from clinical to subclinical mastitis status; $P_{SCM \rightarrow CM}$ = probability of change from subclinical to clinical mastitis status; $P_{CM \rightarrow H}$ = probability of change from clinical mastitis status to healthy status; $P_{SCM \rightarrow H}$ = probability of change from subclinical mastitis status to healthy status and P_{CullCM} = probability of culling due to clinical mastitis.

The risk or probability of clinical mastitis (**PCM**) and subclinical mastitis (**PSCM**) for each cow (i) at each time period (t) was adjusted for by each lactation stage (j) (early, mid and late) and parity (k) based on the following equations. The risk of clinical mastitis was also adjusted for by the number of clinical cases in the previous month (Steeneveld et al., 2008) (r):

$$\mathrm{PCM}_{ijkrt} = \begin{cases} t = 1 \rightarrow \mathrm{IRCM}_t \times \mathrm{AdjLS}_j \times \mathrm{AdjPar}_k \\ \mathrm{Cull}_{it-1} > 0 \rightarrow 0 \\ \mathrm{CM}_{it-2} = 1 \text{ and } \mathrm{SCM}_{it-1} = 1 \rightarrow P_{SCM \rightarrow CM} \times \mathrm{AdjLS}_j \times \mathrm{AdjPar}_k \times \mathrm{Adjc}_{r2} \\ \mathrm{CM}_{it-2} = 1 \text{ and } \mathrm{CM}_{it-1} = 0 \text{ and } \mathrm{SCM}_{it-1} = 0 \rightarrow P_{H \rightarrow CM} \times \mathrm{AdjLS}_j \times \mathrm{AdjPar}_k \times \mathrm{Adjc}_{r2} \\ \mathrm{CM}_{it-2} = 1 \text{ and } \mathrm{CM}_{it-1} = 1 \rightarrow P_{H \rightarrow CM} \times \mathrm{AdjLS}_j \times \mathrm{AdjPar}_k \times \mathrm{Adjc}_{r3} \\ \mathrm{CM}_{it-2} = 0 \text{ and } \mathrm{SCM}_{it-1} = 1 \rightarrow P_{SCM \rightarrow CM} \times \mathrm{AdjLS}_j \times \mathrm{AdjPar}_k \times \mathrm{Adjc}_{r1} \\ P_{H \rightarrow CM} \times \mathrm{AdjLS}_j \times \mathrm{AdjPar}_k \times \mathrm{Adjc}_{r1} \end{cases} \quad [6]$$

$$P_{SCM_{ijkt}} = \begin{cases} t = 1 \text{ or } Cull_{it-1} > 0 \text{ or } D_{SCM_{it-1}} = 3 \rightarrow IR_{SCM_t} \times Adj_{LS_j} \times Adj_{Par_k} \\ CM_{it-1} = 1 \rightarrow P_{CM \rightarrow SCM} \times Adj_{LS_j} \times Adj_{Par_k} \\ SCM_{it-1} = 1 \text{ and } D_{SCM_{it-1}} < 3 \text{ period} \rightarrow P_{SCM \rightarrow SCM} \times Adj_{LS_j} \times Adj_{Par_k} \\ P_{H \rightarrow SCM} \times Adj_{LS_j} \times Adj_{Par_k} \end{cases} \quad [7]$$

where IR_{CM_t} and IR_{SCM_t} are the risk of clinical and subclinical mastitis at each time period (t). Adj_{LS_j} and Adj_{Par_k} represent the adjustment factors of each lactation stages and parity numbers for the risk of mastitis at each time period, respectively. CM_{it-1} and SCM_{it-1} are the status of clinical and subclinical mastitis in the previous one time period, respectively whereas CM_{it-2} represents the status of clinical mastitis in the previous two time periods. Adj_{C_r} is the adjustment factor for the effect of the number of clinical cases in the previous month on the risk of clinical mastitis at the current time period. D_{SCM} stands for maximum duration (**D**) of subclinical mastitis (**SCM**). This duration was calculated based on the 40% recovery rate of subclinical mastitis per two weeks (Abaineh and Sintayehu, 2001). $Cull_{it-1}$, $P_{H \rightarrow CM}$, $P_{SCM \rightarrow CM}$, $P_{CM \rightarrow SCM}$ and $P_{H \rightarrow SCM}$ were explained previously (Figure 1). Based on the probabilities of the occurrence of mastitis, that were adjusted for each lactation stages, parity numbers (k) and the number of clinical cases in the previous month/ two periods (r), the different status of the mastitis at each time period (t) were determined using discrete distribution as follows:

$$CM_{ijkrt} = \begin{cases} t = 1 \rightarrow \text{Discrete}\left(\left[P_{CMjkt=1}, 1 - P_{CMjkt=1}\right], [1,0]\right) \\ Cull_{it-1} > 0 \rightarrow 0 \\ SCM_{it-1} = 1 \rightarrow \text{Discrete}\left(\left[P_{(SCM \rightarrow CM)jkrt}, 1 - P_{(SCM \rightarrow CM)jkrt}\right], [1,0]\right) \\ \text{Discrete}\left(\left[P_{(H \rightarrow CM)jkrt}, 1 - P_{(H \rightarrow CM)jkrt}\right], [1,0]\right) \end{cases} \quad [8]$$

$$SCM_{ijkt} = \begin{cases} t = 1 \text{ or } Cull_{it-1} > 0 \text{ or } D_{SCM_{it-1}} = 3 \rightarrow \text{Discrete}\left(\left[P_{SCMjkt=1}, 1 - P_{SCMjkt=1}\right], [1,0]\right) \\ CM_{it-1} = 1 \rightarrow \text{Discrete}\left(\left[P_{(CM \rightarrow SCM)jkt}, 1 - P_{(CM \rightarrow SCM)jkt}\right], [1,0]\right) \\ SCM_{it-1} = 1 \text{ and } D_{SCM_{it-1}} < 3 \text{ period} \rightarrow \text{Discrete}\left(\left[P_{(SCM \rightarrow SCM)jkt}, 1 - P_{(SCM \rightarrow SCM)jkt}\right], [1,0]\right) \\ \text{Discrete}\left(\left[P_{(H \rightarrow SCM)jkt}, 1 - P_{(H \rightarrow SCM)jkt}\right], [1,0]\right) \end{cases} \quad [9]$$

where CM and SCM represent the status of clinical and subclinical mastitis at each time period. The rest were described previously.

Culling and replacement can happen at each time period. It was assumed that culling is applied at the beginning of the period and the replaced heifer is ready to start lactation on the next period. A cow recovered from clinical mastitis don't attain a full production level for the rest of its lactation period. As a result of this the base risk of culling increased until a certain period of time and we call this effect a carryover effect of clinical mastitis on culling. It was assumed that the carry-over effect of clinical mastitis on culling. It was assumed that the carry-over effect of clinical mastitis on culling lasted for a maximum of 8 periods. When a new clinical mastitis event occurred, the carry-over effect of the previous event ended and the main effect of clinical mastitis on culling was applied. The status of culling ($Cull_{it}$) for each cow (i) at each time period (t) was determined using discrete distribution:

$$\mathrm{Cull}_{it} = \begin{cases} \mathrm{CM}_{it} = 1 \rightarrow \mathrm{discrete}\left(\left[1 - \mathrm{BRCull}_{it} - \mathrm{P_{CullCM}}_{it}, \mathrm{BRCull}_{it}, \mathrm{P_{CullCM}}_{it}\right], \left[0, 1, 2\right]\right) \\ \mathrm{TCO}_{it} > 0 \rightarrow \mathrm{discrete}\left(\left[1 - \mathrm{BRCull}_{it} - \mathrm{P_{CullCOCM}}_{it}, \mathrm{BRCull}_{it}, \mathrm{P_{CullCOCM}}_{it}\right], \left[0, 1, 2\right]\right) \\ \mathrm{discrete}\left(\left[1 - \mathrm{BRCull}_{it}, \mathrm{BRCull}_{it}\right], \left[0, 1\right]\right) \end{cases} \quad [10]$$

where BRCull_{it}, $\mathrm{P_{CullCM}}_{it}$ and $\mathrm{P_{CullCOCM}}_{it}$ are the base risk of culling, the risk of culling due to main effect of clinical mastitis and the risk of culling due to the carry-over effect of clinical mastitis at each time period, respectively. **TCO** represent the carry-over time since a clinical mastitis occurs. In the model 0 means no culling, 1 means culling other than mastitis and 2 means culling due to clinical mastitis. CM_{it} was explained previously. Under Ethiopian dairy farming system, farmers do not cull a cow due to subclinical mastitis. The different risks of culling and the carry-over time were modeled in the present study as shown in Annex 1.

In our model, the milk losses associated with clinical and subclinical mastitis were modeled according to the following equation:

$$\mathrm{MPL}_{it} = \begin{cases} \mathrm{Cull}_{it} > 0 \rightarrow \mathrm{B_{MY}}_{it} \\ \mathrm{CM}_{it} = 1 \rightarrow \%\mathrm{MPL_{CM}} \times \mathrm{B_{MY}}_{it} \\ \mathrm{SCM}_{it} = 1 \text{ and } \%\mathrm{MPL_{COCM}}_{it} < \%\mathrm{MPL_{CMT}}_{it} \rightarrow \%\mathrm{MPL_{CMTQ1}} \times \mathrm{B_{MY}}_{it}/4 + \\ \qquad \%\mathrm{MPL_{CMTQ2}} \times \mathrm{B_{MY}}_{it}/4 \\ \mathrm{SCM}_{it} = 1 \text{ and } \%\mathrm{MPL_{COCM}}_{it} > \%\mathrm{MPL_{CMT}}_{it} \rightarrow \%\mathrm{ML_{COCM}}_{it} \times \mathrm{B_{MY}}_{it} \\ \mathrm{SCM}_{it} = 0 \text{ and } \mathrm{CM}_{it} = 0 \text{ and } \%\mathrm{MPL_{COCM}}_{it} > 0 \rightarrow \%\mathrm{MPL_{COCM}}_{it} \times \mathrm{B_{MY}}_{it} \\ 0 \end{cases}$$

$[11]$ where, $\mathrm{B_{MY}}_{it}$ and MPL_{it} indicate the basic milk yield and the milk production losses (associated with mastitis) in kg for each cow (i) at each time period (t), respectively. For the purpose of calculating the milk losses associated with subclinical mastitis, $\mathrm{B_{MY}}_{it}$ was divided by 4 to get the basic milk yield per quarter. $\%\mathrm{MPL_{CM}}$ and $\%\mathrm{MPL_{COCM}}_{it}$ represent the percent milk loss of clinical mastitis and carry-over effect of clinical mastitis, respectively. When a new clinical mastitis event occurred, it was assumed that the carry-over effect of the pervious event ended and the full effect of clinical mastitis on milk loss was applied. $\%\mathrm{MPL_{CMTQ}}$ indicates the percent milk loss associated with different California mastitis test (**CMT**) scores of a quarter (**Q**) in the case of subclinical mastitis. It was assumed that on average two quarters were infected for each cow with subclinical mastitis. The rest of the acronyms in the equation were explained previously. The carry-over effect of clinical mastitis on milk production and its carry-over time since clinical mastitis occurred, and the CMT score status of a quarter infected with subclinical mastitis were modeled as indicated in Annex 2.

Based on the basic milk production, milk loss and culling status of each cow (i) during each time period (t), the real milk production $(\mathrm{REAL_{MP}}_{it})$ was modeled as follows:

$$\mathrm{REAL_{MP}}_{it} = \begin{cases} \mathrm{Cull}_{it} > 0 \rightarrow 0 \\ \mathrm{LS}_{it} = \mathrm{dry} \rightarrow 0 \\ \mathrm{B_{MY}}_{it} - \mathrm{MPL}_{it} \end{cases} \quad [12]$$

where "dry" indicates the dry period of each cow. Others were explained previously.

Economic effects

The economic losses associated with mastitis were calculated according to Halasa et al. (2007). Production losses, culling, veterinarian costs, treatment, discarded milk, and labour were the main cost factors associated with mastitis. The model was built and run using values in Ethiopian Birr (1 ETB = 0.0417 Euro) and the default values of prices used in this model are given in Table 3 together with the references. Only production losses were included in estimating the total costs of subclinical mastitis. This is because farmers in Ethiopia neither cull (Dego and Tareke, 2003) nor treat cows for subclinical mastitis.

A pert distribution was used to estimate the costs of 1 kg milk production loss and discarded milk based on the minimum, most likely and maximum values obtained from the experts. Costs of milk production losses at each time period for both clinical and subclinical mastitis were calculated by multiplying the amount of milk production losses (kg) caused by clinical or subclinical mastitis at that time period with the costs per kg of production losses. All clinical mastitis cases were assumed to be treated for 3 days and the costs were calculated based on the price of the drug (treatment) per case. The total costs associated with discarded milk were calculated by multiplying the real milk production of a cow at the time of treatment and the waiting time (3 treatment days + 3 withholding days = 6 days) with the selling price of milk (here cost of feed was included unlike the calculation of costs of production losses). The costs of veterinary service were calculated using the price of veterinary visits per case taking into account that 50% of the clinical cases are visited by a veterinarian. The costs of mastitis associated with labour were calculated using the hourly wage of labour per treated case. When the cow was culled due to clinical mastitis, the costs of milk losses until the replaced heifer arrived were also considered in our model and these costs were calculated by multiplying the average expected basic milk production of the cow at the time period of culling with the costs per kg of production losses. The estimated costs of culling were based on the price of purchasing a new heifer (direct cost) and the revenue gained from selling the cow for slaughtering. In addition to these, the loss of future returns from the culled cow was taken in to account by a depreciation factor based on the parity of the culled cow. The estimated price of purchasing a new heifer and the slaughter values are given in Table 3. The costs of culling were modeled as follows:

$$\mathrm{Ccull}_{ikt} = \begin{cases} \mathrm{Par}_{it} < \mathrm{Ago} \rightarrow \left(\mathrm{Ago} - \mathrm{Par}_{it}\right) \times \left({(\mathrm{RV} - \mathrm{SV})} / {\mathrm{Ago}} \right) \\ 0 \end{cases} \qquad [13]$$

Where, $\mathrm{C}_{\mathrm{Cull}\ ikt}$ denotes the costs of culling for each cow i and parity k at each time period t. Ago is the age of old cow in the farm after first calving. Par_{it} stands for the parity of each cow i at each time period t. RV and SV represent the purchasing value of a new heifer and the selling value of a cow for slaughter, respectively. In our model the costs of culling due to clinical mastitis ($\mathrm{CCull}_{\mathrm{CM}\ ikt}$) were differentiated from other culling Eq.(14).

$$\mathrm{CCull}_{\mathrm{CM}\ ikt} = \begin{cases} \mathrm{Cull}_{it} = 2 \rightarrow \mathrm{CCull}_{ikt} \\ 0 \end{cases} \qquad [14]$$

where, the number 2 indicates culling due to clinical mastitis. The rest were explained previously.

All the costs which are explained above were calculated every two weeks and summed up to a year for each cow to get the total costs of mastitis (TCM) Eq.(15).

$$\text{TCM} = \text{TCCM} + \text{TCSCM} \qquad [15]$$

Where TCCM and TCSCM indicate the total costs of clinical and subclinical mastitis, respectively and they were modeled as follows:

$$\text{TCCM} = \sum_{i=1}^{8}\sum_{t=1}^{26} \text{CCMPL}_{\text{it}} + \text{CCullCM}_{\text{ikt}} + \text{CLab}_{\text{it}} + \text{CV}_{\text{it}} + \text{CT}_{\text{it}} + \text{CDM}_{\text{it}} \qquad [16]$$

$$\text{TCSCM} = \sum_{i=1}^{8}\sum_{t=1}^{26} \text{CSMPL}_{\text{it}} \qquad [17]$$

where CCMPL_{it} and CSMPL_{it} represent the costs of milk production losses due to clinical (including milk production loss due to culling clinical mastitic cow, i.e. until replacement takes place in the next period) and subclinical mastitis, respectively. $\text{CCull}_{\text{CM ikt}}$, CLab_{it}, CV_{it}, CT_{it}, CDM_{it} indicate the costs of culling due to clinical mastitis, costs of labour, costs of veterinarian, costs of treatment and costs of discarded milk for each cow (i) at each time period (t), respectively.

Input values

At first, a detailed literature search was carried out. Peer reviewed papers in scientific journals on incidence, prevalence and recovery rate of mastitis, and production performance of dairy cows in Ethiopia were analyzed. Only those studies conducted on cross-breed cows were included. Because literature did not contain complete information, knowledge from experts (local livestock extension workers, veterinarians and the authors) were included. The experts were asked to estimate values of those parameters that were not found in the literature. The values obtained from the experts on a given parameter were merged in to a single value by taking the mean of the values. In rare cases data from other countries were also used. Input data for the herd and mastitis dynamics are given in Tables 1 and 2, respectively. For the economic part input data are presented in Table 3.

Table 1. Input data for cow characteristics along with their reference.

Parameters	Estimates (standard deviation	Distribution	Reference
Farm size	8	Constant	Experts
Distribution parities		Discrete	Million and Tadelle (2003), Experts
Parity 1	0.3		
Parity 2	0.26		
Parity 3	0.19		
Parity 4	0.11		
Parity 5	0.08		
Parity 6 and above	0.06		
Lactational Milk yield[1]	2612 (869)	Normal	Yoseph et al. (2003)
Calving interval		Pert	Experts
Minimum	365		
Most likely	415		
Maximum	465		
Parity adjustment factors for lactational milk yield		Constant	Million and Tadelle (2003)

Parity 1	0.92	
Parity 2	0.98	
Parity 3	1.08	
Parity 4 and above	1.04	
Calving season adjustment factors for lactational milk yield	Constant	Gabriel et al (1983)
Season 1	0.98	
Season 2	0.96	
Season 3	1.04	

[1]The value given for lactational milk yield is the average milk yield for 305 days.

Validation

Because actual data from farms were not available to conduct an external validation, only an internal validation of the model was conducted. The rationalism method, tracing method and face validity method are the different methods that were used to check the validity of the model (Law, 2007; Sorensen, 1990). By the rationalism method the input values were changed and the consistency of the model output was checked, whereas in the face validity method four experts were consulted concerning the assumption and credibility of the model and model output.

Table 2. Input probabilities for the dynamics of mastitis with their reference.

Variables	Probabilities	Distribution	Reference
Incidence of clinical mastitis/year (IR_{CM})	0. 21	Constant	Almaw et al. (2012)
Prevalence of subclinical mastitis (P)	0.52	Constant	Mungube et al. (2005)
Incidence of subclinical mastitis/year (IR_{SCM})	[a] P/([b] D_{SCM} -P* D_{SCM}) = 0.36	Constant	Dohoo et al. (2009)
Probabilities of occurrence at t=1		Constant	
Clinical mastitis	[c] IR_{CM} /26		Calculated
Subclinical mastitis	IR_{SCM} /26		Calculated
Transition probabilities per two weeks		Constant	
Clinical mastitis from healthy state	IR_{CM} /26		Calculated
Subclinical mastitis from healthy state	IR_{SCM} /26		Calculated
Cure rate of subclinical mastitis	0.4		Experts
Cure rate of clinical mastitis	0.85		Experts
Clinical from sub clinical mastitis	0.03		Abaineh and Sintayehu (2001)
Subclinical from clinical mastitis	0.14		Calculated
Subclinical mastitis stay as a subclinical more than one time period	0.57		Calculated
Adjustment factors of lactation stages on the occurrence of mastitis		Constant	Dego and Tareke (2003)
Early lactation (1-120 days)	1.2		
Mid lactation (120-240 days)	0.8		
Late lactation (>240 days)	0.96		
Adjustment factors of Age/parity on the occurrence of mastitis		Constant	Dego and Tareke (2003)
Parity 1	0.88		
Parity 2	0.96		
Parity 3	1		
Parity 4	1.05		
Increased risk of clinical mastitis based on previous		Constant	Steeneveld et al. (2008)

clinical mastitis				
Cumulative previous month clinical mastitis ($AdjC_{r1}$)[d]	0	1		
Cumulative previous month clinical mastitis ($AdjC_{r2}$)[e]	1	2.49		
Cumulative previous month clinical mastitis ($AdjC_{r3}$)[f]	2	3.45		
General culling/year		0.2	Constant	Experts
Odd ratio of culling when there is CM		2	Constant	Experts
Maximum duration of sub clinical mastitis		35 days (~3 period)	Constant	Calculated

[a] Prevalence
[b] Maximum duration (D) of subclinical mastitis (SCM)
[c] Incidence rate (IR) of clinical mastitis (CM)
[d] Adjustment factor when the number of clinical mastitis case that occurred in the previous one month (two periods) is 0
[e] Adjustment factor when the number of clinical mastitis case that occurred in the previous one month (two periods) is 1
[f] Adjustment factor when the number of clinical mastitis cases that occurred in the previous one month (two periods) are 2

Model run and sensitivity analysis

The model was run for one year (26 time steps) and each iteration represented a farm with 8 lactating cows. During the simulation, 10,000 iterations were carried out, where for each iteration a value is drawn from relevant distributions. All outcomes of the simulation were expressed at the farm level. A sensitivity analysis was conducted to show the level of importance of the value of input factors on the average total costs of mastitis at the farm level. All input variables were checked for -10 and +10% of the base value. The average 305-day production per cow was increased and decreased by 375 kg.

Table 3. Default input values for calculating the costs of mastitis with their Reference.

Input variable	Value	Distribution	Reference
Milk production loss %		Constant	
Clinical mastitis	5		Huijps et al. (2008)
Subclinical mastitis (at quarter level)			Mungube et al. (2005)
CMT 1+	1.2		
CMT 2+	6.3		
CMT 3+	33		
Distribution of CMT scores		Discrete	Mungube et al. (2005)
CMT 1+	0.28		
CMT 2+	0.25		
CMT 3+	0.46		
Probability of a clinical mastitis case to be treated by vet.	0.5	Constant	Authors' expertise
Time to treat clinical case, hours	2	Constant	Experts
Age of old cow (Ago) in the farm after first calving (year)	6	Constant	Authors' expertise
Costs for milk production losses, Birr/kg		Pert	Experts
Minimum	7.5		
Most likely	9		
Maximum	10.5		
Costs for discarded milk, Birr/kg		Pert	Experts
Minimum	11		
Most likely	13		
Maximum	15		
Labour hourly wage, Birr	8	Constant	Experts
Veterinary service, Birr/case	150	Constant	Authors' expertise
Price antibiotics, Birr/case		Constant	Experts
Minimum	50		
Most likely	75		
Maximum	100		
Replacement costs, Birr/culled cow		Pert	Experts
Minimum	20,000		
Most likely	23,000		
Maximum	25,000		
Average selling value of a cow for slaughter	7000	Constant	Experts

Results

The simulated annual incidence of clinical mastitis varied from 0 to 50% with a mean annual incidence of 21.6%, whereas the mean annual incidence of subclinical mastitis was 36.2% which varied between 0 and 75%. The simulated number of culled cows due to mastitis (clinical mastitis) was 0.47, varying from 0 to 2 and this number accounts on average for 28% of the overall culling within the farm. The mean annual incidence of both clinical and subclinical mastitis varied with stage of lactation. For clinical mastitis, the incidence at the farm level was 0.63 (36.4%), 0.43 (24.8%) and 0.67 (38.7%) for early, mid and late lactation, respectively and each varying from 0 to 2. Whereas, for subclinical mastitis the incidence at early, mid and late lactation was 1.04 (35.8%), varying from 0 to 3, 0.71 (24.5%), varying from 0 to 2, and 1.12 (38.6%), varying from 0 to 3. The incidence of mastitis also varied with parity number (Table 4).

Table 4. The average output number of mastitis (per year , lactation stages and parity) and culling rate per year along with 0.05 and 0.95 percentiles in parentheses).

Item	Clinical	Subclinical
Number of mastitis cases per farm	1.73 (0-4)	2.9 (0-6)
Number of mastitis cases per farm per lactation stage		
Early	0.63 (0-2)	1.04 (0-3)
Mid	0.43 (0-2)	0.71 (0-2)
Late	0.67 (0-2)	1.12 (0-3)
Number of mastitis cases per farm per parity		
Parity 1	0.44 (0-2)	0.75 (0-2)
Parity 2	0.5 (0-2)	0.8 (0-2)
Parity 3	0.4 (0-2)	0.67 (0-2)
Parity 4 and above	0.39 (0-2)	0.65 (0-3)
Number of culled cow due to mastitis per farm	0.47 (0-2)	-

Based on the model output, the milk losses associated with clinical mastitis were 51 kg per farm per year (30 kg per case), varying from 0 to 148 kg per farm per year (0 to 85 kg per case). The milk losses due to subclinical mastitis were 47 kg per farm per year (17 kg per case), varying from 0 to 132 kg per farm per year (0 to 46 kg per case).

The total costs due to mastitis for a default farm with 8 cows were 6,709 ETB per year (838 ETB per cow per year). The costs varied considerably with 5th and 95th percentiles of 109 ETB and 22,009 ETB, respectively (Tale 5). Similarly, the variation in the total costs of mastitis among different farms were indicated in Figure 2. The total annual costs of clinical mastitis per farm were on average 6,282 ETB, varying from 0 to 21,454. Of these total costs of clinical mastitis, costs of culling comprised the highest proportion, followed by discarded milk and milk production losses. The total average costs associated with subclinical mastitis were 426 ETB per farm per year (53 ETB per cow per year), ranging from 0 to 1,196. These costs were solely due to milk production losses. On average a clinical case costs 3,631 ETB, varying from 0 to 12,401, whereas a sub clinical case costs 147 ETB ,varying from 0 to 412.

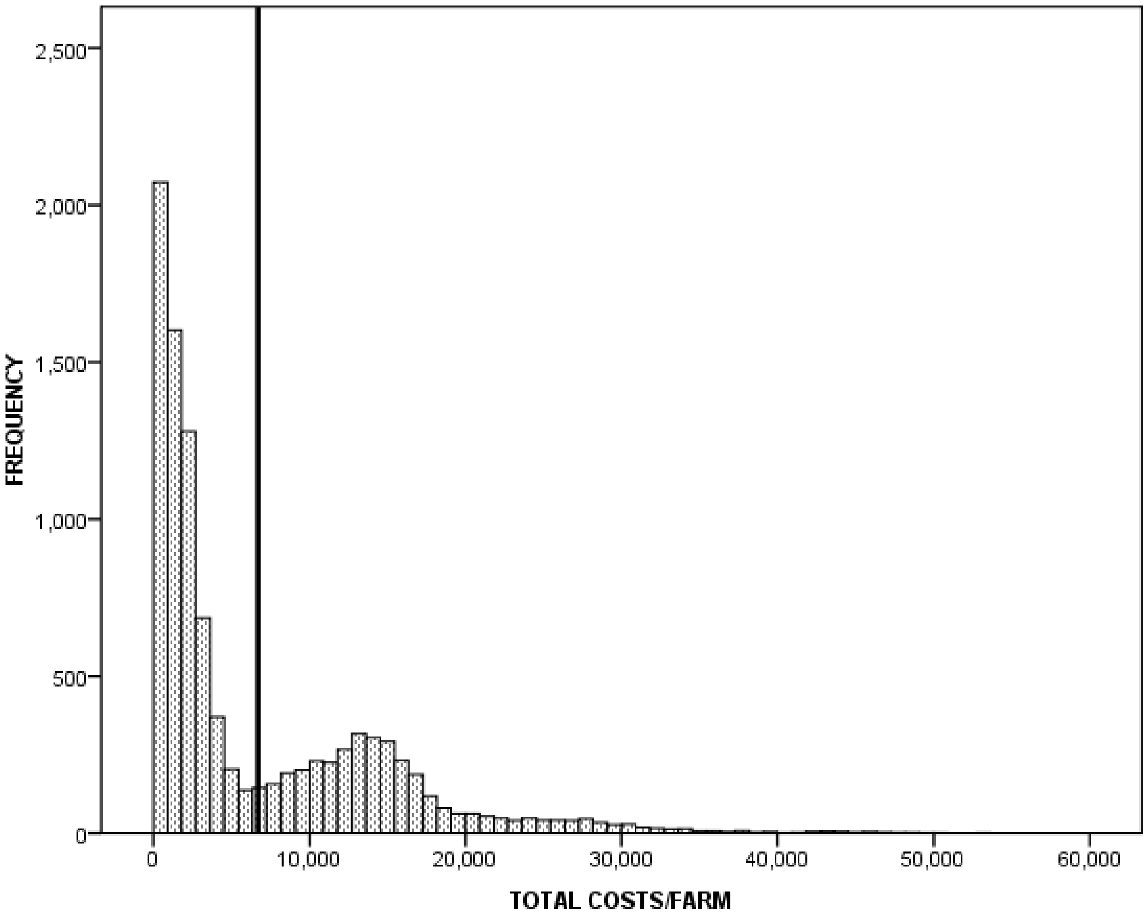

Figure 2.The variation in the total costs of mastitis between farms with a herd size of 8 cows. The vertical line represents for average costs of mastitis.

The costs of a clinical case varied with parity number and the highest costs were when clinical mastitis occurred in parity 1. On average, a clinical case costs 4,248 ETB (varying from 0 to 14,870) , 4,082 ETB (varying from 0 to 12,842), 3,435 ETB (varying from 0 to10,021) and 2,558 ETB (varying from 0 to 8,633) when it occurred in parity one, parity two, parity three and parity four and older cows, respectively.

Table 5. The output economic consequences of clinical and subclinical mastitis at the farm and cow level (ETB/year along with 0.05 and 0.95 percentiles in parentheses)

Cost factors	Item	Cow
Production losses		
Clinical	456 (0-1,338)	57 (0-167)
Subclinical	426 (0-1,196)	53 (0-150)
Discarded milk	854 (0-2,479)	107 (0-310)
Antibiotic	119 (0-310)	15 (0-39)
Veterinarian	119 (0-300)	15 (0-37)
Culling	4281 (0-17,102)	535 (0-2035)
Labour	25 (0-64)	3 (0-8)
Milk loss due to culling mastitis cases	426 (0-1,993)	53 (0- 249)
Total costs of mastitis	6,709 (109-22,009)	838 (15-2,751)

Sensitivity analysis

The results of the sensitivity analysis for parameters that affected the total average annual costs of mastitis are shown in Figure 3. Total costs due to mastitis on the farm were most sensitive to variation in the probability of occurrence of clinical mastitis and the probability of culling. Increasing the occurrence probability of clinical mastitis by 10% resulted in an increase of the total costs per year to 7,260 ETB and decreasing this probability with the same percentage reduced the total costs to 6,000 ETB. Decreasing the probability of culling by 10% resulted in a decrease of the total costs per year to 6,082 ETB whereas a 10% increase on culling probability raised the total costs to 7,119 ETB. The other influential factor that affects the total costs was the costs of culling. A 10% increase and decrease of the costs of culling brought the total costs to 7,040 ETB and 6,329, respectively. The recovery rate of subclinical mastitis, costs of production losses, milk production level, costs of antibiotic, the transition from subclinical to clinical mastitis, costs of the discarded milk and costs of veterinarian were also important contributors to the total costs of mastitis.

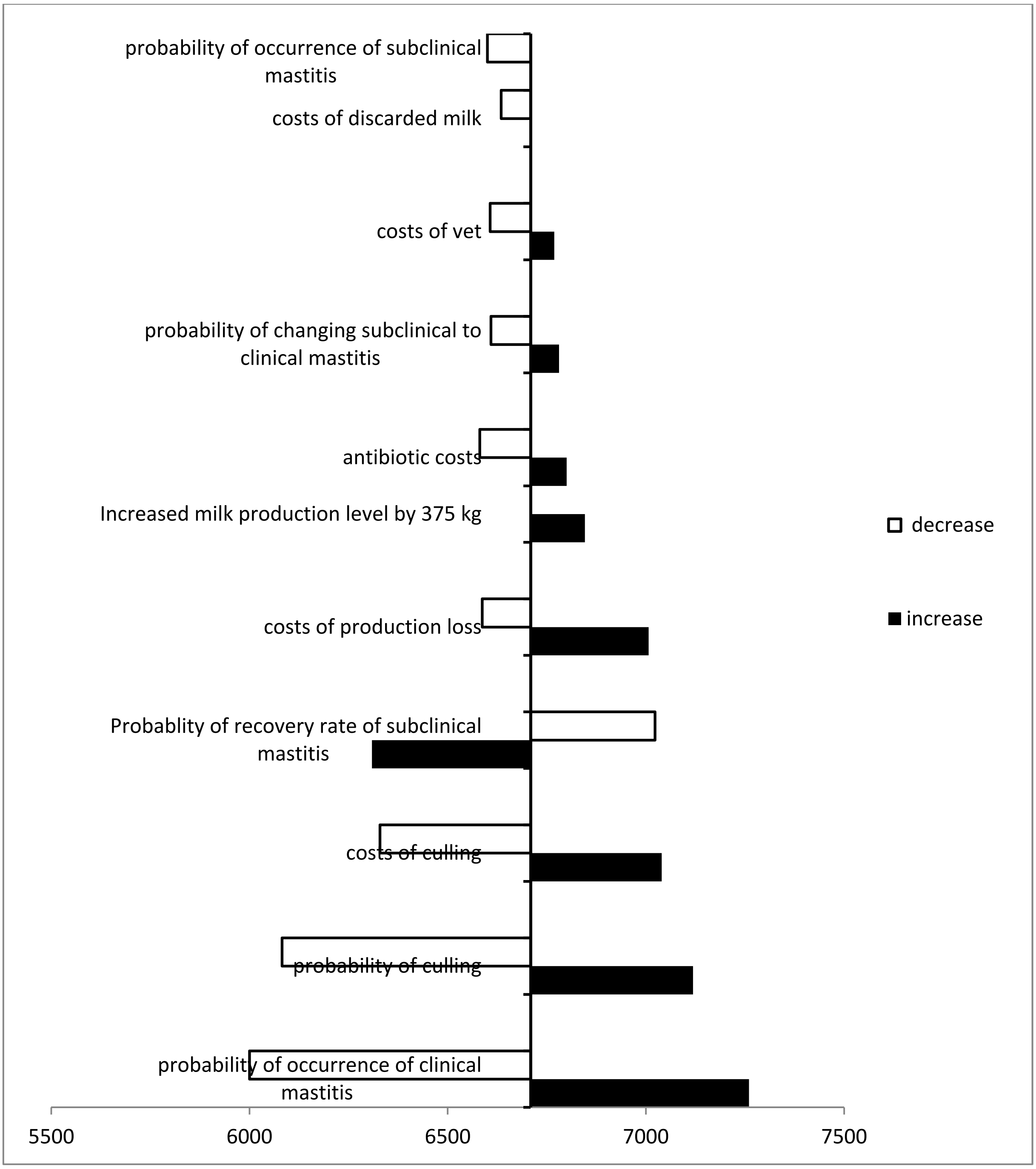

Figure 3. Results from the sensitivity analysis which showing an increase and decrease effect of the changed input variables (± 10%) on the total costs of mastitis per farm.

Discussion

The objective of this study was to estimate the costs associated with both clinical and subclinical mastitis in cross-breed dairy cows which are found on market-oriented dairy farms in Ethiopia. The purpose of the economic calculations was to support decision making. Before considering changes in management on a certain disease, it is important to have insight in the room for investment for that particular disease (Huijps et al., 2009). However, there was no study, so far, regarding the costs of mastitis in Ethiopia. Only two studies were reported on the costs of subclinical mastitis (Mungube et al., 2005; Tesfaye et al., 2010) in Ethiopia. The economic losses per cow per lactation reported by Mungube et al. (2005) were US$38 and that of Tesfaye et al. (2010) were US$78. In those two studies the average milk production was used to estimate the costs associated with milk production losses due to subclinical mastitis. However, when estimating the costs of a disease effect on milk yield, it is important to look (model) at which stage of lactation (days in milking) the disease occurs rather than using a single summary for the 305-d lactational milk yield. This is important because milk loss will vary with both stage of lactation and time after the occurrence of the disease (Grohn et al., 2004). The other limitation of those two studies was that, they did not take into account clinical mastitis, which is one of the most frequent diseases in Ethiopian cross breed cattle (Lemma et al., 2001).

The dynamic stochastic Monte-Carlo simulation model (bio-economic model) in the present study is the first economic model for Ethiopian milk production circumstances to calculate the economic effect of mastitis. In fact, there are several models regarding costs of mastitis which were conducted in different parts of the world (Bar et al., 2008; Huijps et al., 2008; Halasa et al., 2009; Cha et al., 2011). However, applying these models directly to Ethiopian circumstances may not properly indicate the losses associated with mastitis as there is a great variation in the farming systems and methods of detecting mastitis. For instance, in European studies, the milk losses associated with subclinical mastitis were determined based on the somatic cell count (**SCC**) (Huijps et al., 2008; Halasa et al., 2009). However in Ethiopia, unlike European countries, the CMT is the most widely used method to detect subclinical mastitis rather than SCC. Therefore, it is important to have a model which enables the association of subclinical mastitis milk losses with the CMT score. In our model, economic effects of mastitis, both for clinical and subclinical cases, can be estimated. The model can also be used in different countries with similar farming systems and where CMT is used for detection of subclinical mastitis. In our model, because usually farmers in Ethiopia neither cull (Dego and Tareke, 2003) nor treat cows for subclinical mastitis, milk production losses were the only cost factor that we took into account for subclinical mastitis. Whereas for clinical mastitis all cost factors were accounted. In our model, we assumed that the carry-over effect of a reduced milk production due to clinical mastitis lasted the entire lactation period. This is because once cows are infected with mastitis, they often never recover their potential yield (Grohn et al., 2004). The default inputs used in our model were representative for farms with an intensive/ relatively intensive farming system that have only cross-breed cattle (Cross between local cattle and Holstein-Friesian).

The simulated annual incidences of clinical and sub clinical mastitis were 21.6% (0 to 50%) and 36.2% (0 to 75%), respectively. The outcome of the model incidence for clinical mastitis was similar with Almaw et al., (2012). This can be seen as a validation of the estimated input values for input parameters on the dynamics of mastitis that we could not take directly from the literature. The incidences of both clinical and subclinical mastitis were adjusted for by stage of lactation and parity (Table 4). Consequently, a higher incidence of both clinical and subclinical mastitis was observed in early and late lactation as compared to mid lactation. The explanation for this is that the mammary gland is more susceptible during early and late period of lactation (Radostits et al., 2000). Similarly, the incidence of mastitis varied with parity number. Variation in the occurrence of mastitis between parities was also reported by Dego and Tareke (2003) and Mungube et al. (2004). The simulated number of culled cows due to mastitis (clinical mastitis) on the farm was 0.47, varying from 0 to 2 and this number accounts for 28% of the overall culling within the farm. Workineh et al. (2002) reported that mastitis accounts for 27% from the overall culling on the farm and our result was very close to this report. The result in the present study indicated that clinical mastitis causes 30 kg milk

production loss per case, whereas subclinical mastitis causes 17 kg per case. This was low compared to studies in developed countries (Houben et al., 1993; Halasa et al., 2009). This is caused by the lower production level of cows in Ethiopia compared to European breed. In addition, the cross-breed cattle in the present study have a higher resistance and recovery rate for mastitis as compared to pure exotic breeds under the same production circumstances (Radostits et al., 2007).
The total costs due to mastitis for a farm with 8 cross-breed cows were 6,709 ETB per year (838 ETB per cow). The total costs at the farm level varied considerably with 5th and 95th percentiles of 109 ETB and 22,009 ETB, respectively. Similarly Figure 2 indicated a clear variation of the total costs of mastitis among farms. The bimodal distribution in the figure related to the discrete outcome of culled cows. The majority of the total costs were due to clinical mastitis (6,282 ETB per farm). Subclinical mastitis contribution was only minor to the total costs (426 ETB per farm). This is because culling, which is one of the highest cost factor for mastitis, and treatment were not accounted for in the subclinical mastitis costs. The costs for subclinical mastitis in our study were much lower than that of Mungube et al. (2005) and Tesfaye et al. (2010). The main reason for this difference is, in those two studies the authors assumed that there is a constant infection throughout the year (for every recovered teat there is always a new infection in the other quarter). As a result of this they used the 328 days quarter based average milk to calculate the loss associated with subclinical mastitis. However Abaineh and Sintayehu, (2001) reported a higher recovery rate of subclinical mastitis at the cow level (40%). Based on this, in our model the maximum duration of a subclinical mastitis case was 6 weeks and so the model used the milk production decrease of a cow (by assuming two quarters affected on average) only during this period of time to calculate the production loss rather than assuming a constant infection throughout the lactation period.
Because of the absence of an economic study in Ethiopia for clinical mastitis, we could not compare our study with others with the same farming system within the country. Our result for total costs of mastitis was much lower than results from European countries (Huijips et al., 2008; Halasa et al., 2009). One of the reason for this difference is, much lower milk production level of cross-bred cattle in our study than European pure breed cattle. According to Radostits et al. (2000), high yielding cows are more susceptible to mastitis than low yielding ones. Other factors that contributed include lower incidence of both clinical and subclinical mastitis, higher recovery rate and lower culling rate of cross-breed cattle in our study as compared to the European pure breed cattle.
The sensitivity analysis showed the importance of different parameters on the total costs of mastitis at the farm level. Figure 3, shows the change in total costs per year per farm when varying the different input values by 10% of the base value. According to the sensitivity analysis, the model was most sensitive for changes in the probability of occurrence of clinical mastitis, the probability of culling and culling costs in descending order. This may indicate the importance of reducing the occurrence of mastitis in the farm. By reducing the occurrence of clinical mastitis on the farm or by providing good care in the farm, the farmer can reduce the probability of culling. In addition, discarded milk and production losses can be reduced by reducing the occurrence of clinical mastitis. Another influential factor was the recovery rate from subclinical mastitis. Subclinical mastitic cows can be a constant source of infection leading to higher annual costs when the recovery from subclinical mastitis is low. The importance of the recovery rate from subclinical mastitis, can provides awareness for dairy farmers in Ethiopia in which they do not treat subclinical mastitis in most cases. One of the main reason why the farmers do not paid attention to subclinical mastitis is the absence of clinical symptom (Tolosa et al.,

2013). According to Swinkels et al. (2005) the treatment of subclinical intramammary infection might be a good way to decrease the costs of mastitis. Treatment of subclinical mastitis can reduce the costs of mastitis by reducing the number of subclinical mastitis cases flaring up to clinical mastitis and also by shortening the duration of the disease.
The total costs of mastitis which were reported in the present study are significant to the Ethiopian farmers and this can cause a negative impact on the development of the dairy sector within the country. Management in the farm is one of the main problems in relation to the occurrence of mastitis in Ethiopia (Dego and Tareke, 2003). Unhygienic milking procedures, wet and muddy stalls, especially during the rainy season, persistent tick infections, the absence of post-milking teat dipping and the absence of dry cow therapy may be possible predisposing factors of cows on the farm. Therefore, there may be a possibility for Ethiopian farmers to reduce the costs of mastitis by following proper management practice within the farm.

Conclusions

The bio-economic model developed in this study was used to estimate the economic impact of mastitis in dairy herds. The simulated annual incidence of clinical mastitis varied between 0 and 50%. For subclinical mastitis, the incidence varied between 0 and 75%. Total costs due to mastitis on the default farm under Ethiopian circumstances were 6, 709 ETB per year (838 ETB per cow) ranging from 109 to 22,009 ETB. Culling was the most important factor that contributes to the total annual costs of mastitis. The total costs at the farm level were most sensitive for variation in probability of occurrence of clinical mastitis, the probability of culling and the costs of culling, respectively. The sensitivity of the model to the recovery rate of subclinical mastitis and to the transition probability from subclinical to clinical implied that subclinical mastitis, which is almost all of the time not treated/detected by the farmers, has an impact on the total costs in the farm. Awareness by the farmers concerning mastitis could reduce the economic consequence and this can improve the development of dairy sector within the country.

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Annex 1: Modelling of the different risks of culling and the carry-over time of the effect of clinical mastitis on culling.

Based on the status of mastitis at each time period, the annual general culling, relative risk (RR) of culling when there is mastitis and the assumption made on carry-over period (CO_P), the base risk of culling and the risk of culling when a new clinical mastitis occurs for each cow (i) at each time period (t) were modeled according to the following three equations: $$BR_{Cull} = \frac{G_{Cull}}{(1 - IR_{CM}) + (IR_{CM} \times RR)} \quad [1]$$

$$BR_{Cull_{it}} = \frac{BR_{Cull}}{26} \quad [2]$$

$$P_{CullCM_{it}} = \frac{BR_{Cull} \times RR}{\left(CO_P - \frac{(CO_P \times (CO_P + 1)/2)}{CO_P + 1}\right)} \quad [3]$$

where IR_{CM}, G_{Cull} and BR_{Cull} are the incidence rate of clinical mastitis per year, the annual general culling (culling due to any reasons including mastitis) and the annual base risk of culling (culling other than mastitis), respectively. $BRCull_{it}$ represents the base risk of culling(other than mastitis) for each cow (i) at each time period (t). $PCullCM_{it}$ stands for the risk of culling when a new clinical mastitis is occurs for each cow (i) at any time period (t)

A cow recovered from clinical mastitis does not attain a full production level through the lactation period. As a result of this the base risk of culling increases until a certain period of time and we call this effect a carry-over effect of clinical mastitis on culling. It was assumed that the carry-over effect of clinical mastitis on culling lasts for a maximum of 8 periods. The risk of the carry-over effect of clinical mastitis on culling and its carry-over time since clinical mastitis occurred were also modeled according to Eq. (4) and (5), respectively.

$$P_{CullCOC_{it}} = \begin{cases} CM_{it} = 1 \rightarrow P_{CullCM_{it}} \\ 0 < TCO_{it} < CO_P + 1 \rightarrow P_{CullCM_{it}} - \frac{P_{CullCM_{it}}}{CO_P + 1} \times TCO_{it} \\ 0 \end{cases} \quad [4]$$

$$TCO_{it} = \begin{cases} t = 1 \text{ or } Cull_{it-1} > 0 \text{ or } CM_{it} = 1 \rightarrow 0 \\ CM_{it-1} = 1 \text{ or } TCO_{it-1} > 0 \rightarrow TCO_{it-1} + 1 \\ 0 \end{cases} \quad [5]$$

where, $P_{CullCOC_{it}}$ and TCO_{it} are the probability of carry-over effect of clinical mastitis on culling and its carry-over time since clinical mastitis occurred and until a new event of clinical mastitis occurs, respectively. The rest were explained previously.

Annex 2. Modelling the carry-over effect of clinical mastitis on milk production and its carry-over time since a new clinical mastitis occurs and, the CMT (California mastitis test) score status of a quarter infected with subclinical mastitis.

A cow recovered from clinical mastitis does not attain a full production level throughout the lactation period and we call this effect a carry-over effect of clinical mastitis on milk production.

In our model the carry-over effect of clinical mastitis on milk production and its carry-over time since a new clinical mastitis occurs were modeled as follows:

$$TCO_{it} = \begin{cases} t = 1 \text{ or } Cull_{it-1} > 0 \text{ or } CM_{it} = 1 \rightarrow 0 \\ CM_{it-1} = 1 \text{ or } TCO_{it-1} > 0 \rightarrow TCO_{it-1} + 1 \\ 0 \end{cases} \qquad [6]$$

$$\%MPL_{COC_{it}} = \%MPL_{CM} - \left(\frac{\%MPL_{CM}}{26}\right) \times TCO_{it} \qquad [7]$$

where, (TCO_{it}) indicates the period of carry-over effect after the occurrence of clinical mastitis until a new event of clinical mastitis occurs. (TCO_{it} = 1,…, 25). When a new clinical mastitis event occurs, it was assumed that the carry-over effect of the previous event ends and the full effect of clinical mastitis on milk loss was applied. The rest were described in the material and methods part.

When a subclinical mastitis occurs in each cow (i) at any time period (t), a CMT (California mastitis test) score status of a quarter was assigned using discrete distribution Eq. (8).

$$CMT_{it} = \begin{cases} D_{SCM_{it}} = \text{one or} > \text{three time periods} \rightarrow \text{Discerete}([P_{CMT1}, P_{CMT2}, P_{CMT3}], [1,2,3]) \\ D_{SCM_{it}} = \text{one or three time periods} \rightarrow CMT_{it-1} \\ 0 \end{cases} \qquad [8]$$

where, P_{CMT1}, P_{CMT2} and P_{CMT3} represent the probability of a subclinically infected quarter to have a CMT score of 1, 2 or 3, respectively. D_{SCM} is duration of subclinical mastitis (the maximum duration is approximately three time periods).

Chapter 5

Failure costs associated with mastitis in smallholder dairy farms keeping Holstein Friesian × Zebu cross breed cows

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Submitted

Abstract

Mastitis is a costly disease and in many areas of the world; these costs have been quantified to support farmers in their decision making with regard to prevention of mastitis. Although for subsaharan circumstances estimates have been made for the costs of subclinical mastitis (**SCM**), farm specific cost estimations comprising both clinical mastitis (**CM**) and SCM are lacking. In this paper, we quantified failure costs of both CM and SCM on 150 Ethiopian market oriented dairy farms keeping Holstein Friesian × Zebu breed cows. Data about clinical mastitis (CM) was collected by face-to-face interviews and the prevalence of subclinical mastitis (SCM) was estimated for each farm using the California mastitis test. All other relevant information needed to calculate the failure costs, such as the consequences of mastitis and price levels, was collected during the farm visits, except for the parameter for milk production losses due to SCM, which was based on literature estimates and subjected to sensitivity analyses. The average total failure costs of mastitis were estimated to be 4 765 Ethiopian Birr (**ETB**) (1 ETB = 0.0449 USD) per farm per year of which SCM contributed 54% of the costs. The average total failure costs per lactating cow per farm per year were 1 961 ETB, with a large variation between farms (range 0 to 7 357 ETB). This large variation in failure costs between farms was mainly driven by variation in incidence of CM and prevalence of SCM. Milk production losses made the largest contribution (80%) whereas culling contributed only 13-17% to the total failure costs. In our estimates, costs of veterinary services, drugs, discarded milk and labour made a minor contribution to the total costs of mastitis. Relative to the income of dairy farmers in North Western Ethiopia; the total failure costs of mastitis are high. Ethiopian farmers are, in general terms, aware of the negative consequences of CM, but creating awareness of the high costs of SCM and showing the large variation between farmers may be instrumental in motivating farmers to take preventive measures also for SCM.

Keywords
Clinical mastitis, dairy, failure cost, smallholder farms, subclinical mastitis.

Introduction

Mastitis is a common production disease affecting dairy cows worldwide. In many countries and for many circumstances, it has been calculated how costly mastitis is to a farmer (e.g., Huijps *et al.*, 2008; Geary *et al.*, 2012; Guimarães *et al.*, 2017). Often, such studies estimate failure costs (costs resulting from having mastitis), rather than preventive costs (costs associated with prevention of mastitis) (van Soest *et al.*, 2016; Hogeveen and van der Voort, 2017). Estimating farm specific failure costs is instrumental in motivating farmers to control mastitis and to support on-farm decision making as it helps evaluate the profitability of preventive measures (Heikkilä *et al.*, 2012).

Although many articles have been published on the costs of mastitis, specific information on mastitis costs in developing countries is limited. Of the 37 studies recently reviewed by Hogeveen and van der Voort (2017), only one was about a developing country (Getaneh *et al.*, 2017). As the management systems, income of farmers and other related features are different, generally, it is not possible to generalize data from studies conducted in industrialized countries to developing countries (Hogeveen and Østerås, 2005; Food and Agricultural Organization, 2014). Dairy production is of great importance for the Ethiopian economy (Tangka *et al.*, 2002). Urban and peri-urban dairy farmers serve as the major milk suppliers for the fast growing urban milk market (Gebre-Wold *et al.*, 1998; Ayenew *et al.*, 2009). In Ethiopia, mastitis is a common problem on dairy farms. Farmers often notice cases of clinical mastitis (**CM**), but generally are less aware of subclinical mastitis (**SCM**), despite its high prevalence (Almaw *et al.*, 2008; Tolosa *et al.*, 2015; Mekonnen *et al.*, 2017). Good estimates of mastitis costs, including costs of SCM may help motivate farmers to take preventive measures. Particularly, the estimation of farm specific costs can be helpful, as it provides farmers with a benchmark against which to assess their own situation.

The costs of mastitis are not well studied in Ethiopian dairy farms nor for other subSaharan contries. Two Ethiopian studies have been published on the economic impact of SCM: Mungube *et al*. (2005) reported a financial loss of US$38 and Tesfaye *et al*. (2010) of US$79 per cow per lactation. In those studies, the calculation was based on a relatively old estimation (1985) of milk yield at the early days of cross-breeding of Holstein Friesian × Zebu breed cows (Mekonnen *et al.*, 1985). In the meantime, the average milk yield of cross breed cows has increased because of increased Holstein Friesian blood level, resulting in higher milk yield, but likely also in increased prevalence of SCM, as the Holstein Friesian blood level was found to be positively associated to SCM prevalence in Ethiopia (Mekonnen *et al.*, 2017). In addition, costs related to CM such as costs related to treatment, culling and labour in managing sick cows have, in previous studies, not been included despite the fact that these costs are expected to be substantial. Recently, the total costs of CM and SCM were estimated for Ethiopian market oriented dairy farms by using a normative bio-economic simulation model (Getaneh *et al.*, 2017), using available knowledge from the literature. Because hardly any data from Ethiopia was available, almost all parameters had to be based on knowledge from other countries and experts' opinions (Getaneh *et al.*, 2017). Moreover, although a normative study with limited data can give insight in the average costs of mastitis, insight in the real farm-to-farm variation in costs is difficult to obtain with such models. In this study, we collected the farm specific parameters needed to calculate per-farm failure costs of mastitis through questionnaires and by testing individual cows for SCM on 150 farms. Our aims were to estimate the average total failure costs of mastitis in North-West

Ethiopian urban and peri-urban dairy farms and to quantify the between-farm variation in these costs.

Material and Methods

Studied Herds and sampling design

The studied herds kept Holstein Friesian × Zebu breed cows. The herds were selected from a list of urban or peri-urban dairy farms in the area of Bahir Dar and Gondar in North-Western Ethiopia. By collecting lists of dairy farms from artificial insemination records, from dairy cooperatives, and from veterinary clinics, a longlist of 1 209 dairy farms from Gondar and of 272 from Bahir Dar was made. Computer generated random numbers were assigned, stratified for Gondar and Bahir Dar, to all farms on the longlist. Farms were enrolled in the order of the assigned random numbers.
If a farmer was not willing to participate in the study, had stopped dairy farming, if the farm could not be found, or if a farm only had cows of indigenous breeds, that farm was skipped from the list and the farm with the next number was approached. For these reasons, 230 farms in Gondar and five farms in Bahir Dar were skipped. Finally, we could include a total of 150 farms, 89 from Gondar (27% of the listed farms) and 61 from Bahir Dar (26% of the listed farms). The studied herds and the sampling design have been described in more detail in Mekonnen *et al*. (2017).

Design of the questionnaire and on farm data collection

A questionnaire aiming to collect data required to estimate the farm-specific failure costs of mastitis was prepared based on literature and our own expertise. Questions were designed to collect data in such a way that farm-specific calculations of the failure costs of mastitis (after Huijps et al. 2008) could be made. Data included the average milk yield and lactation length, the occurrence of CM during the last year (365 days), the impact of mastitis on milk yield, treatments and the duration of treatments, the withholding periods, costs of veterinary service, costs of labour, and costs of culling.
The order of the questions was carefully chosen in order to avoid leading questions. 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 questions used in the data collection are included (Annex). In addition, the questionnaire was tested by performing five pilot interviews in a convenience sample of farmers. Selection of participants and the data collection took place together with data collection for another study in which the data collection procedure was described in more detail (Mekonnen *et al.*, 2017). Since there is no routine testing program for SCM in place, milk from each quarter of each cow was tested with the California mastitis test (**CMT**). Quarter milk CMT scores were classified as negative (0), trace (T), weakly positive (1+), distinctively positive (2+), and strongly positive (3+), in accordance with National Mastitis Council recommendations (National Mastitis Council, 1999). A quarter was considered to have SCM if it scored trace (T), +1, +2, or +3.

Calculation of failure costs

Failure costs of mastitis were calculated based on the prevalence of SCM by the CMT score at quarter level and the incidence of CM obtained from the questionnaire, in combination with information obtained from the questionnaire and the literature. When a

farmer did not have information on specific parameters, these were estimated. For instance, the quantity of discarded milk during treatment was estimated by assuming that all quarters of a cow contribute equally to the average milk yield of a cow and was furthermore based on the farmer's response on the number of quarters from which milk was withheld or discarded; the time that was spent in managing a sick cow was estimated by our experience to be 10 minutes per day; sick cows need special management for five days and animal attendants working time was estimated to be 10 hours per day. Calculations were carried out at the farm level. The calculations were done, based on the framework described by Halasa *et al*. (2007) and the methodology used by Huijps *et al*. (2008), with the costs of SCM based on CMT scores. Costs of milk production losses due to CM, treatment (antibiotics, labour and discarded milk), veterinary visits and culling were summed to estimate the total costs of CM. As farmers did not have information on the effect of SCM on milk yield, we used data from a study conducted on crossbred dairy cows in Ethiopia (Mungube *et al.*, 2005) which showed that udder quarters with CMT scores of 1+, 2+ and 3+ had a reduced milk production of respectively 1.2%, 6.3% and 33% compared to CMT score 0.
To estimate the total failure costs per farm, we made the following assumptions:

1. Because there is no routine testing of SCM in Ethiopia, we assumed a constant SCM prevalence at the herd level over the year, based upon the single cross sectional CMT measurement during the farm visit.
2. Milk production losses from quarters with a CMT score T and a CMT score 1+ were considered to be equivalent.
3. The value of milk suckled by calves while CM cows were not milked because of treatment and drug withdrawal was considered to be insignificant.
4. Only one quarter was assumed to be affected in a cow with CM.
5. Because parity of cows was generally unknown to the farmers, all CM cases were considered to occur in the same average parity.
6. Because dairy farmers did not have any cow records, lactation length was uncertain. Therefore, the failure costs of mastitis per lactating cow were estimated at an average lactation length (314 days).
7. If farmers could not estimate the duration of milk production losses due to CM after the drug withdrawal period, milk production losses due to CM were considered to last for half a lactation.
8. Milk production level was considered constant throughout lactation.
9. Losses in milk production due to CM and SCM were calculated independent from each other.
10. When a farmer had veterinary service from a governmental veterinary clinic as well as from a private veterinary service, it was assumed that half of the cases were treated by the government veterinary clinic and half by the private veterinarian.

Costs of subclinical mastitis

Costs of SCM is estimated as:

$$C_i^{SCM} = [(N_{\mathrm{T}1i}^{SCM} * 0.012) + (N_{2i}^{SCM} * 0.063) + (N_{3i}^{SCM} * 0.33)] * AvM_i/4 * l_i * P_i^M \qquad [1]$$

Where:

C_i^{SCM} = costs of SCM in farm *i*

$N_{\mathrm{T}1i,2i,3i}^{SCM}$= number of SCM quarters with CMT score T, 1+, 2+ or 3+ in farm *i*

$AvM_i =$ average daily milk yield of cows in kg in farm *i*

l_i = lactation length in days in farm *i*

P_i^M= price per kg of milk in farm i.

Costs of withholding or discarding milk due to clinical mastitiss

In this paper, we define withholding milk as milk not being delivered to the market for a certain period of time. If a cow has a quarter with CM, milk can be withheld from all quarters, or only of the affected quarter, while milk of the unaffected quarters is delivered. In some farms a cow with a CM quarter was not milked at all, but was suckled by a calf during treatment, while the cow was milked again after the end of the treatment period. In other farms, only CM quarter(s) were not milked during the period of treatment and drug withdrawal. The number of days that milk was withheld or discarded for drug withdrawal differed between farms. Therefore, the calculations were made specific to the individual farm situation as follows:

$$C_i^{WD} = N_i * AvM_{i/4} * (T_i^T + T_i^W) * P_i^M \quad [2]$$

Where:

C_i^{WD} = costs of withholding or discarding milk due to CM in farm i

$N_i =$ number of quarters not milked in farm i during treatment and for variable drug withdrawal period (days)

$AvM_i =$ average daily milk yield per cow in farm i

T_i^T = Number of days milk withheld because of treatment in in farm i

T_i^W = number of days milk withheld because of drug withdrawal in farm i

P_i^M = price per kg of milk in farm i.

Costs of milk production losses due to clinical mastitis

Costs of milk production losses due to CM after the drug withdrawal period were estimated based on the number of CM cases, the milk production losses per day, and duration of milk production losses:

$$C_i^{ML} = N_i^{CM} * L_{di}^M * T_i^{ML} * P_i^M \quad [3]$$

Where:

C_i^{ML} = costs of milk production losses after drug withdrawal period in farm i

N_i^{CM} = number of CM cases in farm i

L_{di}^M = milk production losses in kg per day due to CM after drug withdrawal period in farm i

T_i^{ML} = duration of milk production losses (days) due to CM after the drug withdrawal period in farm i

P_i^M= price per kg of milk in farm i.

Costs of veterinary services and drugs

The costs of veterinary services and drugs were calculated using the number of CM cases and the price for veterinary services and drugs:

$$C_i^{VSD} = N_i^{CM} (P_i^{VS} + P_i^D) \quad [4]$$

Where:

- C_i^{VSD} = costs of veterinary services and drugs in farm *i*
- N_i^{CM} = number of CM cases in farm *i*
- P_i^{VS} = price of veterinary service per case in farm *i*
- P_i^D = price of drugs per case in farm *i*.

Costs of labour

The amount of labour that the farmer spent on CM differed, depending on the source of the veterinary service. In farms that obtain veterinary services from a private veterinarian or treat mastitis themselves, CM cases cost them extra labour for managing the sick cow. This was estimated to be 50 minutes per case. In farms that use government veterinary service, there were additional labour costs, because the cow had to be taken to and from the governmental veterinary clinic. In farms that use private veterinary service and in farms where the farmer treats CM himself, the costs were calculated as:

$$C_i^L = N_i^{CM} * P_i^{Lh} * \frac{50}{60} \quad [5]$$

In farms that use government veterinary service as

$$C_i^L = (N_i^{CM} * P_i^{Lh} * \frac{50}{60}) + (N_i^{CM} * P_i^{Lh} * T_i^{TR}) \quad [6]$$

In farms that use both, private and government veterinary services as

$$C_i^L = \frac{1}{2}(N_i^{CM} * P_i^{Lh} * \frac{50}{60}) + \frac{1}{2}[(N_i^{CM} * P_i^{Lh} * \frac{50}{60}) + (N_i^{CM} * P_i^{Lh} * T_i^{TR})] \quad [7]$$

Where:

- C_i^L = costs of labour because of CM in farm *i*
- N_i^{CM} = number of CM cases in farm *i*
- P_i^{Lh} = price of labour per hour in farm *i*
- T_i^{TR} = time spent to take a cow to and from a governmental veterinary clinic in farm *i*.

Costs of culling

The costs of culling because of mastitis were calculated based on the price of a replacement cow and the slaughter value of the culled cow:

$$C_i^{Cu} = N_i^{Cu} * (C_i^R - P_i^{Cu}) \quad [8]$$

Where:

- C_i^{Cu} = costs of mastitis-related culling in farm *i*
- N_i^{Cu} = number of cows culled because of mastitis in farm *i*
- C_i^R = costs of a replacement cow in farm *i*
- P_i^{Cu} = slaughter value of a culled cow in farm *i*.

Total failure costs of mastitis

The total farm-specific failure costs of mastitis in farm *i* were calculated by summing the individual cost factors:

$$FCM_i = C_i^{SCM} + C_i^{WD} + C_i^{ML} + C_i^{VSD} + C_i^L + C_i^{Cu} \quad [9]$$

Where:

- FCM_i = total failure costs of mastitis in farm *i*
- C_i^{SCM} = costs of SCM in farm *i*
- C_i^{WD} = costs of withholding or discarding milk due to CM in farm *i*
- C_i^{ML} = costs of milk production losses after milk discarding in farm *i*
- C_i^{VSD} = costs of veterinary services and drugs
- C_i^L = costs of labour in farm *i*
- C_i^{Cu} = costs of culling in farm *i.*

Farm-specific failure costs of mastitis

The farm-specific failure costs of mastitis per cow were estimated by dividing the FCM_i by the number of lactating cows on the farm:

$$FCM_i^c = \frac{FCMi}{N_i^{Co}} \quad [10]$$

Where:

- FCM_i^c= failure costs of mastitis per cow in farm *i*
- FCM_i = total failure costs of mastitis in farm *i*
- N_i^{Co} = the number of lactating cows in farm *i.*

Because apart from production losses due to SCM, all other costs are related to CM, the failure costs per case of CM per farm could be calculated as

$$FCM_i^{ca} = \frac{FCM_i - C_i^{SCM}}{N_i^{CM}} \qquad [11]$$

Where:

- FCM_i^{ca}= failure costs per case of CM in farm *i*
- FCM_i = total failure costs in farm *i*
- C_i^{SCM} = costs of SCM in farm *i*
- N_i^{CM} = the number of CM cases in farm *i.*

Sensitivity analysis

The milk production losses due to SCM were based on Mungube *et al*. (2005), who estimated losses from SCM by comparing milk yield from mastitic quarters with mastitis-free quarters within a cow. It is known, however, that mastitis-free quarters partially compensate the reduced milk yield of mastitic quarters (McDougall, 2002; Green *et al.*, 2006). Thus, the default calculation of milk production losses, based on Mungube *et al*. (2005) can be seen as maximum production losses. Therefore, we considered a scenario where 50% of the milk production losses reported by Mungube *et al*. (2005) were used in the calculations. Because the price of milk was highly variable between farms, depending on their market circumstances, the calculations were also carried out with a fixed, average, milk price instead of the individual farmer reported milk prices.

Results

All farms used a tied housing system and farmers hand milked their cows twice a day. The studied herds had, on average, 5.6 head of cattle, varying from 1 to 37, and 2.8 lactating cows, varying from 1 to 17. Dairy farmers sold the milk they produced directly to families (68%), restaurants and hotels (23%), or through a farmers' cooperative (9%). Forty one percent of farms used a government veterinary clinic, another 41% of farms used a private veterinary service, 9% used both and 9% of the farmers used no veterinarian and treated their cows with antibiotics themselves.

The average quarter level incidence rate of CM was 0.13 per farm per year. Most farms (75 out of 150 farms) did not experience CM at all in the last year. Of the farms that did experience CM, the incidence varied largely, due to the small herd sizes.

The average within farm prevalence of positive CMT (≥T) score was 30%, with a large variation between farms (Figure 1). On 30 farms, no positive CMT scores were found.

The minimum, average and maximum values of the most important input items are summarized in Table 1. Milk price, duration of milk production losses after clinical recovery and costs of culling varied largely between farms.

Failure costs of mastitis

The average farm-specific failure costs of mastitis per year in North-Western Ethiopian dairy farms were 4 765 Ethiopian Birr (**ETB**) (1 ETB = 0.0449 USD) at the baseline scenario and 3 485 ETB at the low milk production losses scenario (Table 2). The average farm-specific failure costs per lactating cow per farm per year, respectively, were 1 961 ETB and 1 554 ETB at the baseline and at low milk production losses scenarios.

Large differences were seen between farms in the total failure costs of mastitis, costs of SCM and costs of CM per lactating cow per farm (Figure 2). The failure costs of CM per lactating cow were higher than the failure costs of SCM per lactating cow in the majority of the farms. Also, the costs of CM per cow per farm varied more than the costs of SCM per cow per farm.

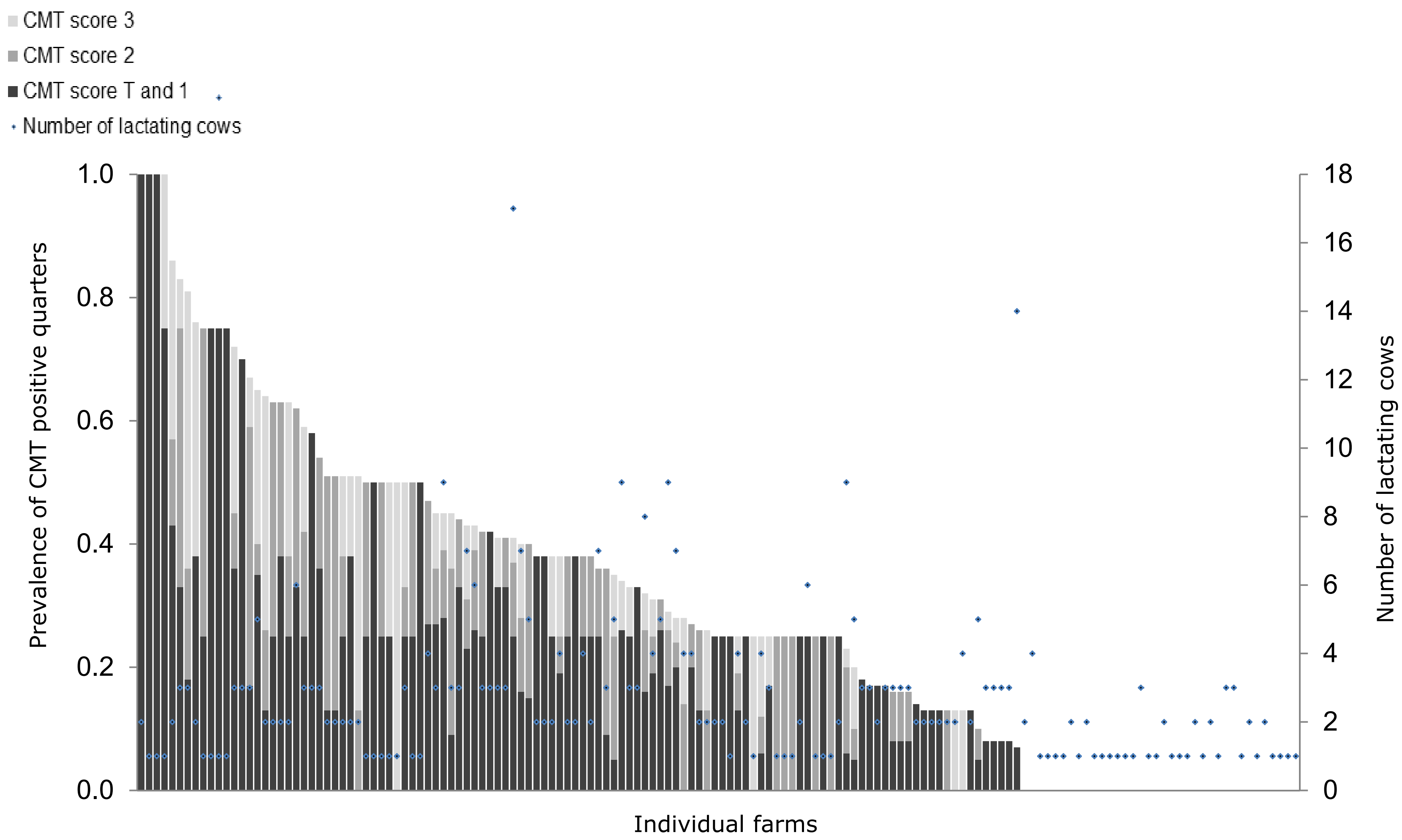

Figure 1. The prevalences of quarters of positive CMT (California mastitis test) score T and 1, CMT score 2 and CMT score 3 in 150 market oriented dairy farms in North-Western Ethiopia.

Table 1. Descriptive statistics of model input parameters based on a questionnaire administered at 150 market oriented dairy farms in North-Western Ethiopia.

Model parameter	Minimum	Average	Maximum
Number of quarters with CMT[1] score T and 1	0	2.04	17
Number of CMT score 2	0	0.87	8
Number of CMT score 3	0	0.54	5
Average daily milk yield per cow (kg)	4	10.4	22
Lactation length (days)	150	314	365
Price per kg of milk (ETB[2])	8	11.2	15
Treatment duration (days)	1	3.6	6
Number of clinical mastitis cases	0	0.99	10
Drug withdrawal period (days)	1	4.8	15
Milk production losses per day due to clinical mastitis after drug withdrawal period (kg)[3]	0.5	1.75	7
Milk production losses per day due to clinical mastitis after drug withdrawal period (%)[4]	25	48	60
Duration of milk production losses due to clinical mastitis after drug withdrawal period (days)	3	58	195
Price of veterinary service per case (ETB)	30	132	500
Price of drugs per case (ETB)	15	64	180
Time to take and return a cow to veterinary clinic (minutes)	20	96	240
Price of labour per hour (ETB)	0.5	4	11
Costs of a replacement cow (ETB)	5 000	10 500	17 000
Slaughter value of a cow (ETB)	10 000	24 642	35 000

[1] California mastitis test.

[2] Ethiopian Birr (1 ETB = 0.0449 USD).

[3] Response of 60 dairy farmers who experienced CM last year for the milk losses due to clinical mastitis after drug withdrawal period.

[4] Response of 15 dairy farmers who experienced clinical mastitis last year for the milk losses due to clinical mastitis after drug withdrawal period.

Table 2. Average farm-specific failure costs of mastitis in ETB (1 ETB = 0.0449 USD) per farm per year and per lactating cow per farm per year at a baseline scenario and at a low milk production losses scenario, based on farmers' reported milk prices, and at an average milk price 11.20 ETB on 150 market oriented dairy farms in North-Western Ethiopia.

Cost factors	Calculation scenarios						Average milk price[2]		
	Baseline			Low milk losses[1]					
	Average	5%	95 %	Average	5%	95%	Average	5%	95 %
FCM[3] per farm (ETB/year)									
Subclinical mastitis	2 569	0	12 469	1 284	0	6 234	2 706	0	12 580
Clinical mastitis	2 196	0	5 680	2 196	0	5 680	2 206	0	12 123
Total	4 765	0	20 281	3 480	0	14 383	4 912	0	23 075
FCM per cow per farm (ETB/year)									
Subclinical mastitis	814	0	2 939	407	0	1 470	838	0	3 251
Milk production losses	814	0	2 939	407	0	1 470	838	0	3 251
Clinical mastitis	1 147	0	12 290	1 147	0	12 290	1 128	0	5 041
Withheld milk	64	0	236	64	0	236	61	0	230
Milk production losses after drug withdrawal	688	0	4 438	688	0	4 438	672	0	4 238
Veterinary services and drugs	109	0	418	109	0	418	109	0	418
Labour	29	0	199	29	0	199	29	0	199
Culling	257	0	2 740	257	0	2 740	257	0	2 740
Total	1 961	0	7 357	1 554	0	6 402	1 966	0	7 515
FCM per case (ETB) of clinical mastitis	1 793	80	9 720	1 793	80	9 720	2 151	76	11 088

[1] An alternative scenario where 50% of the milk production losses as reported by Mungube et al. [10] were used.
[2] Average milk price across farms, instead of the individual farm milk prices were used to calculate failure costs for individual farms.
[3] Failure costs of mastitis.

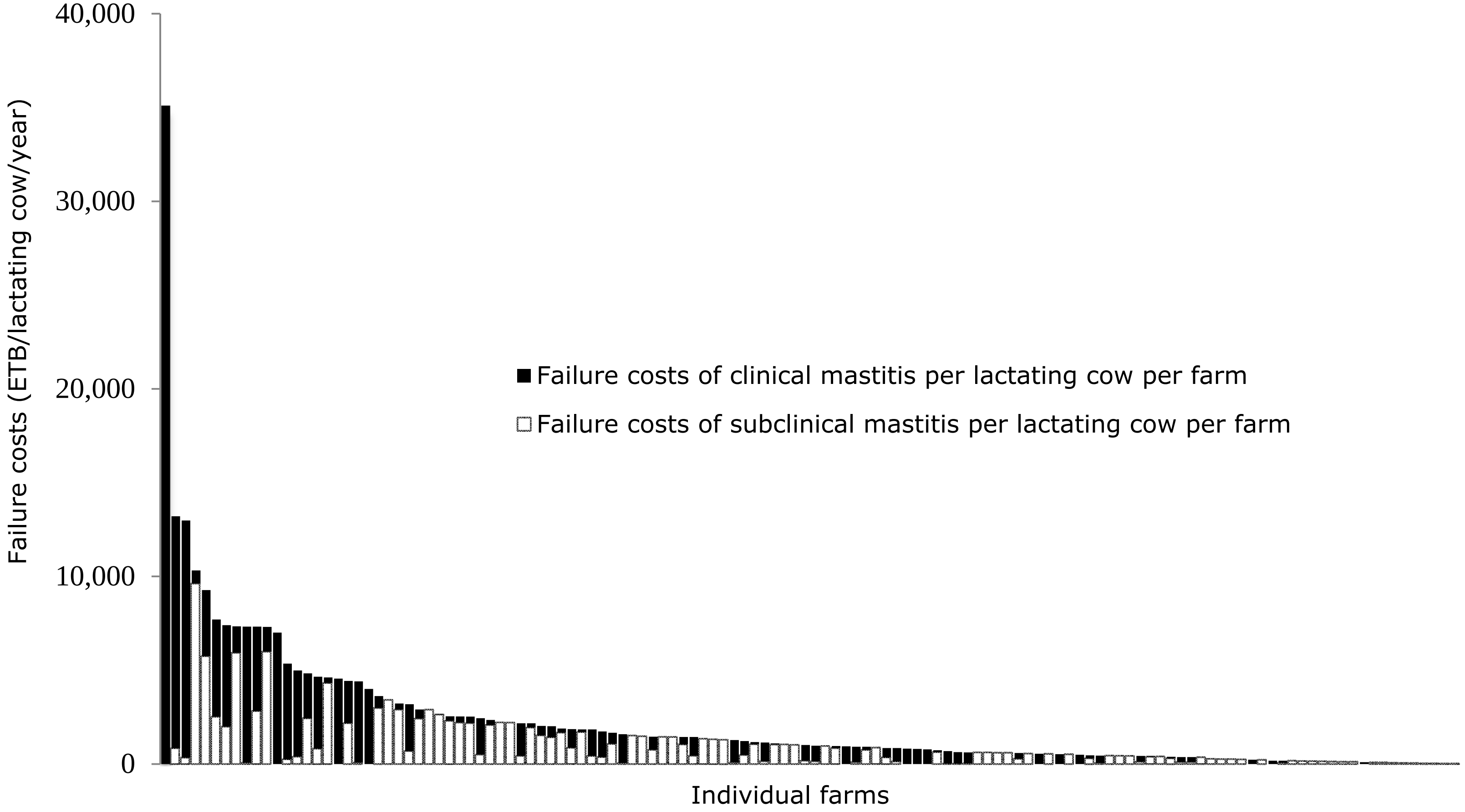

Figure 2. Failure costs of mastitis in ETB (Ethiopian Birr) per lactating cow per farm summed for subclinical mastitis and clinical mastitis on 150 market oriented dairy farms in North-Western Ethiopia.

Discussion

Although there is a reasonable number of papers on failure costs of mastitis, to our knowledge, only a few studies estimated the costs of mastitis at individual farms (Huijps *et al.*, 2008; van Soest *et al.*, 2016; Guimarães *et al.*, 2017). Specifically for developing countries this type of knowledge, although valuable, is lacking. Through estimating farm-specific failure costs of mastitis in dairy herds of small sizes in general and more specifically farm-specific failure costs of mastitis referring to North-Western Ethiopian dairy farms, our study gives more insight in these economic effects for both, individuals farmers as well as veterinarians and other advisors in different roles.

Total failure costs of mastitis

The average farm-specific failure costs were 4 765 ETB and 3 480 ETB per farm, and 1 961 ETB and 1 554 ETB per lactating cow per year at the baseline and the low milk losses scenario, respectively. The average farm-specific failure costs per farm were lower while the costs per lactating cow were higher than previously estimated for Ethiopian Holstein Friesian × Zebu breed cows (Getaneh *et al.*, 2017). Besides the failure costs described in this paper, it is known that additional costs related to mastitis may result from associations with other diseases and reproductive failure (Heravi Moussavi *et al.*, 2012; Gunay and Gunay, 2008; Boujenane *et al.*, 2014). In this study, as in almost all other economic studies, such associations were not taken into account. This might have led to an underestimation of the failure costs of mastitis.

In absolute terms, the failure costs of mastitis estimated per cow were lower than the failure costs estimated for Western dairy systems (van Soest *et al.*, 2016; Huijps *et al.*, 2008; Geary *et al.*, 2012). In relative terms, however, comparing the estimated failure costs of mastitis per cow to the milk returns per cow, the costs of mastitis in Ethiopia are high. Comparing 1 961 ETB failure costs per cow per year, an average milk price, an average number of days in lactation and an average daily milk yield, revealed that the failure costs of mastitis per cow per year equal to 5.2 % of the average milk returns per cow. If we do a similar calculation, for instance for Dutch dairy farms, the failure costs of mastitis per cow per year were 3.9 % of the average milk returns per cow (Huijps *et al.*, 2008). This indicates that, in relative terms, these Ethiopian dairy farmers are losing larger income due to mastitis, as compared to Dutch dairy farms.

Great variations were seen in the average farm-specific failure costs and in the failure costs per cow between farms. The variation in milk price from (8 ETB to 15 ETB), explains part of the high variation in failure costs between farms. When using the average milk price instead of the farm specific milk price, the 95^{th} percentile of the failure costs increased from 20 281 ETB to 23 075 ETB and the variation in failure costs of mastitis between farms is mainly caused by the difference in incidence of CM and/or prevalence of SCM. When the average duration of milk production losses after clinical recovery was used, instead of the farmers' reported duration of milk production losses after clinical recovery, the average failure costs of mastitis per lactating cow doubled (data not shown). This indicates that farmers underestimated the duration of milk production due to CM. The variation in failure costs due to mastitis between farms indicates that it is possible to gain additional income by controlling mastitis in North-Western Ethiopia.

All in all, the largest part of the variation in failure costs can be explained by differences in incidence of CM and prevalence of SCM. The large variation in incidence of CM and prevalence of SCM is partly due to the small sizes of the farms in the study, with a huge impact of one case of mastitis in small herds, with for instance only one lactating cow. In our study SCM was measured at one point in time, assuming it was constant throughout the year. Would SCM have been measured more frequently, the estimated SCM per farm would be closer to the mean, and variation between farms would be smaller. Therefore we may have overestimated the variation in SCM.

Although we wanted to base the estimate of failure costs of mastitis as much as possible on actual farm data, it was not possible to retrieve all needed data from the field. Therefore we had to make a number of assumptions, for instance on lactation length and parity of cows with CM. Because of these assumptions, there may be discrepancies between the real failure costs of mastitis and our estimates. In the sensitivity analysis, the effect of these assumptions were studied but were found to be small. For instance, the effect of a different lactation length was 18 ETB and 70 ETB on the average failure costs of mastitis per farm and per cow per year, respectively (data not shown). This indicates that the lack of information on these parameters, only had a minor effect on the estimates of the total failure costs of mastitis.

Failure costs of subclinical mastitis

A relatively large part (54%) of the total failure costs was due to SCM, which contradicts the results of Getaneh *et al*. (2017), who found a lower contribution of SCM to the failure costs of mastitis. One explanation could be differences in the prevalence of positive CMT score quarters. Getaneh *et al*. (2017) used a 40% cow level recovery rate of SCM while in the current study, prevalence of positive CMT score quarters were assumed to be constant throughout the year. The measurements in our study were, generally, better than the approach of Getaneh *et al*. (2017) because most of the input values in the current study were based on data directly collected from dairy farmers.

The large contribution of SCM to the total failure costs of mastitis is caused by the high prevalence resulting from the generally chronic nature of SCM cases (Petrovski *et al.*, 2006; Tolosa *et al.*, 2013). Ethiopian dairy farmers lack awareness about the existence of SCM because there is no routine measurement of somatic cell count or any other parameter to monitor SCM (Mungube *et al.*, 2005; Almaw *et al.*, 2008; Tolosa *et al.*, 2013). In a situation where farmers are neither aware of the prevalence, nor of the economic consequences of SCM, it is unlikely that farmers will make efforts to prevent SCM. Therefore, counselling dairy farmers about the impacts of SCM and the potential profit of mastitis control measures, as well as training them by showing applications of mastitis prevention and management measures, is important to motivate a change in behaviour of dairy farmers to improve udder health (Lam *et al.*, 2011).

Failure costs of clinical mastitis

The total failure costs of CM, both per farm and per cow, estimated in our study were lower than costs of CM estimated by Getaneh *et al*. (2017). Differences between the previous and the current cost estimates can be explained by the differences in incidence of CM and duration of milk production losses between the current study and the study by Getaneh *et al*. (2017). The current estimates were based on farmers' responses by recalling the number of cases of CM in the last year. Therefore, cases might have been missed due to recall bias. The estimates of Getaneh *et al*. (2017) were based on an incidence obtained from a previous study of Almaw *et al*. (2012) conducted in Ethiopia. Of the dairy farmers who experienced CM in the last year, 55% believed that, after clinical recovery, the milk production of a cow is equivalent to the milk production before CM. Based on this, we think it likely that dairy farmers underestimated the duration of milk production losses due to CM. Milk production losses, however, contributed most to the total failure costs of CM. This underlines our finding that North-Western Ethiopian market oriented dairy farmers underestimate failure costs of mastitis.

In conclusion, mastitis in North-Western Ethiopian market oriented dairy farms was associated with higher failure costs than costs of mastitis previously reported for Ethiopian Holstein Friesian × Zebu breed cows' farms. On average, farm-specific failure costs of mastitis were 1 961 ETB per lactating cow per year and showed large variation between farms. Relative to the milk returns per cow per year, these costs are high as compared to developed countries. Total failure costs of mastitis were mainly due to costs of milk production losses both for CM and SCM. Although a large proportion (54%) of the total failure costs of mastitis was due to SCM, Ethiopian dairy farmers seem to lack knowledge on the occurrence of SCM. This showed that mastitis and specifically SCM deserve more attention in livestock development extension service and in dairy health training programs.

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Annex: Questionnaire on failure costs of mastitis

Dairy farm number: ___________

Name of the dairy farm ……………………………………..

1. What was the average herd size during the last year? __________________
   Lactating cows: _________________ Non lactating cows: ____________________
2. Do you know what mastitis is? O Yes O No
3. Do you know whether different types of mastitis exist? O Yes O No
   If yes, could you describe the different types of mastitis: ____
   ____
4. How do you recognize clinical mastitis? ____
5. Did any of the cows in your farm experience clinical mastitis during the last year?
   O Yes O No If yes, how many of them? ___________________________
6. Do you treat cows when affected by clinical mastitis? O Yes O No
7. How many of the cows that were affected by clinical mastitis in the last year were treated? ______ How many were not treated? ________
8. Do you treat clinical mastitis yourself? O Yes O No
   - If yes, how many of them are treated by you: _________
   - How many are treated by an expert: ______________
   - If you treat clinical mastitis cases yourself, how do you treat them? ____
9. In t cows affected by clinical mastitis, how many quarters were affected in each cow?

No. of quarters per cow affected at the same time	Number of cows
1	
2	
3	
4	

10. What do you do with the milk immediately after milking? O Stored O Selling
11. Who are your milk customers?
    O People in the neighbourhood O Cooperative
    O Shops, kiosks, and supermarkets O Restaurants, coffee and tea sales and hotels
12. How much does the income from the dairy farm contribute to your family income?
    O 25% O 25-50% O 50-75% O > 75%
13. Do you have other additional source of income for your family?
    O Yes O No
    If yes, how much of your daily income comes from the additional source of income (in ETB)? ___________ ETB
14. What is the milk yield of the cows in your farm in liter per day)?

Production level	**Milk yield in Liters**
Highest yield	
Average yield	
Lowest yield	

15. What is the lactation length of the cows in your farm (in months)?

Length per lactation (month)	**Duration in month**
Minimum	

Most likely
Maximum

16. What is the average value of a liter of milk? ________________ETB/liter
17. Does milk price vary with season? O Yes O No
    If yes, what is the lowest, average and maximum price in different season?

Season	Milk price lowest	Average	Maximum

18. Do you use family labour or hired labour for your dairy activities?
    O Family labour O Hired labour All by myself
19. If you use hired labour, how much do you pay the labourer (in ETB)? ___________ birr/day/month/year.
    What are the costs of feeding the employed labourer (ETB/month)? ___________
20. If you would get your cows treated for mastitis, how much money would you be willing to pay per cow? _______ ETB
21. Where do you get veterinary service?
    O By calling a private veterinarian when a cow gets sick O From public Veterinary service O Other ------------------------
22. How much do you pay in average for the treatment of a single case of clinical mastitis?
    When working with a private veterinarian: costs -------------------/case of clinical mastitis
    Government veterinary clinic: costs -------------------/case of clinical mastitis
23. If you are using governmental veterinary service, how long does it take you to take the mastitis cow to the clinic, get treated and bring her back home? ____________________________ hours.
24. What are the costs of the drug used to treat one case of clinical mastitis in a veterinary drug shop? ___________________________________ETB.
25. Do you milk your cow while she is treated for mastitis, or do you stop milking for some time? O I continue milking O I stop milking for some time
    If you stop milking, for how long do you stop milking? _____________________ days
    If you continue milking, what is the milk used for?
    O For human consumption use O To feed calves O No use/will be discarded Other --------------
26. Do you think a cow loses milk production when she suffers from clinical mastitis?
    O Not at all O Not significant O Substantial O She loses almost all her milk

If you say clinical mastitis causes milk reduction, how much do you think the reduction in milk yield is per cow with clinical mastitis per day? __________ liters.
For how many days? ----------------------

27. After a cow with clinical mastitis cured, does she give an equivalent quantity of milk as that she did before the case of clinical mastitis? O Yes O No
    If no, how much do you estimate the reduction in milk yield (in liter per day) is after being cured from clinical mastitis? _________________________.
28. Did you remove/cull cows from your farm because they were unfit for milk production?

O Yes   O No

What is the main reason for culling cows on your farm?

O Mastitis   O Old age
O For selection of cows of better yield   O Other

29. How many cows you removed/culled because of mastitis?

O In the last year ____________   O In the last two years ____________

30. What was the price of a cow culled due to mastitis ?

Minimum __________,   Most likely__________,   Maximum__________.

31. How much does it cost you to buy a similar cow (free of mastitis) to replace a culled cow?

Minimum __________,   Most likely__________,   Maximum__________.

Chapter 6

The intention of North-Western Ethiopian dairy farmers to control mastitis

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Abstract

Understanding the intentions of dairy farmers towards mastitis control is important to design effective udder health control programs. We used the Theory of Planned Behavior (TPB) to explore the intentions of North-Western Ethiopian dairy farmers towards implementing non-specified mastitis control measures (nsMCMs) and towards implementing 4 specific MCMs. Face to face interviews were held with 134 dairy farmers to study associations between their intentions and any of three factors (attitude, subjective norm and perceived behavioral control) that, according to the TPB, determine intentions. The majority of the farmers (93%) had a positive intention to implement nsMCMs, whereas a smaller majority of farmers had the intention to implement the specific MCMs to improve udder cleaning (87%), to improve stall hygiene (78%), to improve feeding of cows (76%), and to perform foremilk stripping (74%). Farmers had a more positive attitude, but lower subjective norm and lower perceived behavioural control towards implementing nsMCMs compared with implementing most specific MCMs, although the subjective norms for stall hygiene and perceived behavioural control for improving feeding of cows were also low. Attitude was positively associated with intentions to implement nsMCMs, to improve cleaning of the udders, to improve stall hygiene and to implement foremilk stripping. Both the intention to improve udder cleaning and to implement foremilk stripping, were positively associated to subjective norms towards these MCMs. Our data can help tailor intervention programs aiming to increase the intention of Ethiopian dairy farmers to implement MCMs and thus to improve udder health in this country. We show that such programs should primarily focus on changing attitude and secondarily on improving the farmers’ subjective norms.

Keywords

Theory of planned behavior, mastitis, intention, attitude, subjective norms, perceived behavioral control.

Introduction

Recently, a remarkable increase in milk production is seen in African countries like Egypt, Ethiopia, Uganda and Namibia (Ndambi et al., 2007). Market infrastructure and increasing populations in combination with urbanization have led to the intensification of dairy farming around urban areas (Ndambi et al., 2007; Ayenew et al., 2009).
There are three dairy production systems in Ethiopia; urban, peri-urban and rural. The urban and peri-urban dairy production is market-oriented and include smallholder and specialized commercial dairy farms mainly concentrated in and around Addis and other regional towns (Yilma et al., 2011). These urban and peri-urban dairy productions keep Holstein-Friesian and Zebu cross-breed cows and are expanding and serving as the major milk supplier to the fast growing urban market (Gebre-Wold et al., 1998; Holloway et al., 2000; Ayenew et al., 2009). The rural dairy production system predominantly uses indigenous breeds with a low milk yield. This rural dairy production system is part of the subsistence farming system and includes pastoralist, agro pastoralist and mixed crop-livestock producers (Ahmed et al., 2004). Unlike the urban and peri-urban dairy production, the rural dairy production system is not market-oriented and most of the milk produced is used for home consumption. Currently, this dairy production system is being substituted by the urban and peri-urban dairy production systems.
Mastitis is the most prevalent production disease in dairy herds worldwide (Seegers et al., 2003). It is a well-documented disease with a heavy burden in both, developed and developing countries (FAO, 2014; Getaneh et al., 2017). In Ethiopia, mastitis is one of the most frequent (Mekonnen et al., 2006) diseases of dairy cows reported with high prevalence (Dego and Tareke, 2003; Mekonnen and Tesfaye, 2010; Tolosa et al., 2013). Recently, we showed that a higher Holstein-Friesian blood level is associated with more mastitis (Mekonnen et al., 2017). This is important given the growing numbers of urban and peri-urban dairy farmers that use such cross-breeds, aiming for higher milk yields. In Holstein-Friesian and Zebu cross-breed dairy herds, mastitis was one of the two major clinically manifested health problems (Lema et al., 2001) and the second cause of culling (Workineh et al., 2002) reported to be an economically important problem (Getahun et al., 2008). Associated with loss in milk yield due to subclinical mastitis alone, Mungube et al. (Mungube et al., 2005) reported a financial loss of US$38 and Tesfaye et al. (Tesfaye et al., 2010) of US$79 per cow per lactation. Several other diseases such as internal parasites, lumpy skin disease, heartwater, blackleg, hypocalcaemia and trypanosomosis are also prevalent in Ethiopia, but their importance largely differs between regions and with cattle breeds (Duguma et al., 2012; Tesfaye et al., 2012; Fasil et al., 2016; Asmare et al., 2017; Mekonnen et al., 2017). In the Holstein-Friesian and Zebu cross-breed cows in the urban and peri-urban dairy farms in the North-West of Ethiopia, mastitis likely is one of the most important diseases.
Improving udder health requires the application of appropriate mastitis control measures (MCMs) by those involved in managing dairy herds and the milking process (Garforth, 2011). Success of implementation of udder health programs depends on the willingness of farmers to change their behavior (van den Borne et al., 2014). Strategies that enhance farmers' motivation to improve udder health in their herds might, therefore, be an important part of effective mastitis control programs. In large dairy herds in the developed world, farmers have been shown to be motivated to improve mastitis control based on their perception of the economic losses of mastitis (Valeeva et al., 2007; Huijps et al., 2008). Money, however, is not the only factor that motivates farmers in their decisions to improve mastitis management (Valeeva et al., 2007). Non-monetary factors,

such as farmer characteristics, and psychological factors such as attitude (AT), subjective norms (SN) and perceived behavioral control (PBC) also the motivation of farmers to improve mastitis and to implement MCMs (Jansen et al., 2009; van den Borne et al., 2014).

Understanding the drivers of dairy farmers' motivation is, therefore, essential for implementing effective intervention strategies (Valeeva et al., 2011; Bruijnis et al., 2013). However, only a limited number of studies have investigated factors motivating farmers to improve animal disease control in Ethiopia or other sub Saharan countries (Kairu-Wanyoike et al., 2014; Jemberu et al., 2015), none of them focusing on mastitis. The theory of planned behavior (TPB) framework has been used in several studies to obtain insight in the psychological factors that influence intentions related to animal health. This approach has, for instance, also been used by Lind et al. (Lind et al., 2012) to investigate farmers' participation in herd health programs and their behavior concerning treatment of mild clinical mastitis and by Bruijnis et al. (Bruijnis et al., 2013) to gain insight on dairy farmers' intention to improve dairy cow foot health.

The present study was conducted with the goal to identify what determines the motivation of North-Western Ethiopian dairy farmers towards controlling mastitis, and specifically to study how their AT, SN and PBC towards MCMs are related to their intention to implement these MCMs. A second goal was to identify socio-demographic characteristics potentially affecting farmers' intentions trough associations with their AT, SN and PBC.

Materials and methods

Theoretical framework

According to the TPB, a behavioral intention indexes a person's motivation to perform (or not perform) a behavior and is the immediate determinant of an action (Ajzen, 2005). The theory also postulates that a person's intention to perform (or not perform) behavior of different kinds can be predicted with high accuracy from his AT, SN, and PBC (Figure 1) (Ajzen, 1991). By changing these three 'predictors', it is possible to increase the person's intention to implement a desired action and thus to increase the chance that a person will actually execute that behavior (Francis et al., 2004; Ajzen, 2005). The variables in the TPB model are psychological (internal) constructs that can be seen as latent variables which can be approximated by interviewing (Francis et al., 2004).

With regard to mastitis control, the AT is determined by the farmer's beliefs about the consequences of performing MCMs (behavioral beliefs) and the corresponding positive or negative judgements the farmer gives to this effect (outcome evaluation). Subjective norms are determined by beliefs about how other people, who may be in some way important to the dairy farmer, would like him to behave (normative beliefs) and the motivation to comply with these referents (motivation to comply). Perceived behavioral control is determined by the dairy farmer's belief whether he has the necessary resources (control belief) including knowledge, money, time and labor and how confident he feels about being able to perform or not perform the MCM (perceived power of control) (Ajzen, 1991; Francis et al., 2004).

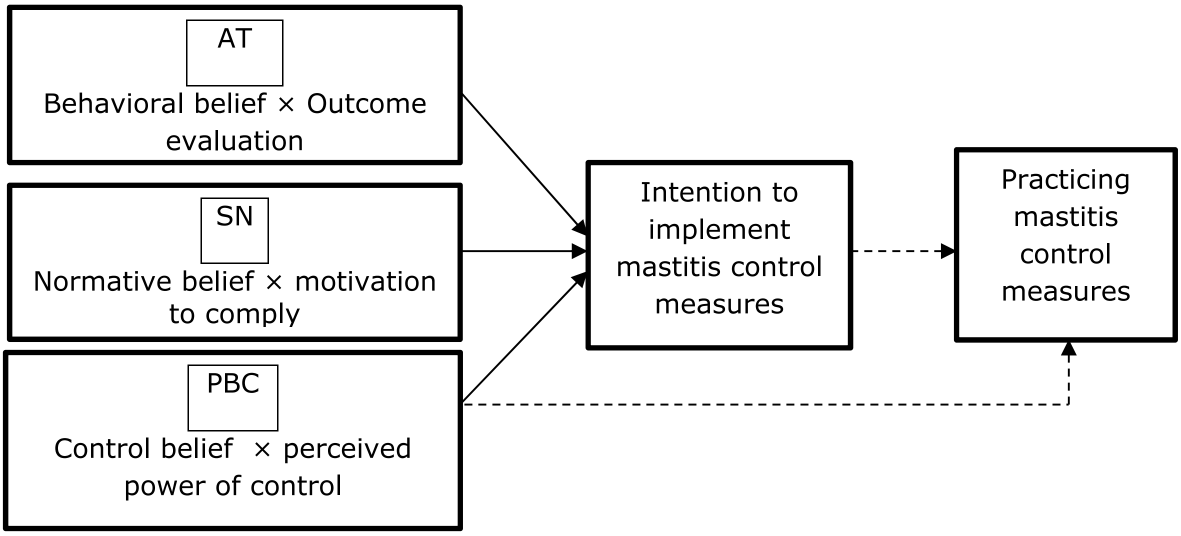

Figure 1. Framework of the theory of planned behavior model as applied in the performed analyses on the intention to participate in mastitis control measures (adapted from Ajzen (Ajzen, 1991)). AT = Attitude, SN = Subjective norm, PBC = Perceived behavioral control. Dotted lines indicate associations that are not studied in this paper.

Questionnaire design

The questionnaire design was tailored to MCM under Ethiopian circumstances and on data requirements of the TPB model. The first step in constructing the questionnaire was identifying potential MCMs for the Ethiopian peri-urban dairy farming situation. One statement was aimed at obtaining the intentions towards MCMs in general, that we refer to in this paper as non-specified MCMs (nsMCMs). Four statements were designed about intentions towards specific MCMs (Table 1).

Table 1. Statements used in the face to face interviews to quantify farmers' intentions towards implementing non-specified mastitis control measures and 4 specific mastitis control measures, using a 7 points bipolar Likert scale (-3 to 3).

Intention statements
In the near future I plan to implement one or more MCM(s) to reduce mastitis in my farm
In the near future I plan to improve the cleaning of the udders to reduce mastitis in my farm
In the near future I plan to improve stall hygiene to reduce mastitis in my farm
In the near future I plan to improve feeding of my cows to reduce mastitis in my farm
In the near future I plan to foremilk strip to reduce mastitis in my farm

MCM(s) = mastitis control measure(s).

Behavioral, normative, and control beliefs that can affect dairy farmers' behavior and corresponding evaluations were identified based on our experience. These latent variables were measured by asking respondents about statements concerning their beliefs and the corresponding evaluations.

The behavioral beliefs of AT (bbAT) towards the intention to implement nsMCMs was measured by 4 items, all referring to the impact of mastitis: treatment costs, milk production losses, risk of culling, and impact on milk quality. Behavioral beliefs of attitudes towards the specific MCMs were measured by asking dairy farmers about the importance of the specific MCMs when it would be implemented on their farm in the near future. To measure the bbAT towards improving udder cleaning, the opinion on the effect of udder cleaning on their farms was studied by asking about the effect of cleaning on milking hygiene, spread of infection and diagnosis of clinical mastitis. To measure the bbAT towards improving stall hygiene, the opinion on the effect of stall hygiene on udder health in general, spread of infection and exposure of teats to infection was quantified. The bbAT towards improving feeding of cows was studied by asking about the expected effect on the cows' nutritional balance and the cows' susceptibility for mastitis. To measure the bbAT towards implementing foremilk stripping, the farmer's opinion on the effect of foremilk stripping on diagnosis of mastitis and on the possibility to give treatment early was asked. Corresponding outcome evaluations of the behavioral beliefs of both the specific and the nsMCMs were measured by statements asking the farmer to give his own positive or negative judgements about the MCMs.

To measure the SNs, we considered veterinarians, artificial inseminators, milk customers, neighbors and other dairy farmers most important to the dairy farmer to approve or disapprove MCMs. Normative beliefs were evaluated by statements asking the farmer about the opinion of these 5 groups of people to approve or disapprove MCMs. Motivations to comply were measured by statements asking the farmer whether the opinion of those people influences his intention to implement MCMs.

Perceived behavioral control was evaluated in relation to the ability of the farmer, and to time and money needed to implement MCMs. Perceived behavioral control beliefs were measured by statements evaluating whether MCMs were difficult to implement, were time consuming and were considered expensive by the farmer. The corresponding perceived power of control was measured by statements evaluating how confident a farmer feels about being able to implement, having time to implement and having money to cover the costs to implement a MCM.

Participants gave their level of agreement for each statement on a 7 point Likert's scale. Statements referring to beliefs were evaluated using a unipolar Likert scale (1 to 7). Statements referring to outcome evaluations, motivation to comply and perceived power of control questions were evaluated using a bipolar 7 point Likert scale (-3 to 3). An illustration of the questionnaire structure is given for udder cleaning in Table 2. The English version of the whole questionnaire is available (Annex).

Table 2. Statements used to measure behavioral, normative and control beliefs and outcome evaluation, motivation to comply and perceived power of control for udder cleaning on 134 North-Western Ethiopian dairy farmers.

	Latent variables	Statements	Measurement
Attitude	Behavioral belief	What is your opinion about udder cleaning?	
		1. Udder cleaning reduces mastitis	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
		2. Cleaning the udder minimizes spread of bacteria causing mastitis	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
		3. Clean udders facilitate diagnosis of clinical mastitis	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
	Outcome evaluation	What is your opinion about the importance of udder cleaning?	
		1. Milking hygiene by cleaning the udder is important to reduce mastitis	Strongly disagree-3-2-10 1 2 3 Strongly agree
		2. Minimizing spread of mastitis causing bacteria by cleaning the udder is important to reduce mastitis	Strongly disagree-3-2-10 1 2 3 Strongly agree
		3. Facilitating diagnosis of mastitis by cleaning the udder is important to reduce mastitis	Strongly disagree-3-2-10 1 2 3 Strongly agree
Subjective norm	Normative belief	What, according to your knowledge, is the opinion of the following people?	
		1. Veterinarians think that udder cleaning is ...	Very unimportant 1 2 3 4 5 6 7 Very important
		2. Artificial inseminators think that udder cleaning is ...	Very unimportant 1 2 3 4 5 6 7 Very important
		3. Milk customers think that udder cleaning is ...	Very unimportant 1 2 3 4 5 6 7 Very important
		4. My family thinks that udder cleaning is ...	Very unimportant 1 2 3 4 5 6 7 Very important
		5. Other dairy farmers think that udder cleaning is ...	Very unimportant 1 2 3 4 5 6 7 Very important
	Motivation to comply	Does the opinion of the following people influence your intention to improve udder cleaning?	
		1. Veterinarians	Not at all -3 -2 -1 0 1 2 3 Very much
		2. Artificial inseminators	Not at all -3 -2 -1 0 1 2 3 Very much
		3. Milk customers	Not at all -3 -2 -1 0 1 2 3 Very much

		4. Neighbors	Not at all -3 -2 -1 0 1 2 3 Very much
		5. Other dairy farmers	Not at all -3 -2 -1 0 1 2 3 Very much
Perceived behavioral control	Control belief	What is your opinion on the implementation of udder cleaning?	
		1. Udder cleaning is difficult	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
		2. Cleaning of the udder is time consuming	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
		3. Cleaning the udder is expensive	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
	Perceived power of control	What is your capability to implement udder cleaning?	
		1. I know how to clean the udder	Strongly disagree-3-2-10 1 2 3 Strongly agree
		2. I have time to clean the udder	Strongly disagree-3-2-10 1 2 3 Strongly agree
		3. I can afford to cover costs of cleaning the udder	Strongly disagree-3-2-10 1 2 3 Strongly agree

Information that provides the basis for dairy farmers' beliefs about the consequences of implementing MCMs, about the normative expectations of important people, and about the obstacles that may prevent them from performing a behavior can affect behavioral, normative, and control beliefs towards the intention of performing MCMs. Similarly, different groups of people can have experiences and information that differ in important ways from the experiences of others (Francis et al., 2004; Ajzen, 2005). Therefore, additional data were collected about social and informational background factors expected to influence behavioral, normative, or control beliefs of Ethiopian dairy farmers. Open-ended or closed questions were used to collect information on these factors.
The questionnaire was prepared in English, and translated into the farmers' language (Amharic). The questionnaire was then translated back to English by an external translator, to validate the translation. The final questionnaire was tested by pilot interviews with five farmers to check whether the farmers had any difficulty in answering the questions. The farmers used for the pilot interviews were selected based on convenience. Questions which were difficult to understand by farmers were modified.

Data collection

The data was collected from selected dairy farmers in two regions in North-Western Ethiopia: Gondar and Bahir Dar. These two sites are areas where there are a large number of market-oriented dairy farmers who kept Holstein Friesian × Zebu breed dairy cows. We made an extensive list of dairy farms at the sites before selecting our respondents. Artificial insemination records from private and government artificial insemination centers, lists of dairy farmers from two dairy associations, and records from government veterinary clinics were used to prepare the list. Finally, 1,209 dairy farms in Gondar and 272 in Bahir Dar were listed. Then, a random number was given to each of the farms. Data collection started based on the order of the random numbers, separately for the Gondar and the Bahir Dar list of farms. The list for Gondar was expected to contain many records of farms that did not exist anymore or that could not be included in the study for other reasons. For that reason a much longer list was used. We are not aware of previous work related to Ethiopian or any other sub-Saharan countries dairy farmers' intentions to control mastitis. Therefore, the required sample size could not be determined based on work done previously. However, in a multiple regression approach with a response rate of 50%, a sample size of 80 is acceptable for TPB studies (Francis et al., 2004). With this in mind, we had a sampling scheme based on the time and logistics available, with the goal to include a representative sample from the population of dairy farmers. In total, 369 farms (321 from Gondar and 48 from Bahir Dar) were visited. As expected the number of farms in Gondar that could be used in the study was much smaller than the number of farms on the list; 178 dairy farms had indigenous breeds only, 30 had stopped dairy farming, of 16 farms the address could not be found and 6 farms were not willing to be interviewed (230 farms in total). When a farmer was not willing to be interviewed, had quit dairy farming or the address could not be found, that farm was skipped and the farm with the subsequent number on the list was approached. For these reasons, 230 farms in Gondar and 5 farms in Bahir Dar were not included, leaving 91 farms from Gondar and 43 from Bahir Dar to be included in the study.
For the questionnaire-based study described here, no formal approval by the Institutional Review Board of the University of Gondar or Utrecht University was required. Participating farmers were instructed about the content and goal of the questionnaire before the start of the interview (informed consent). All data were analyzed and reported anonymously. The data was collected by the first author and two final year students in Veterinary Medicine who were specifically trained for this purpose.

Statistical analysis

The data was inspected for data entry errors by checking whether all responses were within the range of the response format. The score given for each of the behavioral, normative and control belief statements was multiplied by the corresponding outcome evaluation, motivation to comply, or perceived power of control, respectively, to create a new variable that represented the product score. In this way, product composites were created for the three TPB factors (AT, SN and PBC).

Cronbach's alpha was calculated among the product composites of AT, of SN and of PBC to test internal consistency. The product composites were considered to have internal consistency and, therefore, to measure similar construct, if Cronbach's alpha was >0.7 (Field, 2009). In that case, product composites were averaged to obtain one single measure (mean score) for AT, SN and PBC (Eq.1-3). For those constructs where the Cronbach's alpha was <0.7, product composites were used as separate TPB factors.

$$\mathrm{AT} = \frac{\sum_{i=1}^{n}(bb_i * oe_i)}{n} \qquad \text{[Eq.1]}$$

Where i = the product composite item i and n = number of items for AT, bb = behavioral belief, oe = outcome evaluation.

$$\mathrm{SN} = \frac{\sum_{i=1}^{n}(nb_i * mc_i)}{n} \qquad \text{[Eq.2]}$$

Where i = the product composite item i and n = number of important referents for SN, nb = normative belief, mc = motivation to comply.

$$\mathrm{PBC} = \frac{\sum_{i=1}^{n}(cb_i * ppc_i)}{n} \qquad \text{[Eq.3]}$$

Were i = the product composite item i and n = number of relevant control belief items, cb = control belief, ppc = perceived power of control.

We did not find an established rule in literature to categorize TPB factors into levels. However, a number of factors influence the beliefs people hold such as ethnicity, socio-economic status, nationality, religious affiliation, general attitudes and values (Ajzen, 2005). Based on these reasons, it seems impossible to have standardized categorization criteria. Therefore, the TPB factors were classified into three categories based on the distribution of the product composites. Weak AT, weak SN and weak PBC (product composite ≤ 0), moderate AT, moderate SN and moderate PBC (>0 to <19), and strongly positive AT, strongly positive SN and strongly positive PBC (≥19). When the category weak or the category strong had <10 observations, categories were merged, resulting in two categories (weak and moderate or moderate and strong). For AT, SN and PBC of nsMCMs, two categories were created, below and above the median. A dependent variable was constructed for the intention towards each of the MCMs by classifying dairy farmers into low intenders (Likert score for intention ≤0) versus high intenders (Likert score for intention >0). The categorized intention variables were used as dependent variable in logistic regression models (one model for each intention) with AT, SN and PBC as predictors and, as is commonly done in TBC studies, the full models were presented. First, we did univariable analysis with region (Bahir Dar and Gondar) as fixed effects and intentions as dependent variable. The analysis didn't show any association between region and intentions. Therefore, we evaluated associations for Gondar and Bahir Dar in the same model.

To investigate the effect of background factors on TPB variables, all the background factors were used as binary variables in logistic regression models with TPB factors (AT, SN and PBC) as dependent variables. Variables significant at P<0.15 by Chi-squared test, or Fisher's exact test, when the number of observations in a cell was <5, were used in multivariable models. Variables which were significant at P<0.10 were retained in the final models using a backward stepwise procedure. The statistical analyses were performed using SPSS version 22.0 (IBM SPSS for Windows, Armonk, NY: IBM Corp).

Results

Descriptive statistics

On average, dairy farmers that participated in the study had 15 years of dairy farming experience, 5 animals in the herd and were 50 years of age. The majority of dairy farmers had a positive intention to implement nsMCMs. More farmers (93%) intended to implement nsMCMs than any of the specific MCMs. A large number of dairy farmers had a positive intention to improve cleaning of the udder, while the lowest proportion of farmers had a positive intention to improve feeding of cows (Fig 2).

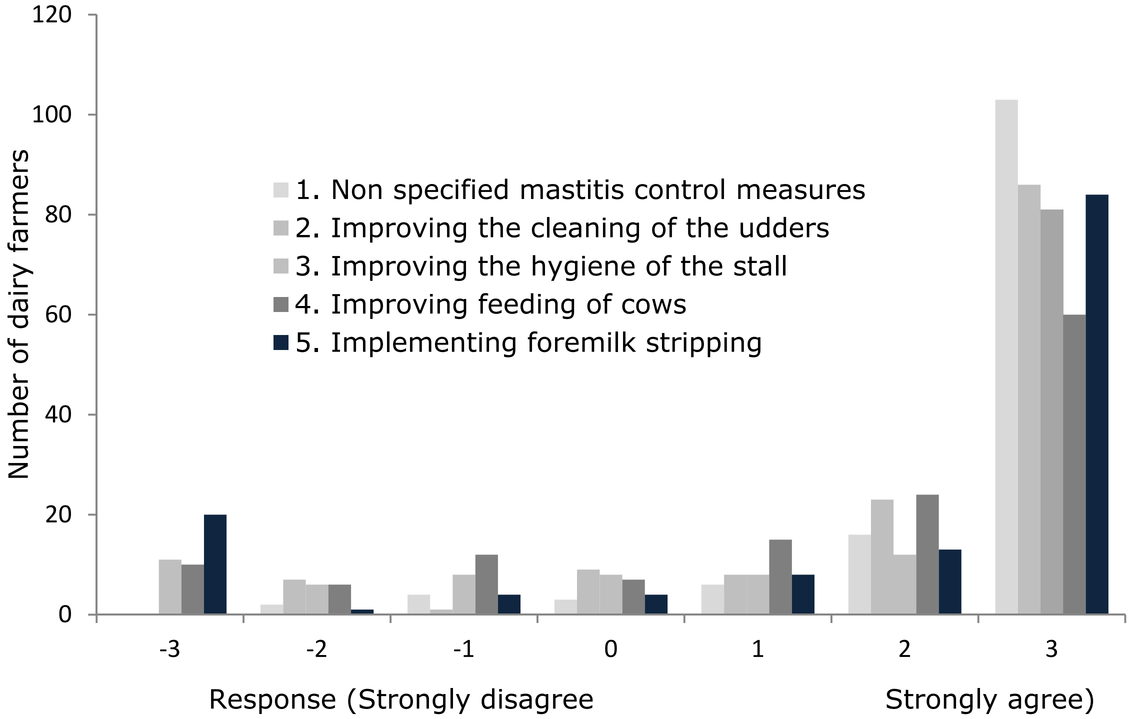

Figure 2. Dairy farmers' intentions to implement mastitis control measures based on 134 farmers in North-Western Ethiopia.

Table 3 shows the descriptive statistics of the TPB factors. Overall, the farmers often had a positive AT and positive SN, except for nsMCMs and improving stall hygiene, where respectively, 50% and 64% of farmers had a positive SN. With respect to PBC, farmers scored very high for 3 out of 5 MCMs, but the percentage of farmers positive for PBC was very low for nsMCMs and for improving feeding of cows compared to the percentage of farmers positive for PBC in other MCMs.

Table 3. Descriptive statistics of attitude (AT), subjective norms (SN) and perceived behavioral control (PBC) measured with respect to the MCM intentions in 134 farmers in North-Western Ethiopia.

Mastitis control Measure	Variable	Cronbach's alpha	Weak (n)	Moderate (n)	Strong positive (n)	Positive responses (n (%)
Non-specified mastitis control measures	AT (bb)	0.91	0	43	91	134 (100)
	SN (nb*mc)	0.71		67	67	67 (50%)
	PBC_1 (cb*ppc)	0.57		70	64	64 (48)
	PBC_2 (cb*ppc)			76	58	58 (43)
	PBC_3 (cb*ppc)			74	60	60 (45)
Improving cleaning of the udders	AT (bb*oe)	0.90	35	67	32	99 (74)
	SN (nb*mc)	0.80	26	98	10	108 (81)
	PBC (cb*ppc)	0.79	34	69	31	100 (75)
Improving stall hygiene	AT (bb*oe)	0.72	39		95	95 (71)
	SN_{1-3} (nb*mc)	0.77	19		115	115 (86)
	SN_4 (nb*mc)		74		60	60 (45)
	SN_5 (nb*mc)		52		82	82 (61)
	PBC (cb*ppc)	0.80	124		10	131 (98)
Improving feeding of cows	AT (bb*oe)	0.87	20	99	15	114 (85)
	SN (nb*mc)	0.78	36		98	98 (73)
	PBC (cb*ppc)	0.70	65		69	69 (51)
Foremilk stripping	AT (bb*oe)	0.80	21	40	73	113 (84)
	SN (nb*mc)	0.87	33	90	11	101 (75)
	PBC (cb*ppc)	0.78	11	109	14	123 (92)

AT = Attitude, bb = behavioral beliefs, oe = outcome evaluation, SN = subjective norm, nb = normative belief, mc = motivation to comply, PBC = perceived behavioral control, cb = control belief, ppc= perceived power of control, 1-3 = the 1^{st}, the 2^{nd} and the 3rd items that had Cronbach's alpha value >0.7 and averaged for SN of stall hygiene, but for non-specified mastitis control measures, it was used as separate variable without averaging them, 4-5 = the 4^{th} and 5^{th} SN items used as separate variables for tall hygiene.

The number of farmers grouped on the levels of social and informational background factors is presented in Table 4.

Table 4. Social and informational background factors expected to influence behavioral, normative and control beliefs of dairy farmers in Noth-Western Ethiopia.

Group	Background factor	Groups (n)	
Social	Gender	Male (107)	Female (27)
	Age	≤ 50 years (77)	>50 years (57)
	Level of education	≤ 8 grade (65)	>8 grade (69)
	Membership to dairy association	No (64)	Yes (70)
	Herd size	≤ 3 cows (63)	>3 cows (71)
Informational	Experience of dairy farming	≤15 years (73)	>15 years (61)
	Knowledge whether mastitis exists	No (8)	Yes (126)
	Training about dairy cows management	No (98)	Yes (36)
	Experience of mastitis in own farm last year	No (65)	Yes (69)

TPB factors associated with intentions to control mastitis

The behavioral belief statements of the intention for nsMCMs were highly correlated to the corresponding outcome evaluations, suggesting that they address the same construct. Therefore, only the scores given for behavioral belief were used in this analysis. Dairy farmers' ATs were significantly and positively associated with the intention to implement nsMCMs and specific MCMs: to improve cleaning of the udders, to improve stall hygiene and to implement foremilk stripping. Subjective norms were significantly associated with the intention to improve udder cleaning and implement foremilk stripping (Table 5). PBC was not significantly associated to any of the intentions.

Table 5. Logistic regression model results describing the association of dairy farmers' attitude, subjective norms and perceived behavioral control with intention towards implementing non-specified mastitis control measures, improving udder cleaning, improving stall hygiene, improving feeding of cows and implementing foremilk stripping, based on interviews with 134 farmers in North-Western Ethiopia. Significant odds ratios (P<0.05) are given in bold.

Mastitis control intention	Variable	Weak	Moderate OR[a] (95%CI[b])	Strong positive OR (95%CI)
Non-specified mastitis control measures	AT (bb)		Ref[c].	**18.6 (3.3-103.1)**
	SN (nb*mc)		Ref.	4.5(0.5-43.4)
	PBC_1 (cb*ppc)		Ref.	2.0 (0.3-12.3)
	PBC_2 (cb*ppc)		Ref.	0.3 (0.1-2.1)
	PBC_3 (cb*ppc)		Ref.	1.2 (0.2-6.2)
Improving cleaning of the udders	AT (bb*oe)	Ref.	**5.0 (1.0-25.5)**	6.6 (0.8-56.4)
	SN (nb*mc)	Ref.	**4.5 (1.2-16.2)**	4.6 (0.4-54.4)
	PBC (cb*ppc)	Ref.	1.1 (0.2-5.7)	1.1 (0.2-8.1)
Improving stall hygiene	AT (bb*oe)	Ref.		**2.5 (1.1-5.9)**
	SN_{1-3} (nb*mc)	Ref.		2.9 (0.9-9.9)
	SN_4 (nb*mc)	Ref.		0.6 (0.2-1.5)
	SN_5 (nb*mc)	Ref.		0.7 (0.3-2.0)
	PBC (cb*ppc)	Ref.		3.3 (0.4-28.5)
Improving feeding of cows	AT (bb*oe)	Ref.	1.6 (0.5-4.5)	2.1 (0.4-10.5)
	SN (nb*mc)	Ref.		1.0 (0.4-2.5)
	PBC(cb*ppc)	Ref		1.0 (0.4-2.2)
Foremilk stripping	AT (bb*oe)	Ref.	**4.0 (1.2-13.4)**	**8.0 (2.7-30.4)**
	SN (nb*mc)	Ref.	**4.9 (1.8-13.3)**	4.6 (0.5-47.4)
	PBC (cb*ppc)	Ref.	2.3 (0.5-9.9)	1.7 (0.2-14.5)

[a] Odds ratio, [b] 95% confidence interval, [c] Reference category, AT = Attitude, bb = behavioral beliefs, oe = outcome evaluation, SN = subjective norm, nb = normative belief, mc = motivation to comply, PBC = perceived behavioral control, cb = control belief, ppc= perceived power of control, 1-3 = the 1st, the 2nd and the 3rd items that had Cronbach's alpha value >0.7 and averaged for SN of stall hygiene, but for non-specified mastitis control measures, it was used as separate variable without averaging them, 4-5 = the 4th and 5th SN items used as separate variables for tall hygiene.

Background factors

Of the nine background factors evaluate, two were associated with SN and two other factors were associated with AT. The background factors associated at P<0.10 with TPB factors of the intentions to control mastitis in the multivariable analyses are presented in Table 6. Perceived behavioral control had no association with any of the background factors.

Table 6. Social and informational background factors significantly associated ($P<0.10$) with dairy farmers' (n=134) attitude and subjective norms that were significantly associated with intentions to implement mastitis control measures based on 134 farmers in North-Western Ethiopia.

Mastitis control measure	Background factors	TPB factors	level	OR[a] (95% CI[b])
Non-specified mastitis control measures	Experience with mastitis during the last year	AT[d]	No	Ref.[c]
			Yes	2.43 (1.07-5.53)
Improving cleaning of the udder	Gender	SN[e]	Female	Ref.
			Male	0.28 (0.08-0.98)
Foremilk stripping	Dairy farming experience	AT	≤15 years	Ref.
			>15 years	2.37 (1.01-5.56)
	Education level	SN	≤8 grades	Ref.
			>8 grades	0.44 (0.22-0.87)

[a] Odds ratio, [b] 95% confidence interval, [c] Reference category, [d]Attitude, [e]Subjective norm.

Discussion

This study was conducted to obtain insight in the determinants of the intentions of North-Western Ethiopian dairy farmers towards controlling mastitis and to identify socio-demographic characteristics associated with the farmers' AT, SN and PBC in controlling mastitis. The study was done based on the theory of planned behavior (TPB) framework to explore intentions towards nsMCMs and 4 specific MCMs. According to the TPB, behavior can be predicted at high accuracy by using behavioral intention as a proxy measure of an action (Ajzen, 2005). The theory assumes rational behavior and the intention of a person to perform a certain behavior is assumed to be influenced by their AT, SN and PBC (Ajzen, 1991). If we take udder cleaning as an example, the actual cleaning of the udder is a behavior whereas intention is readiness to clean the udder (Ajzen, 2011). Attitude refers to whether a farmer is in favor of udder cleaning, SN is dairy farmer's perception of social pressure to clean the udder and PBC represents the sense of self-efficacy or ability to clean the udder.

Intentions of dairy farmers to control mastitis

In this study, intentions towards nsMCMs and towards 4 specific MCMs were compared, which, to our knowledge, is a novel approach. We found that the majority of farmers had the intention to implement the suggested MCMs, but interestingly, the intention to practice nsMCMs was higher than the intentions to implement the specific MCMs. Apparently, farmers have a strong intention to do "something", which is quite an intangible measure, but the intention decreases when the measures become more tangible. The reason for this could be a perceived lack of evidence of effectiveness of the specific MCMs as well as a drawback due to a lack of ease of implementation, because of the time it takes, and costs (Garforth et al., 2013). However, as the question on nsMCMs essentially comprises all 4 specific MCMs, only farmers who are not intending to perform any of the 4 specific MCMs should disagree with the statement about intentions towards nsMCMs. Therefore, it may reflect that farmers had multiple MCMs in mind when answering the generic question. Because we asked the nsMCMs questions first, we believe that the farmers were not biased by the specific MCMs that we mentioned later on, so the question on nsMCMs truly reflects the high intention of the farmers to do something about mastitis on their farm. By then asking about more specific MCMs, we could identify that farmers intend to work on udder cleaning, stall hygiene and foremilk stripping, but much less to improve feeding, possibly because many of the farmers perceived low behavioral control towards improving feeding.

TPB factors

Towards all MCMs, the majority of the dairy farmers had a positive AT. The AT questions for nsMCMs were referring to the impact of mastitis, but AT questions of the specific MCMs were referring to the importance of those MCMs. This shows that dairy farmers recognize both the economic impact of mastitis and the importance of the specific MCMs. The percentage of farmers that had a positive SN was high except for nsMCMs. The reason may be due to the fact that nsMCMs are not a tangible measure, and therefore farmers may not have a clear idea to enact normative beliefs of referents.

The PBC of dairy farmers was high for three of the specific MCMs, but low for nsMCMs and for improving feeding of cows. The PBC was evaluated using statements referring to the ability of the dairy farmer to implement the MCM, availability of time and money to cover costs of the control measure. On average, 70% of the dairy farmers reported they have the ability, time and money to implement specific MCMs, but for nsMCMs and for improving feeding of cows, this was only 56% and 53%, respectively. Costs are the most important factor limiting the PBC of improving feeding. Feeding is very expensive so it is perceived that the profits are not justified by the costs. About the reasons behind the low PBC of nsMCMs, we speculate that this is a reflection of the fact that it is not a tangible measure and dairy farmers may assume it being impractical. A comparable study by Gunn et al. (Gunn et al., 2008) reported that cattle and sheep farmers, who had a specific disease problem, but who assumed that methods were impractical or time consuming, were unwilling to practice biosecurity measures. If a measure is easy to implement, farmers are more motivated to implement the measure (Garforth et al., 2013).

Associations between TPB factors and intentions

Dairy farmers who had a positive AT had higher odds of intention to implement nsMCMs. We mentioned that, for nsMCMs, AT was evaluated by questions referring to the impact of mastitis, such as treatment costs and milk production losses. This is in line with Valeeva et al. (2007), who described that monetary factors affect dairy farmers' motivation to mastitis management. Similarly, dairy farmers who had a positive AT had higher odds of intention to improve stall hygiene and to implement foremilk stripping. Attitudes for the intentions to these specific MCMs were evaluated by questions referring to the benefits the farmer expected to have if he would implement these measures. Farmers who have evidence of effectiveness of a measure were motivated to implement those measures (Garforth et al., 2013). From the positive association between AT and intentions towards several MCMs, we conclude that information directed at improving farmers' understanding of the impact of mastitis and of the effect of interventions will likely increase practicing of MCMs.

Dairy farmers who had positive SN had higher odds of a positive intention towards udder cleaning and to implement foremilk stripping (Table 5). Many of the farmers were unfamiliar with the concept of foremilk stripping, those farmers may not have sufficient knowledge to develop their own beliefs and may therefore only have positive intention if the referents approve that foremilk stripping is important to improve udder health. Less experienced farmers, are more likely to be proactive in looking for up-to-date information on livestock disease (Garforth et al., 2013). In a comparable study on the operators' non-compliance behavior to conduct emergency operating procedures, Park and Jung (Park and Jung, 2003) found that relatively less experienced senior reactor operators' who did not have sufficient knowledge to build up their own beliefs tend to rely on training they received and showed higher compliance. A reason for the association between SN and intention to perform udder cleaning may be due to the fact that in North-Western Ethiopian dairy farmers clean the udder not only for mastitis control but also to attract milk customers. Therefore, dairy farmers respond positively to the statement about their intention to clean udders and also have a strong positive SN. The way the milk is sold may therefore be a confounder in this association, but we have no data to support this. In this regard, veterinarians probably had the greatest influence on intention of dairy farmers towards cleaning of the udder.
The PBC of dairy farmers was high for udder cleaning, stall hygiene and foremilk stripping, but it was not significantly associated with any of the intentions. This shows that, when trying to improve mastitis control behavior, improving the PBC of the farmers may have less effect than, for instance, improving AT or SN. Still, the PBC is also a proxy for actual control, which has a direct link to behavior (Fishbein and Ajzen, 2010). The PBC is always of importance, but as it was generally high, it is likely not the limiting factor in the Ethiopian situation. Therefore, interventions to improve udder health should be targeted at AT and SN rather than at PBC. Farm specific costs of mastitis should be calculated to show dairy farmers the extent mastitis costs them. Training and promotion on the advantages of mastitis control measures to dairy farmers themselves, to milk customers and neighbors as well as informing veterinarians and artificial inseminators about their influence on changing dairy farmers intention towards mastitis control are important. In addition, farmers often identify 'other farmers' as a major source of new ideas to uptake new technology (Garforth et al., 2013). Furthermore, farmers are strongly influenced by the perception whether a given measure is actually applicable on the farm (Casal et al., 2007). Therefore, forming farmer groups as an opportunity for exchanging ideas and accessing advice and practical demonstration of the control measures may also be important to improve udder health in North-Western Ethiopia.

Socio-demographic characteristics associated with TPB factors

Dairy farmers who experienced mastitis in their own farm in the previous year had more often a positive AT to implement nsMCMs compared to dairy farmers who did not experience this, indicating that the farmers' behavior is influenced by experience of the disease, as was also reported by Garforth et al. (Garforth et al., 2013). Dairy farmers who had >15 years dairy farming experience had higher odds of a positive AT to implement foremilk stripping compared to less experienced dairy farmers. Since foremilk stripping is propagated by governmental extension workers, more experienced dairy farmers have a higher chance to have learned that foremilk stripping leads to earlier detection of mastitis enabling the farmer to take subsequent action. This may illustrate the effect of communication programs, as was also shown in the Netherlands, where planned communication about MCMs was shown to affect the ATs of farmers towards these measures (Jansen et al., 2010). Dairy farmers who had >8 grade level of education had lower odds of SN with regard to foremilk stripping. It is likely that farmers who have had more education feel more confident to implement mastitis control by themselves and be less susceptible to the advice of referents. Male farmers had lower SN to improve udder cleaning than female farmers. Our finding supports the work by Mekonnen et al. (2006) who showed that women-owned farms had better animal care than men-owned farms.

We tested for associations between 9 background factors and the 3 TPB factors (AT, SN and PBC) for each of the 5 MCMs. The large number of comparisons (135) made in this way, results in a high chance of making type I errors. Still, the actual number of significant associations was only four, suggesting that social and demographic factors were hardly linked to the TPB factors. We discussed the factors that showed statistically significant associations, but for all of these, we should mention the fact that they may well have resulted from chance and have no biological interpretation.

Conclusions

Our study identifies the determinants of dairy farmers' motivation towards controlling mastitis. The majority of dairy farmers has a positive intention, and has a positive AT towards MCMs. However, SN and PBC for nsMCMs, PBC for feeding and SN for stall hygiene were low. Attitude and SN were positively associated with intentions towards several specific MCMs. Therefore, to increase the intention of dairy farmers to implement MCMs interventions directed at changing their AT and their SN are expected to give the largest improvement in mastitis control behavior in North-Western Ethiopian dairy farmers.

Conflict of interest statement

The authors declare that they have no conflicts of interest. One of the corresponding authors, TJGML, is employed in GD animal health. However, this does not alter our adherence to PLOS ONE policies on sharing data and materials.

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Annex: Questionnaire on attitudes, subjective norms, perceived behavioral control and intentions of dairy farmers to control mastitis

I. Intention, attitude, subjective norms and perceived behavioural control to implement non-specified mastitis control measures

Intention: Implementation of non-specified mastitis control measures

Intention	
In the near future I will implement one or more measures to reduce mastitis in my herd	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Attitude	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Behavioural beliefs	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Mastitis is expensive; therefore mastitis control is important	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Mastitis causes milk production losses, therefore mastitis control is important	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Mastitis is a risk of culling, therefore mastitis control is important	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Mastitis milk cannot be sold, therefore mastitis control is important	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Outcome evaluation	
What is the importance of the following facts on your intention regarding preventive mastitis management	
Increased costs for treatment of mastitis (more expensive drugs or veterinarians)	Very unimportant 1 2 3 4 5 6 7 Very important
Increased milk production losses due to mastitis	Very unimportant 1 2 3 4 5 6 7 Very important
Increased risk of culling due to mastitis	Very unimportant 1 2 3 4 5 6 7 Very important
Unable to sell mastitis milk	Very unimportant 1 2 3 4 5 6 7 Very important
Subjective norm	
Strength of normative belief	
What, according to your knowledge, is the opinion of the following people regarding the control of mastitis	

Veterinarians think that control of mastitis is	Very unimportant -3 -2 -1 0 1 2 3 Very important
The artificial inseminator thinks that control of mastitis is	Very unimportant -3 -2 -1 0 1 2 3 Very important
My milk customers think that control of mastitis is	Very unimportant -3 -2 -1 0 1 2 3 Very important
My neighbours think that control of mastitis is	Very unimportant -3 -2 -1 0 1 2 3 Very important
Other dairy farmers think that control of mastitis is	Very unimportant -3 -2 -1 0 1 2 3 Very important

Motivation to comply

Does the opinion of the following people regarding reduction of mastitis influence your intention to implement preventive measures?

Veterinarians	Not at all 1 2 3 4 5 6 7 Very much
The artificial inseminator	Not at all 1 2 3 4 5 6 7 Very much
My milk customers	Not at all 1 2 3 4 5 6 7 Very much
My neighbours	Not at all 1 2 3 4 5 6 7 Very much
Other dairy farmers	Not at all 1 2 3 4 5 6 7 Very much

Perceived behavioural control

Strength of control beliefs

Management measures to reduce mastitis will be effective on my farm	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Management measures to reduce mastitis are expensive	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Management measures to reduce mastitis are time consuming	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Management measures to reduce mastitis are difficult to carry out	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree

Power to influence behaviour

I have money available to implement preventive management measures to reduce mastitis in my farm	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
I have time to implement preventive management measures to reduce mastitis in my farm	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
I have the ability/skill to implement preventive management measures to reduce mastitis in my farm	Strongly disagree 1 2 3 4 5 6 7 Strongly agree

II. Intentions, attitudes subjective norms and perceived behavioural control to implement specific mastitis control measures

Intention: Udder cleaning

In the near future I plan to improve the cleaning of the udders to reduce mastitis in my farm	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree	

Attitude for udder cleaning

Behavioural beliefs

Udder cleaning improves milking hygiene	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Cleaning the udder minimizes spread of bugs causing mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Clean udders facilitate detection of mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree

Outcome evaluation

Milking hygiene by cleaning the udder is important to reduce mastitis	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
Minimizing spread of bugs causing mastitis by cleaning the udder is important to reduce mastitis	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
Easy detection of mastitis is important to reduce mastitis	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Subjective Norm for udder cleaning

What, according to your knowledge, is the opinion of the following people regarding the control of mastitis

Veterinarians think that udder cleaning is	Very unimportant	1	2	3	4	5	6	7	Very important
The artificial inseminator thinks that udder cleaning is	Very unimportant	1	2	3	4	5	6	7	Very important
My milk customers think that udder cleaning is	Very unimportant	1	2	3	4	5	6	7	Very important
My neighbours think that udder cleaning is	Very unimportant	1	2	3	4	5	6	7	Very important
Other dairy farmers think that udder cleaning is	Very unimportant	1	2	3	4	5	6	7	Very important

Motivation to comply

Does the opinion of the following people regarding udder cleaning influence your intention to improve udder cleaning?

Veterinarians	Not at all	-3	-2	-1	0	1	2	3	Very much
The artificial inseminator	Not at all	-3	-2	-1	0	1	2	3	Very much
My milk customers	Not at all	-3	-2	-1	0	1	2	3	Very much

My neighbours	Not at all	-3	-2	-1	0	1	2	3	Very much
Other dairy farmers	Not at all	-3	-2	-1	0	1	2	3	Very much

Perceived Behavioural Control for udder cleaning

Strength of Control Beliefs

Udder cleaning will be effective on my farm to reduce mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Udder cleaning is difficult	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Cleaning the udder is time consuming	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Cleaning the udder is expensive	Strongly disagree	1	2	3	4	5	6	7	Strongly agree

Power of Factors to Influence the Behaviour

I know how to clean the udder	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
I have time to clean the udder	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
I can afford to cover costs of cleaning the udder	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Intention: Hand Cleaning

In the near future I plan to improve cleaning my hands while milking to reduce mastitis in my farm	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Attitude for hand cleaning

Behavioural beliefs

Milking with clean hands improves milking hygiene	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Milking with clean hands minimize spread of bugs causing mastitis among cows	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Outcome evaluation	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Milking hygiene by cleaning hands is important to reduce mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Minimizing spread of mastitis bugs by cleaning hands is important to reduce mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree

Subjective Norm for hand cleaning

Strength of normative beliefs

What, according to your knowledge, is the opinion of the following people regarding the control of mastitis

Veterinarians think that hand cleaning is	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
The artificial inseminator thinks that hand cleaning is	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
My milk customers think that hand cleaning is	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
My neighbours think that hand cleaning is	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
Other dairy farmers think that hand cleaning is	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Motivation to comply

Does the opinion of the following people regarding hand cleaning influence your intention to improve hand cleaning?

Veterinarians	Not at all	1	2	3	4	5	6	7	Very much
The artificial inseminator	Not at all	1	2	3	4	5	6	7	Very much
My milk customers	Not at all	1	2	3	4	5	6	7	Very much
My neighbours	Not at all	1	2	3	4	5	6	7	Very much
Other dairy farmers	Not at all	1	2	3	4	5	6	7	Very much

Perceived Behavioural Control for hand cleaning

Strength of Control Beliefs

Cleaning hands will be effective on my farm to reduce mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Hand cleaning is difficult	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Cleaning the hands is time consuming	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Cleaning the hands is expensive	Strongly disagree	1	2	3	4	5	6	7	Strongly agree

Power of Factors to Influence the Behaviour

I know how to clean the hands	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

I have time to clean the hands	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
I can afford to cover costs of cleaning the hands	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree

Intention: Improving stall hygiene

In the near future, I plan to improve stall hygiene to reduce mastitis in my farm	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree

Attitude for stall hygiene

Behavioural beliefs

A clean stall reduces mastitis	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
Cleaning the stall minimizes transmission of mastitis agents	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
Improving the stall hygiene reduce exposure of teats to mastitis pathogens	Strongly disagree 1 2 3 4 5 6 7 Strongly agree

Outcome evaluation

Stall hygiene is important to reduce mastitis	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Minimizing transmission of mastitis agents by cleaning the stall is important to reduce mastitis	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree
Minimizing exposure of teats to mastitis pathogens is important to reduce mastitis	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree

Subjective Norm for stall hygiene

Strength of normative beliefs

What, according to your knowledge, is the opinion of the following people regarding the control of mastitis?

Veterinarians think that stall hygiene is	Very unimportant -3 -2 -1 0 1 2 3 Very important
The artificial inseminator thinks that stall hygiene is	Very unimportant -3 -2 -1 0 1 2 3 Very important
My milk customers think that stall hygiene is	Very unimportant -3 -2 -1 0 1 2 3 Very important
My neighbours think that stall hygiene is	Very unimportant -3 -2 -1 0 1 2 3 Very important

Other dairy farmers think that stall hygiene is	Very unimportant -3 -2 -1 0 1 2 3 Very important	

Motivation to comply

Does the opinion of the following people regarding stall hygiene influence your intention to improve stall hygiene?

Veterinarians	Not at all	1	2	3	4	5	6	7	Very much
The artificial inseminator	Not at all	1	2	3	4	5	6	7	Very much
My milk customers	Not at all	1	2	3	4	5	6	7	Very much
My neighbours	Not at all	1	2	3	4	5	6	7	Very much
Other dairy farmers	Not at all	1	2	3	4	5	6	7	Very much

Perceived Behavioural Control for stall hygiene

Strength of Control Beliefs

An optimal stall hygiene will be effective on my farm to reduce mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Cleaning the stall is difficult	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Cleaning the stall is time consuming	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Cleaning the stall is expensive	Strongly disagree	1	2	3	4	5	6	7	Strongly agree

Power of Factors to Influence the Behaviour

I know how to clean the stall	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
I have time to clean the stall	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
I can afford to cover costs of cleaning the stall	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Intention: Improve Feeding

In the near future I plan to improve feeding of my cows to reduce mastitis in my farm	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Attitude for feeding Improvement

Behavioural beliefs

Appropriate feeding improves nutritional balance	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Appropriate feeding improves resistance to disease; hence to mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree

Outcome evaluation

Balanced feeding is important to reduce mastitis	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
Improving resistance to mastitis is important to reduce mastitis	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Subjective Norm for feeding Improvement

What, according to your knowledge, is the opinion of the following people regarding the control of mastitis

Veterinarians think that improving feeding is	Very unimportant	1	2	3	4	5	6	7	Very important
The artificial inseminator thinks that improving feeding is	Very unimportant	1	2	3	4	5	6	7	Very important
My milk customers think that improving feeding is	Very unimportant	1	2	3	4	5	6	7	Very important
My neighbours that improving feeding is	Very unimportant	1	2	3	4	5	6	7	Very important
Other dairy farmers think improving feeding is	Very unimportant	1	2	3	4	5	6	7	Very important

Motivation to comply

Does the opinion of the following people regarding feeding improvement influence your intention to improve feeding?

Veterinarians	Not at all	-3	-2	-1	0	1	2	3	Very much
The artificial inseminator	Not at all	-3	-2	-1	0	1	2	3	Very much
My milk customers	Not at all	-3	-2	-1	0	1	2	3	Very much
My neighbours	Not at all	-3	-2	-1	0	1	2	3	Very much
Other dairy farmers	Not at all	-3	-2	-1	0	1	2	3	Very much

Perceived Behavioural Control for feeding Improvement

Strength of Control Beliefs

Improvement in feeding will be effective on my farm to reduce mastitis	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Improvement in feeding is difficult	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Improvement in feeding is time consuming	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Improvement in feeding is expensive	Strongly disagree	1	2	3	4	5	6	7	Strongly agree

Power of Factors to Influence the Behaviour

I know how to improve feeding	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
I have time to improve feeding	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
I can afford to cover costs to improve feeding	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Intention: Foremilk stripping

In the near future I plan to foremilk strip to reduce mastitis in my farm	Strongly disagree -3 -2 -1 0 1 2 3 Strongly agree

Attitude foremilk stripping

Behavioural beliefs

Foremilk stripping is important to diagnose mastitis	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
Foremilk stripping is important to decide treatment against mastitis	
Outcome evaluation	Strongly disagree 1 2 3 4 5 6 7 Strongly agree
Diagnosis of mastitis by foremilk stripping is important to reduce mastitis	
Treatment decision against mastitis by foremilk stripping is important to reduce mastitis	Strongly disagree 1 2 3 4 5 6 7 Strongly agree

Subjective Norm for foremilk stripping

Strength of normative beliefs

What, according to your knowledge, is the opinion of the following people regarding foremilk stripping to control mastitis

Veterinarians think that foremilk stripping is	Very unimportant -3 -2 -1 0 1 2 3 Very important
The artificial inseminator thinks that foremilk stripping is	Very unimportant -3 -2 -1 0 1 2 3 Very important
My milk customers think that foremilk stripping is	Very unimportant -3 -2 -1 0 1 2 3 Very important
My neighbours think that foremilk stripping is	Very unimportant -3 -2 -1 0 1 2 3 Very important
Other dairy farmers think that foremilk stripping is	Very unimportant -3 -2 -1 0 1 2 3 Very important

Motivation to comply

Does the opinion of the following people regarding foremilk stripping influence your intention to control mastitis?

Veterinarians	Not at all 1 2 3 4 5 6 7 Very much
The artificial inseminator think	Not at all 1 2 3 4 5 6 7 Very much
My milk customers	Not at all 1 2 3 4 5 6 7 Very much

My neighbours	Not at all	1	2	3	4	5	6	7	Very much
Other dairy farmers	Not at all	1	2	3	4	5	6	7	Very much

Perceived Behavioural Control for foremilk stripping

Strength of Control Beliefs

Foremilk stripping will be effective on my farm to reduce mastitis									
Foremilk stripping is difficult	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Foremilk stripping is time consuming	Strongly disagree	1	2	3	4	5	6	7	Strongly agree
Foremilk stripping is expensive	Strongly disagree	1	2	3	4	5	6	7	Strongly agree

Power of Factors to Influence the Behaviour

I know how to make foremilk stripping	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
I have time to make foremilk stripping	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree
I can afford to cover costs of foremilk stripping	Strongly disagree	-3	-2	-1	0	1	2	3	Strongly agree

Chapter 7

General discussion

Introduction

Mastitis is an expensive cattle disease which has serious economic effects on dairy farms, associated with extra input costs and losses due to less efficient milk production. Mastitis also affects milk quality, and potentially is a food safety problem. Mastitis management has the goal to solve and prevent mastitis problems in order to improve milk quality, the efficiency of milk production and cow welfare. In Ethiopia, mastitis is a highly prevalent disease in Holstein Friesian × Zebu cross breed cows (Lema et al., 2001; Dego and Tareke, 2003; Mekonnen et al., 2006; Almaw et al., 2008; Mekonnen et al., 2010). Mastitis was also reported to be one of the two major clinically manifested health problems (Lema et al., 2001) and the second cause of culling (Workineh et al., 2002). As a response to the increased milk demand in Ethiopia, and stimulated by government interventions, the number of smallholder market-oriented dairy farms is increasing (Mekonnen et al., 2006). There is a growing number of urban and peri-urban dairy farmers in the country that keep these cross-breed cows to produce milk to be sold at the market. Cross breed cows are, however, more prone to mastitis than indigenous breeds (Almaw et al., 2008; Tolosa et al., 2013). Although clinical mastitis (**CM**) is best known and is an important disease, subclinical mastitis (**SCM**) seems to occur more frequently (Dego and Tareke, 2003; Tolosa et al., 2013) and to have bigger economic consequences.

Although worldwide thousands of scientific papers have been published on mastitis in dairy cows, relatively little information is available on mastitis in sub-Saharan countries. The aim of this thesis is to contribute to the knowledge on mastitis in order to improve udder health in Holstein Friesian × Zebu cross breed market-oriented dairy farms in North-Western Ethiopia. To achieve this aim, factors of primary importance to improve udder health, being the prevalence of mastitis, the actual mastitis management, factors associated with the occurrence of mastitis and motivational aspects including economic factors have been studied and described in this thesis. In this final chapter, first the most important findings of this work will be presented. The main body of this chapter will be a description of the practical aspects of these findings related to knowledge from literature with regard to implementation in daily practice in dairy production in Ethiopia. Finally the conclusions of the thesis will be presented as well as recommendations for future research.

Main findings

One of the most important findings in this study is the high prevalence of SCM (62% at cow level and 33% at quarter level) and of intramammary infections (**IMI**) (81% at cow level and 49% at quarter level) with substantial variation between farms. The most important risk factors associated with mastitis were CM history, days in milk, parity, not checking the udders for CM and herd size (chapter 2). Cows with higher Holstein blood level (**HBL**) were found to be more prone to infection by mastitis pathogens than indigenous breeds. *Staphylococcus aureus* and coagulase negative staphylococci (**CNS**) were the dominant mastitis pathogens identified. *Staphylococcus aureus* was found to be associated with herd size and CM experience, while CNS was associated with herd size, the milking method, calves suckling with cow, level of feeding and cleaning the udder before milking (chapter 2). Twenty different *spa* types of *S. aureus* were identified and there was a high degree of similarity between the isolates. *spa* type t042, belonging to ST4550, and the closely related t15786 are a double locus variant of ST97, were most frequently identified. The prevalence of toxin genes *lukMF'*, *pvl* and *tst* was low. All

isolates were susceptible to methicillin, but resistant to both penicillin and ampicillin (86%) and tetracycline (54%) (chapter 3).
When the estimated failure costs of mastitis per cow was compared to the milk returns per cow, the failure costs of mastitis in North-Western Ethiopia were found to be relatively high, being 5.2% of the average milk returns per cow, which is higher than the comparative failure costs of mastitis per cow in western countries. This makes mastitis, from an economic perspective, more important in Ethiopia than in western countries. A large variation between farms was found, varying from 0 to 7,357 Ethiopian Birr per lactating cow per farm per year. The main costs are due to milk production losses, particularly caused by SCM, and to culling (chapter 4 and 5). The majority of the dairy farmers had a positive intention towards nonspecific measures to control mastitis (93%), and to implement specific measures such as improving udder cleaning (87%), improving stall hygiene (78%), improving feeding of the cows (76%), and performing foremilk stripping (74%). Farmers were found to have a more positive attitude, but lower subjective norms and perceived behavioral control towards nonspecific mastitis control measures as compared with implementing specific mastitis control measures. Subjective norms were found to be positively associated with intentions of dairy farmers to implement improved udder cleaning and foremilk stripping (chapter 6).

Improving udder health

Given the findings in chapter 2, improving udder health in Ethiopia should mainly focus on staphylococci, in this thesis subdivided in *S. aureus* and CNS. *Staphylococcus aureus* is considered a contagious pathogen, which is adapted to the environment of the mammary gland and spreads from cow to cow during the milking process (Bi et al., 2016). The contagious character of CNS species is less clear and may vary for different species (Pyörälä and Taponen, 2009; De Visscher et al., 2015). Nevertheless, control measures against contagious mastitis pathogens, such as post-milking teat disinfection (**PMTD**), will decrease not only the number of *S. aureus*, but also CNS infections in a herd (Pyörälä and Taponen, 2009). It therefore seems logic to focus mastitis control measures in North-Western Ethiopia on contagious pathogens in general.
In order to reduce the prevalence of contagious mastitis pathogens, two approaches are important: 1) eliminating existing infections, being the main source of new infections and 2) preventing new infections to occur, through decreasing transmission of pathogens. Once mastitis is optimally controlled, a regular monitoring of udder health is necessary as part of a successful mastitis control program to pick up problems at an early stage so that timely action can be taken. Apart from that, several studies suggest that whether and how mastitis management practices are implemented on a farm depends on the mindset and the behavior of the farmer (Nyman et al., 2007; Wenz et al., 2007). Therefore most mastitis control programs currently focus not only on knowledge transfer, but also give attention to their attitude and motivation while designing effective (mastitis) control programs (Chase et al., 2006; LeBlanc et al., 2006; Jansen et al., 2009).

Eliminating existing infections

Existing infections can be eliminated by treatment of cows with mastitis (CM and SCM). This can be done during lactation or at drying off. Another approach to eliminate infections from a herd is culling of chronically infected mastitis cows (Stott et al., 2002).

Treatment of mastitis during lactation

The objectives of antibacterial treatment of mastitis are clinical recovery, elimination of the infectious agent, and to prevent serious tissue damage in order to maintain future milk production (Constable et al., 2008). Apart from this, treatment helps to prevent new infections by removing existing infections, which can be a source of new IMI.

In general, therapeutic success is improved by a targeted treatment, which is based on identified causative bacteria preferably with insight in their antimicrobial sensitivity (Barkema et al., 2006). In Ethiopia, however, it generally is not possible to identify the causative agent of mastitis in a timely way, due to the limited laboratory facilities and the related costs. An alternative is to collect data at herd level to make herd level decisions on optimal treatment to improve therapeutic success (Pyörälä, 2009). Given the small herd size this is, however, not practically feasible in Ethiopia. Because the vast majority of the culture results in our study yielded CNS or *S. aureus* (Chapter 2), mastitis treatment in North-Western Ethiopia should be primarily targeted at staphylococcus species. The majority of the *S. aureus* isolates found were resistant to penicillin and ampicillin (86% for both) (chapter 3). A drug concentration that exceeds the minimum inhibitory concentration of the pathogen in the udder is important to increase the success of mastitis therapy (du Preez, 2000; Sol et al., 2000; Oliver et al., 2004; Barkema et al., 2006). Therefore, guidelines on therapeutic regimen (indication, type of antibiotic, dose, frequency and duration of treatment) ought to be set, which are preferably evidence based, with the goal to increase therapeutic success in mastitis treatment.

In Ethiopia, there is indiscriminate use of antimicrobials in animal husbandry. Dairy farmers purchase antibiotics without veterinary prescription and treat their own cows (chapter 3). Because farmers lack knowledge on prudent and effective antimicrobial use, this may lead to suboptimal decisions on whether or not to treat a cow, the type of antibiotic chosen and the duration of treatment. A too short treatment duration may for instance be an important reason for poor cure rates in mastitis therapy (Oliver et al., 2004; Deluyker et al., 2005). This may lead to development of antimicrobial resistance which can have consequences for animal health as well as for public health. In order to alter the indiscriminate use of antimicrobials in animals, a policy modification is needed to enhance prudent antimicrobial use. In this study, 86% of the *S. aureus* isolates were resistant to penicillin and ampicillin. This is much higher than in other countries, for example in 10 western countries, 4% to 76% of *S. aureus* were positive for β-lactamase (Oliveira et al., 2000) and this warrants a totally different approach of antimicrobial use on dairy farms. To begin with, they should use other drugs such as amoxicillin for treatment of mastitis instead of penicillin and ampicillin because these drugs probably will not be effective in most cases. Moreover, antimicrobial treatment should preferably be accompanied with regular testing of antimicrobial sensitivity.

Dry cow treatment

In the western world, the dry period is known to be a period in which many new IMI occur (Robert et al., 2008) and therefore is a very important period for udder health. In many parts of the world, blanket dry cow treatment, treating all cows with antibiotics at drying off, is advised with the aim to cure existing IMI and to prevent new IMI to occur during the dry period (Henderson et al., 2016). In several countries blanket dry cow treatment became a concern from the perspective of prudent antibiotic use in recent years, with the use of selective dry cow treatment being promoted. Ethiopian dairy farmers, however, do not practice any dry cow treatment at all. Although no studies have been done on the effect of dry cow treatment in Ethiopia, it may be an important measure to improve the mastitis situation in the country and should be further studied.

The fact that the majority (86%) of *S. aureus* isolates is resistant to penicillin and ampicillin (chapter 3) probably has a negative effect on cure rates and should be included in studies on antibiotic treatment at drying off. Dry cow tubes containing penicillin or ampicillin should preferably not be used to treat cows at drying off.

Culling of chronically infected cows

Dairy cows may be culled voluntary or for non-voluntary reasons. Voluntary culling generally is due to low production, or because a herd has too many animals. Involuntary culling is due to reasons such as diseases, and may also be important from a welfare perspective (Ahlman et al., 2011). If cows do not respond favorably to treatment and continue to flare-up with signs of CM, antimicrobial treatment is no longer a valid approach, both from the perspective of prudent use of antibiotics as from an economic perspective. In that situation, culling of the infected animals often is the only way to eliminate IMI from a herd. This may be cost-effective due to the reduction of future mastitis cases (Stott et al., 2002).

Culling of dairy cows, however, is a complex decision, which involves knowledge on factors such as the cow's parity, stage of lactation, conception status, and the current and predicted milk production level (Ahlman et al., 2011). Generally, low yielding cows, whether diseased or not, are more likely to be culled, while high yielding cows, even if they are diseased, are more likely to be kept in the herd. Similarly, pregnant cows are more likely to remain in the herd than non-pregnant cows. In Ethiopia, dairy cows are not easily culled because of it will decrease the cash flow related to milk production. Additionally, because cross breed cows are very valuable for reproduction in Ethiopia, they likely are maintained in the farm even if they are chronically infected and have repeated CM cases. A chronically infected low yielding cow, however, is expensive to keep, and culling her may potentially increase profits through reduction of costs (Rajala-Schultz and Gröhn, 1999). In that situation, culling may be the preferred approach. That does not take away that culling is an important part of the failure costs. Therefore the first priority obviously is to try and prevent IMI as much as possible.

Preventing new intramammary infections

To improve udder health, prevention of new IMI is at least as important as treating them. Prevalence studies showed that CNS are the most frequently isolated species of bacteria in Ethiopia (chapter 2) with *S. aureus* being the second in frequency of being isolated. Within *S. aureus*, strains differ in frequency (chapter 3), indicating potential differences in characteristics such as spread or persistence in the host. Mastitis control measures should therefore preferably prevent new IMI caused by pathogens with different characteristics. The basic approach to prevent IMI is to reduce transmission of pathogens and by reducing susceptibility of cows to IMI (Sharif et al., 2009).

Reducing transmission of mastitis pathogens

The main source of contagious pathogens is the cow, more specifically the infected udder. Transmission of IMI from one cow to another has been described in western countries to primarily occur during the milking process (Vanderhaeghen et al., 2015), probably due to milk from an infected gland coming into contact with uninfected glands. This phenomenon likely also occurs during hand milking, which is generally practiced in Ethiopia.

Spread of bacteria may occur during milking through contaminated towels that are used in several cows, milkers' hands, and flies, bringing bacteria to the teat end. The greater the pathogen load at the teat end, the greater is the probability of new IMI to occur (Ceballos-Marquez et al., 2013). Proper milking procedures and good milking hygiene helps to decrease the pathogen load at teat ends and therefore the occurrence of new IMI and the spread of mastitis. Proper and hygienic milking procedures include that teats are cleaned and dried before milking (Gleeson et al., 2009). Remarkably, in this thesis, cleaning the udder before milking was positively associated with the prevalence of CNS (chapter 2). This may be related to the way udder cleaning is performed. Almost all farmers (88%) that used a cloth or a towel to dry udders before milking, used only one cloth or towel to dry the udders of all lactating cows in the herd, which are seldom washed between milkings. This way, towels may actually be vehicles for transmission between cows. It is important to make farmers aware of the importance of proper milking procedures such as washing hands, fore-stripping, drying the teats with specific attention to the teat end using a single clean cloth per cow and possibly also PMTD. Although these measures are proven successful in reducing occurrence of mastitis (Pyörälä and Taponen, 2009; Ruegg, 2017), they have not been evaluated in Ethiopia which needs further study.

Milkers' hands can be important vehicles in transferring udder pathogens between cows during the milking process (Capurro et al., 2010; Zadoks et al., 2011). In North-Western Ethiopia, most dairy farmers clean their hands once before milking starts, but do not clean them between cows (chapter 2). They also do not have the intention to improve their hand cleaning (data not shown) (chapter 6). Proper milking procedures often do not cost extra time or money and are a cheap way to decrease the risk of mastitis problems. Therefore, if adapted to Ethiopian circumstances, training dairy farmers on the principles of proper milking procedures accompanied with short practical demonstrations seems to be an easy way to prevent new IMI.

Post milking teat disinfection is a key part in prevention of new IMI caused by contagious pathogens such as *S. aureus* (Lam et al., 1995). Although PMTD is not very expensive, is easy to perform, and is known to be very effective, it is not practiced by Ethiopian dairy farmers (Workineh et al., 2002; Mungube et al., 2004). Therefore there is no experience at all with this measure in Ethiopia. Although it seems likely PMTD has an added value in preventing mastitis, this currently is unknown. In order to evaluate this and to have evidence to convince advisors on the added value of the measure, (case) studies on the effect of PMTD under Ethiopian circumstances are considered to be valuable.

Reducing susceptibility to intramammary infections

Although we try to keep the infectious pressure as low as possible, we will never be fully successful and at some point in time udders will come in contact with mastitis pathogens. Therefore, in addition to pathogen load at the teat end, the risk that a cow develops mastitis depends on the cow's ability to prevent bacterial infection in the mammary gland. In that respect, the host susceptibility is of importance. Unbalanced rations and deficiencies of energy, protein, minerals, or vitamins have repeatedly been associated with increased disease susceptibility as a result of a suboptimal immune function (Valde et al., 2007). Optimal feeding of minerals and vitamins, for example, have an effect on cell regulation, immune function, and thus influence cow health and productivity while they may enhance the nutritional value of milk and other dairy products (Weiss, 2017).

Cows in negative energy balance are at a higher risk of ketosis and clinical ketosis is associated with a two-fold increase in the risk of CM (O'Rourke, 2009). Feeding a well-balanced diet enhances the animal's immune system. For example, deficiencies of selenium and vitamin E in the diet have been associated with an increased rate of new IMI (Valde et al., 2007; O'Rourke, 2009). The immune system, as any physiological system, does not function optimally during periods of malnutrition. In addition, the immune system has specific requirements for specific nutrients and when these nutrients are not provided in adequate amounts, immune function may suffer (Patel, 2016). Also in the work described in this thesis feeding regimen of dairy cows was found to be related to the prevalence of IMI (chapter 2). In Ethiopia, about 73% of producers in urban production systems have problems in feeding their cows due to seasonal variation in availability and the high price of feeds (Yigrem et al., 2008). Training dairy farmers in feed conservation practices, like making hay and silage, may help to make roughage feed supplies available throughout the year. Also organizing groups of feed producers, sharing resources and experiences may be a possible approach to supply these farms with roughage during the dry season.

For many Ethiopian dairy farmers the perceived behavioral control of improving feeding dairy cows is low. Costs are considered to be the most important factor limiting to improve feeding and most dairy farmers perceive that the profits are not justifying the related costs (chapter 6). In genetically high milk producing cows, the actual milk production is highly influenced by the amount and the quality of the feed given to these cows, in which it is important that the nutritional requirements of the dairy cow during the various stages of the lactation cycle are met (O'Rourke, 2009).

An additional factor that is important to reduce susceptibility to IMI is avoiding stress. Stress is an external event or condition that interferes with immunological defense systems (Collier et al., 2017). When stress is severe and not relieved by management changes, an animal will be chronically stressed and may be more susceptible to disease (Collier et al., 2017). Both in warm and in more temperate parts of the world, heat stress may offset much of the genetic progress for increased milk production (Kadzere et al., 2002). Heat and associated body temperature due to high metabolic demand negatively affects feed intake leading to heat-induced depression of milk production in previously high-producing dairy cows (Collier et al., 2017). When the stress is severe and not relieved by management changes, it will ultimately weaken the immune system of the cow which will eventually facilitate udder infections (Pragna et al., 2017). Modification of the environment, through providing shade and air cooling systems, is considered to be the primary way to reduce adverse effects of hot weather conditions (Fournel et al., 2017). Although, market-oriented Ethiopian dairy farmers keep cows in-house, most dairy farmers use housing that does not allow air movement, which may lead to high temperatures in the stable. Due to the high environmental temperature and high metabolic demand associated with higher milk yield, Holstein Friesian × Zebu cross breed cows are more likely to experience heat stress than the lower-producing indigenous Zebu bred cows. Therefore, dairy farmers should be trained about the importance of air movement to relief heat stress (Fournel et al., 2017).

Monitoring udder health

Routine monitoring and record keeping are essential for optimal herd management to keep track of the udder health situation and be able to intervene when the udder health situation worsens. Diagnostics of both CM and SCM are crucial to timely eliminate existing IMI (Barkema et al., 2006). Thus, regular udder health monitoring with the goal to identify cows with mastitis and to take appropriate measures at an early stage, is necessary to successfully implement mastitis control programs. In chapter 2 was described that the prevalence of SCM was lower in farms that checked udders for CM.

This is probably due to the fact that these farmers find new CM cases at an earlier stage and by taking action, reduce the spread of IMI in their herds. In this section, methods applicable in Ethiopia for diagnosis of mastitis will be discussed.

Monitoring clinical mastitis

Clinical signs of mastitis can be detected by manual and visual observation of the udder and the milk in individual animals. As such, the milker has a crucial role in detecting CM. In North-Western Ethiopia, dairy farmers milk by hand (chapter 2), which gives them the opportunity to continuously monitor for mastitis. However, only a limited number of dairy farmers practices foremilk stripping (chapter 2). According to Lam et al. (2009), there is variation between farmers in their ability to diagnose CM because of differences in dairy farmers' definitions of CM and/or differences in observing and identifying clinical cases. This variability in diagnosis apparently also exist in Ethiopia and may be reduced by education. More specifically, practical demonstrations of mild and moderate CM cases seem useful. If foremilk stripping is done poorly, it may contribute to the spread of bacteria from teat-to-teat and from cow-to-cow via the milkers' hands. To reduce the chance of spreading IMI, milkers should take care to avoid ever getting milk on their hands while they strip the foremilk. In chapter 6, it was described that dairy farmers' attitude and subjective norms are positively associated with the intention to implement foremilk stripping. Organizing farm visits and on-farm study group meetings may be helpful to motivate dairy farmers to implement management measures such as foremilk stripping. Dairy farmers were found to be influenced by their colleagues (chapter 6), underlining the possible positive effects of study groups.

Monitoring subclinical mastitis

Subclinical mastitis can be monitored in a variety of ways. The most common are direct measurement of the somatic cell count (SCC) or indirect measurement of SCC by performing a California Mastitis Test (CMT). The sensitivity and specificity of CMT to detect IMI are relatively low, but it is an easy, cheap, and quick cow-side test which is applicable under basically all circumstances. Also in Ethiopian circumstances, the CMT reagent can be easily made available. Although in some countries, people may be reluctant to use CMT because of time constraints, this is no issue in Ethiopia. Labor is cheap in Ethiopia and with proper training, leading to repeatable test results (Ribeiro, 2002) CMT may be a valuable tool to diagnose SCM.

Bacteriological culturing

Bacteriological culture of milk from quarters with SCM or CM can provide valuable information on pathogen prevalence, on the effectiveness of control programs and is a logical way to select appropriate treatment protocols (Lam et al., 2009). In Ethiopia, bacteriological culturing of milk samples is not readily available to dairy farmers, because of shortage of laboratory facilities and of skilled manpower. Although in specific situations, when the prevalence of mastitis is extremely high or for other specific reasons, bacteriological culturing is performed in collaboration between regional veterinary laboratories and universities. In most situations, however, bacteriological culturing is not practiced and will not become available in the near future. Therefore, it is not further discussed here.

Record keeping

Record keeping is useful as a basis for decision making with respect to treatment and prevention of mastitis. Records such as the incidence of CM and SCM, applied treatments and cure rates, withheld milk and culled cows are used in decision to control mastitis and on the type of the control measure to be implemented by the dairy farmer. At this point in time, however, there are no dairy farmers in North-Western Ethiopia that keep any of this type of records. At the same time, we have to realize that most dairy farmers in Ethiopia are smallholders, with a very limited number of dairy cows, with quite a few of them being illiterate. Therefore the added value of record keeping probably is limited and may not be the first priority. Farmers which have more cows in their farms, however, need to rethink record keeping, and might have profit here. For this type of farmers, record keeping needs specific attention in training programs.

Motivating dairy farmers to control mastitis

Addressing farmers' attitude and motivation is necessary to implement effective mastitis control programs (Jansen et al., 2009). Dairy farmers are primarily responsible for the quality of the milk they produce and for mastitis control measures they implement at their farm. In Ethiopia, as in other countries, there is quite some variation in implementation of mastitis control measures. The farmers' use of veterinary services, for instance, varies highly between farmers (Dehinenet et al., 2014). A first prerequisite for dairy farmers to implement mastitis control measures is that they consider mastitis as a problem, either from an animal health, an economic or another perspective (Lam et al., 2013). It has been reported that, although it would benefit their results, some farmers do not implement effective mastitis management practices (Barkema et al., 1999). That may be due to the fact that farmers do not see the suggested approaches as effective or feasible which can be due to ineffective communication and leads to lack of motivation to change behavior related to mastitis management (Lam et al., 2011). Several studies from different countries described that whether or not mastitis management practices are implemented on a farm, highly depends on the human factor: attitude, subjective norms and perceived behavioral control (Nyman et al., 2007; Wenz et al., 2007). Similarly, the data in this thesis shows that North-Western Ethiopian dairy farmers can potentially be motivated to control mastitis by changing their attitude, subjective norms and perceived behavioral control. Such a program should primarily focus on changing attitude and secondarily on improving the farmers' subjective norms (chapter 6).

The costs of mastitis, 5.2 % of the average milk returns per cow, are relatively high in North-West Ethiopia with a large variation between dairy farms. Nevertheless, dairy farmers do not always perceive mastitis as expensive (chapters 4 and 5). This may be due to the fact that important cost factors such as decreased milk production and increased culling are not directly visible (Hogeveen et al., 2011). Economic aspects, however, have an important role in internal motivation to change behavior related to udder health (Jansen and Lam, 2012). Although there are differences between farmers in price of milk and in some of the mastitis related expenditures between farmers, most of the variation in costs of mastitis is associated with the occurrence of CM and SCM (chapters 4 and 5). Estimating costs of mastitis at the farm-level may be helpful in showing the added value of improving mastitis management and to motivate farmers to change their behavior in this respect.

Knowledge and problem awareness are undeniably important to influence behavior of farmers with regard to mastitis management (Kuiper et al., 2005; Jansen et al., 2009; Mankad, 2016). However, behavioral determinants, such as farmers' attitude, internal

normative beliefs and perceived self-efficacy of farmers, are also important aspects and need specific attention (Kuiper et al., 2005; Jansen et al., 2010; van den Borne et al., 2014). Ethiopian dairy farmers basically have positive intentions to control mastitis.
Awareness of the efficacy of preventive measures stimulates the internal motivation to implement the measures (Lam et al., 2013). In order to convince farmers of the added value of certain management measures it is important to show them that added value. That can for instance be done in small study groups where participants can share knowledge and experiences in which the effectiveness of the measures can be demonstrated. In this respect, veterinarians have a great influence on intentions of dairy farmers towards mastitis control measures (chapter 6). However, it is important to realize that a 'one-size-fits-all' approach is not appropriate to be successful in changing intentions and behavior (Elliott et al., 2011). Ideally, for each farmer the specific circumstances should be considered, taking into account both technical as well as motivational aspects.

Conclusions

In this thesis, some epidemiological aspects of mastitis, mastitis pathogens, and mastitis control measures were studied, as were economic effects of mastitis and factors motivating dairy farmers to control mastitis. Based on the findings described in this thesis, the following conclusions can be drawn:

- Subclinical mastitis, although much more prevalent than CM (chapter 2), is mainly caused by contagious mastitis pathogens, is associated with high costs and is not well recognized by most of the dairy farmers (chapter 4 and 5).
- The most important measures to prevent new IMI caused by contagious pathogens in North-Western Ethiopia are improving milking hygiene and feeding of cows, in particular in Holstein Friesian × Zebu cross breed cows (chapter 2).
- The majority of *S. aureus* isolates are resistant to antimicrobials that are used for mastitis therapy in North-Western Ethiopia (chapter 3). Therefore, implementing effective preventative measures aiming to limit transmission is likely more effective than using antimicrobials to control mastitis.
- Dairy farmers underestimate the losses due to mastitis, which can be a reason for lack of motivation to implement mastitis control measures. This asks for specific education in this field.
- Intervention programs aiming to motivate dairy farmers to improve udder health should be performed primarily to change their attitude and secondarily to improve the farmers' subjective norms (chapter 6).

Recommendations for future research

The mastitis studies in North-Western Ethiopia described in this thesis deliver much information, but at the same time reveal that much is still unknown. Consequently, research work still needs to be done. The following subjects for future research are important to further facilitate mastitis control programs in North-Western Ethiopia.

- Although standard mastitis prevention measures such as dry cow treatment, PMTD, and proper milking procedures such as washing hands, fore-stripping and drying the teats are proven successful measures in reducing occurrence of mastitis, they have not been evaluated in Ethiopia. Because the Ethiopian smallholder dairy farms are quite different from the western dairy industry where most of the studies were done. Therefore, studies that evaluate the effects of these mastitis control measures in the Ethiopian situation are important to gain knowledge as well as to locally demonstrate their effect.
- To decrease antimicrobial resistance, antimicrobial usage should be limited as much as possible, which is highly influenced by management practices in the dairy herds. Additional studies are needed to identify management practices that can influence antimicrobial usage and can be applied in dairy herds in Ethiopia.
- *In vitro* tests of antimicrobial susceptibility of mastitis pathogens are often used to guide treatment decisions. However, because of lack of interpretive criteria specific for most mastitis therapeutics, interpretation of the antimicrobial susceptibility data relies on interpretive criteria developed for human data, which may result in over or under estimation of antimicrobial susceptibility. Therefore, further antimicrobial resistance studies and therapy efficacy studies are needed using clinical and field trials.
- When a cow is chronically infected with mastitis, culling is recommended both from the perspective of practical antibiotic use and from an economic perspective. However, the cross-bred cows are also required for breeding. Therefore, further research that optimizes the time of culling is required to make culling more cost effective.
- There are many CNS species causing mastitis, that have different characteristics and may ask for different control measures such as teat disinfection. Therefore, identifying the role of different species of CNS in mastitis is needed to implement targeted control measures.

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Summary

Mastitis is an important disease in dairy cattle worldwide. It causes milk production losses, leads to premature culling of cows and affects cows' welfare. Moreover, mastitis affects milk quality and public health due to an increased risk of antimicrobial residues in milk and the possible emergence of bacteria that are resistant to antimicrobials. In Ethiopia, mastitis is one of the most frequent diseases of dairy cows. Although mastitis control would enable an improvement in quantity and quality of milk production and cows' welfare, currently little attention is given to mastitis control programs in Ethiopia. Lack of adequate knowledge about the occurrence and consequences of mastitis in the country hinders quantification of the potential of improving the udder health situation. Moreover, implementation of mastitis control programs demands epidemiological and economic information and knowledge about factors motivating dairy farmers to control mastitis. As has been described in the first chapter of this thesis, the overall objective was to contribute to the knowledge on mastitis in Holstein Friesian × Zebu breed market-oriented dairy farms in North-Western Ethiopia in order to, in the future, be able to develop mastitis control programs in the region.

In chapter 2 the prevalence of mastitis and of its causative agents, as well as cow and herd level risk factors for mastitis are described, based on data collected from 167 dairy farms and 510 lactating cows. Cows were examined for clinical mastitis (CM) by visual inspection of the udder and the milk and for subclinical mastitis (SCM) by performing the California mastitis test (CMT). Milk samples were collected and cultured from all lactating quarters of a cow with CM or with one or more CMT positive quarters. Additionally, samples were cultured from a random selection of 50% of the cows that had four CMT negative quarters. Based on the data, 33% of the quarters and 62% of the cows were CMT positive. Coagulase negative staphylococci (CNS) (31%) and *Staphylococcus aureus* (9%) were the pathogens most frequently isolated. Statistical associations between several putative risk factors and SCM or a positive culture for any bacteria, CNS or *S. aureus* were tested by using mixed effect survey logistic regression modeling. Clinical mastitis history, higher parity, >150 days in milk (DIM) and herds with owners that have >10th grade level of education were associated with SCM. Culturing of any bacteria was associated with ≥25% Holstein Friesian blood level (HBL), >150 DIM, housing on cemented floors, and milking by squeezing compared to stripping. Likewise, 25–50% HBL, >150 DIM, and milking by squeezing were associated with culturing CNS. History of CM and larger herd size were associated with culturing of *S. aureus.* Checking the udder for CM, 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. The information is important in planning targeted udder health control programs in North-Western Ethiopia.

The genetic background and antimicrobial susceptibility of mastitis related *S. aureus*, isolated from Ethiopian dairy farms has not been studied previously. In chapter 3, 79 *S. aureus* isolates were characterized by *spa*, multilocus sequence typing, virulence factor typing and by assessment of antimicrobial susceptibility. A PCR was used to detect *lukM-lukF'* and *pvl* genes encoding the bovine and human associated bi-component leukocidins, the toxic shock syndrome toxin gene-1 (*tst*) and methicillin-resistance genetic determinant *mecA*. Antimicrobial susceptibility was determined using the broth microdilution method. Twenty different *spa* types were identified, most were t042 (58%), and the closely related t15786 (11%). The proportion of isolates positive for *lukM-lukF'*, *tst* and *pvl* was low at 0.04, 0.10 and 0.09, respectively, with *lukM-lukF'* often co-occurring with *tst*, but not with *pvl*. Methicillin-resistance was not found, but resistance to penicillin/ampicillin (86%) and tetracycline (54%) was very common. A

high degree of similarity was seen among *S. aureus* isolates, suggesting contagious transmission of strains that are often resistant to commonly used antimicrobials, within and between farms. This highlights the urgency of trying to limit transmission of bacteria and the occurrence of new intramammary infections, rather than using antimicrobials to control *S. aureus* mastitis in Ethiopia.

In Chapter 4 and 5, the costs of mastitis (CM as well as SCM) were estimated for Ethiopian market-oriented dairy farms with Holstein Friesian × Zebu cross-breed cows. In chapter 4, the economic effects of mastitis were described for a default farm size of 8 lactating cows by using a normative bio-economic simulation model. Production losses, culling, veterinary costs, treatment, discarded milk, and labor were modeled. The mean annual incidence of CM and SCM were 21.6%, varying between 0 and 50%, and 36.2%, varying between 0 and 75%, respectively. The total costs of mastitis were 6,709 Ethiopian birr (ETB) (1 ETB = 0.0449 USD) per farm per year with considerable variation between farms, 5th and 95th percentiles being 109 ETB and 22,009 ETB respectively. Most of the costs were contributed by culling. The costs of a case of CM and SCM were 3,631 ETB, varying from 0 to 12,401 ETB, and 147 ETB, varying from 0 to 412 ETB, respectively. The estimation of costs was most sensitive for variation in the occurrence of CM and culling.

In chapter 5, failure costs of mastitis (again CM as well as SCM) were quantified by using an economic calculation model. Empirical data were collected from 150 North-Western Ethiopian dairy farms, aiming to estimate the average total failure costs of mastitis and to quantify the between-farm variation in these costs. The data were collected by face-to-face interviews, farm visits and by performing CMT. Scientific literature was used to estimate milk production losses due to SCM. The average total failure costs of mastitis were 4,765 ETB per farm per year of which 54% was contributed to SCM. The average total failure costs per lactating cow per year were 1,961 ETB, varying between 0 and 7,357 ETB. The variation was mainly driven by the variation in incidence of CM and prevalence of SCM. Most of the costs (80%) were due to milk production losses. Culling contributed 13-17%, whereas costs of veterinary services, drugs, discarded milk and labor made only minor contributions to the total failure costs of mastitis. The main cost factors of mastitis in market oriented dairy farms in North-Western Ethiopia are milk production losses (particularly due to SCM) and culling (chapter 4 and 5). Creating awareness of the costs of SCM and the large variation in costs between farms may be important in motivating dairy farmers to take mastitis preventive measures.

In Chapter 6, the intentions of North-Western Ethiopian dairy farmers in mastitis management were studied using the framework of the Theory of Planned Behavior based on data collected from 134 dairy farmers that were collected by face-to-face interviews. The relation between determinants of motivation (attitude (AT), subjective norms (SN) and perceived behavioural control (PBC)) and the intentions of farmers to implement non-specified mastitis control measures (nsMCMs) and 4 specific mastitis control measures (sMCMs) were studied. Additionally the socio-demographic characteristics that were potentially affecting farmers' intentions through associations with their AT, SN and PBC were evaluated. The majority of farmers (93%) had a positive intention to implement nsMCMs. A smaller majority of farmers had the intention to implement one or more of the sMCMs: improving udder cleaning (87%), improving stall hygiene (78%), improving feeding of cows (76%), and performing foremilk stripping (74%). Farmers had a more positive AT, but lower SN and lower PBC towards implementing nsMCMs than towards most sMCMs. The SN for stall hygiene and the PBC for improving feeding of cows were low. Attitude was positively associated with intentions to implement nsMCMs, to improve cleaning of the udders, to improve stall hygiene and to implement foremilk stripping. Both the intention to improve udder cleaning and to implement foremilk stripping, were positively associated with the SN towards these sMCMs. It seems of primary interest to focus interventions to improve the intention of dairy farmers with respect to mastitis control in North-West Ethiopia on influencing dairy farmers’ attitude. A secondary focus could be on improving the farmers' subjective norms. Interventions could for instance be done by training and promotion programs.

Based on the findings described in this thesis, the following conclusions are drawn:

- Subclinical mastitis is more prevalent in North-Western Ethiopia than CM, is mainly caused by contagious mastitis pathogens and is associated with high costs.
- Improving milking hygiene and feeding of cows are the most important measures to prevent new intramammary infections caused by contagious pathogens in North-Western Ethiopia.
- Measures aiming to limit transmission in order to prevent new intramammary infections is likely more effective than using antimicrobials to control mastitis in North-Western Ethiopia.
- Underestimated losses due to mastitis can be a reason for lack of motivation of dairy farmers to implement mastitis control measures. It seems to be valuable to educate farmers on these aspects.
- Motivating dairy farmers to improve udder health seems to be most efficacious by primarily changing the farmers' attitude and by secondarily improving their subjective norm.

Samenvatting

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Wereldwijd is mastitis is een belangrijke aandoening bij melkvee. Het veroorzaakt melkproductieverliezen, voortijdige afvoer van koeien en vermindert het welzijn van de koe. Bovendien beïnvloedt mastitis de melkkwaliteit en de volksgezondheid doordat het risico op de aanwezigheid van antimicrobiële residuen in melk verhoogt en doordat er meer kans is op het ontstaan van bacteriën die resistent zijn tegen antimicrobiële middelen. In Ethiopië is mastitis en van de meest voorkomende ziektes bij melkkoeien. Hoewel een betere uiergezondheid tot een hogere melkproductie, een betere melkkwaliteit en een verbeterd welzijn van de koeien zou leiden, wordt er op dit moment weinig aandacht gegeven aan programma's om de mastitissituatie in Ethiopië te verbeteren. Het kwantificeren van het gunstige effect dat een betere uiergezondheid zou kunnen hebben wordt bemoeilijkt door het feit dat er onvoldoende kennis is over het vóórkomen en de gevolgen van mastitis in dit land. Verder zijn voor het implementeren van een programma om de mastitissituatie te verbeteren, epidemiologische en economische gegevens nodig, evenals kennis over factoren die bijdragen aan de motivatie van boeren om mastitis aan te pakken. In hoofdstuk 1 is beschreven dat het overkoepelende doel van dit proefschrift was om een bijdrage te leveren aan de kennis over mastitis in marktgeoriënteerde melkveebedrijven met Holstein Friesian (HF) × Zebu koeien in Noordwest Ethiopië, zodat in de toekomst effectieve mastitis programma's ontwikkeld kunnen worden in die regio.

In hoofdstuk 2 zijn de prevalentie van mastitis en de veroorzakers daarvan beschreven, evenals risicofactoren voor mastitis op koe- en op koppelniveau, gebaseerd op data die verzameld zijn bij 510 melkgevende koeien op 167 melkveebedrijven. De koeien zijn onderzocht op klinische mastitis (KM) door middel van visuele inspectie van uier en melk en op subklinische mastitis (SKM) door het uitvoeren van de California mastitis test (CMT). Van alle melkgevende kwartieren van koeien met KM en van koeien met één of meer CMT positieve kwartieren werden melkmonsters verzameld en op kweek gezet. Aanvullend werden monsters verzameld en onderzocht van een random steekproef van 50% van alle koeien die vier CMT negatieve kwartieren hadden. Op basis van deze data bleek 33% van de kwartieren en 62% van de koeien CMT positief te zijn. De meest voorkomende bacteriesoorten waren coagulase-negatieve staphylococcen (CNS) (31%) en *Staphylococcus aureus* (9%). Statistische associaties tussen mogelijke risicofactoren en SKM of het kweken van bacteriën ongeacht de soort, of het kweken van CNS of *S. aureus* werden getest middels mixed effect survey logistische regressie modellen. Een geschiedenis van KM, hogere pariteit, >150 dagen in lactatie en koppels van eigenaren die relatief veel onderwijs hebben genoten waren geassocieerd met SKM. Het kweken van bacteriën (ongeacht de soort) was geassocieerd met ≥25% HF bloed, >150 dagen in lactatie, huisvesting op een vloer van cement en met melken door spenen leeg te knijpen in plaats van door te . Vergelijkbaar daarmee was het kweken van CNS bacteriën geassocieerd met 25-50% HF bloed, >150 dagen in lactatie en melken door middel van het leegknijpen van de spenen. Een geschiedenis van KM en grotere koppels waren geassocieerd met het kweken van *S. aureus*. Het controleren van de uiers op KM, het verstrekken van voldoende voer en het bij de koe laten drinken van kalveren waren negatief geassocieerd met respectievelijk SKM, het kweken van bacteriën ongeacht de soort en het kweken van CNS. Op bedrijven van boeren die zijn aangesloten bij een zuivelcoöperatie was de kans op het vinden van SKM en het kweken van CNS hoger dan op andere bedrijven. Deze informatie is belangrijk bij het opstellen van gerichte uiergezondheidsprogramma's in Noordwest Ethiopië.

De genetische achtergrond en antimicrobiële gevoeligheid van mastitis-gerelateerde *S. aureus* stammen gekweekt op Ethiopische melkveebedrijven is niet eerder onderzocht. In hoofdstuk 3 zijn 79 *S. aureus* isolaten gekarakteriseerd door middel van *spa* typering, multilocus sequence typering, detectie van virulentie factoren en het bepalen van antimicrobiële gevoeligheid. Middels PCR werd de aanwezigheid van *lukM-lukF'* en *pvl* onderzocht, genen die voor de boviene en humane leukocidines coderen, en werd gekeken naar het toxic shock syndroom toxine gen-1 (*tst*) en naar het gen dat methicilline-resistentie veroorzaakt, *mecA*. Antimicrobiële gevoeligheid werd bepaald met behulp van de broth microdilution methode. Er zijn twintig verschillende *spa* typen gevonden, waarvan de meerderheid behoorde tot het type t042 (58%) en het nauw verwante t15786 (11%). Het percentage isolaten positief voor *lukM-lukF'*, *tst*, en *pvl* was laag, respectievelijk 4%, 10% en 9%, waarbij *lukM-lukF'* vaak samenging met *tst* maar niet met *pvl*. Methicilline resistentie werd niet gevonden, maar resistentie tegen penicilline/ampicilline (86%) en tetracycline (54%) kwam veel voor. We zagen een grote mate van gelijkenis tussen de onderzochte *S. aureus* stammen, wat suggereert dat contagieuze verspreiding binnen en tussen bedrijven plaatsvindt, van bacteriën die ongevoelig zijn voor veelgebruikte antimicrobiële middelen. Dit onderstreept het belang van het beperken van transmissie van bacteriën om nieuwe infecties te voorkomen, boven het gebruik van antimicrobiële middelen om *S. aureus* mastitis in Ethiopië te bestrijden.

In hoofdstuk 4 en 5 zijn de kosten van mastitis (zowel KM als SKM) geschat voor marktgeoriënteerde melkveehouderijen met HF × Zebu koeien in Ethiopië. In hoofdstuk 4 zijn de economische effecten van mastitis beschreven voor een standaard bedrijf met 8 melkgevende koeien, door gebruik te maken van een bio-economisch simulatie model. Hierbij zijn melkproductie verliezen, afvoer, diergeneeskundige kosten, behandeling, weggegooide melk en arbeid gemodelleerd. De gemiddelde jaarlijkse incidentie van KM en SKM waren respectievelijk 21,6%, variërend tussen 0 en 50%, en 36,2%, variërend tussen 0 en 75%. De totale kosten van mastitis waren 6.709 Ethiopische Birr (ETB) (1 ETB = €0,03) per bedrijf per jaar, met aanzienlijke variatie tussen bedrijven: de 5de en 95ste percentielen waren 109 ETB en 22.009 ETB. De meeste kosten waren het gevolg van afvoer. De kosten van een geval van KM bedroegen 3.631 ETB, variërend van 0 tot 12.401 ETB. Een geval van SKM kostte 147 ETB, variërend van 0 tot 412 ETB. De kostenschatting was het meest gevoelig voor variatie in het vóórkomen van KM en van afvoer.

In hoofdstuk 5 zijn de faalkosten van mastitis (opnieuw KM en SKM) gekwantificeerd met een economisch rekenmodel Op 150 Noordwest Ethiopische melkveebedrijven zijn empirische data verzameld, waarbij het doel was om de gemiddelde totale faalkosten van mastitis in te schatten en de variatie in kosten tussen bedrijven te kwantificeren. De data werden verzameld via interviews, bedrijfsbezoeken en het uitvoeren van CMT op de bedrijven. De wetenschappelijke literatuur is gebruikt om melkproductieverliezen gerelateerd aan SKM in te schatten. De gemiddelde totale faalkosten van mastitis waren 4.765 ETB per bedrijf per jaar, waarvan 54% het gevolg was van SKM. De gemiddelde totale faalkosten per lacterende koe per jaar bedroegen 1.961 ETB, variërend tussen 0 en 7.357 ETB. Deze variatie werd voornamelijk veroorzaakt door variatie in de incidentie van KM en de prevalentie van SKM. De meeste kosten (80%) waren het gevolg van melkproductieverliezen. Afvoer droeg 13-17% bij aan de kosten, terwijl kosten voor diergeneeskundige diensten, diergeneesmiddelen, weggegooide melk en arbeid slechts een kleine bijdrage leverden aan de totale faalkosten van mastitis. The belangrijkste kostenposten op marktgeoriënteerde melkveebedrijven in Noordwest Ethiopië zijn melkproductie verliezen (met name als gevolg van SKM) en afvoer (hoofdstuk 4 en 5). Het ontwikkelen van bewustzijn omtrent de kosten van SKM en de grote verschillen in kosten tussen bedrijven zijn mogelijk belangrijk om melkveehouders te motiveren om preventieve maatregelen tegen mastitis te nemen.

In hoofdstuk 6 zijn de intenties van melkveehouders in Noordwest Ethiopië op het gebied van mastitis management bestudeerd. Hiertoe zijn via interviews gegevens van 134 melkveehouders verzameld, waarbij de Theorie van gepland gedrag als raamwerk werd gebruikt. De relatie tussen de determinanten van motivatie (attitude (AT), subjectieve normen (SN) en ingeschatte beheersing van gedrag (IBG)) en de intentie van boeren om niet-specifieke mastitis beheersmaatregelen (nsMBMs) en 4 specifieke mastitis beheersmaatregelen (sMBMs) te implementeren werd bestudeerd. Daarnaast werden sociaal-demografische karakteristieken geëvalueerd die mogelijk via associaties met AT, SN en IBG van invloed waren op de intentie van melkveehouders. De meerderheid van de melkveehouders (93%) had een positieve intentie om nsMBMs te implementeren. Een iets kleinere meerderheid van de veehouders had de intentie om een of meer sMBMs te implementeren: uiers beter schoonmaken (87%), stalhygiëne verbeteren (78%), beter voeren (76%) en voorstralen (74%). Veehouders hadden een positievere AT, maar een lagere SN en IBG voor het implementeren van nsMBMs dan voor het implementeren van de meeste sMBMs. De SN voor stalhygiëne en de IBG voor het beter voeren van koeien waren laag. Attitude was positief geassocieerd met intenties om nsMCMs te implementeren, om uiers beter schoon te maken, de stalhyiëne te verbeteren en om te gaan voorstralen. Zowel de intentie om de uiers beter schoon te maken als om te gaan voorstralen waren positief geassocieerd met de SN ten opzichte van deze sMBMs. Om de intentie van melkveehouders ten aan zien van een verbetering van het mastitis management te verbeteren lijkt het primair van belang om aandacht te geven aan de beïnvloeding van de attitude van veehouders en secundair aan het verbeteren van de subjectieve normen. Dat zou bijvoorbeeld door middel van training en promotie programma’s bereikt kunnen worden.

Op basis van de bevindingen die beschreven zijn in dit proefschrift zijn de volgende conclusies getrokken:

- In Noordwest Ethiopië komt meer SKM voor dan KM, wordt mastitis vooral veroorzaakt door besmettelijke mastitis verwekkers en gaat gepaard met hoge kosten. Het verbeteren van melkhygiëne en het voeren van koeien zijn de belangrijkste maatregelen om nieuwe intramammaire infecties veroorzaakt door besmettelijke pathogenen in Noordwest Ethiopië te voorkomen.
- Maatregelen om transmissie te beperken om op die manier nieuwe intramammaire infecties te voorkomen zijn waarschijnlijk effectiever dan het gebruik van antimicrobiële middelen om mastitis in Noordwest Ethiopië te beheersen.
- Een onderschatting van melkproductieverliezen ten gevolge van mastitis kan een reden zijn voor een gebrek aan motivatie van melkveehouders om maatregelen te implementeren om mastitis te bestrijden. Het lijkt belangrijk om veehouders hier voorlichting over te geven.
- Melkveehouders lijken vooral gemotiveerd te worden om uiergezondheid te verbeteren door hun attitude te beïnvloeden en door hun subjectieve normen te verbeteren.

ማጠቃለያ

የጡት በሽታ በመላዉ ዓለም የወተት ላሞችን የሚያጠቃ በሽታ ነዉ፡፡ የጡት በሽታ የወተት ምርት እንዲቀንስ፤ የወተት ላሞች የምርት ጊዜአቸዉን ሳይጨርሱ ከእርባታ እንዲወገዱና ደህንነታቸዉ እንዲጓደል ያደርጋል፡፡ በተጨማሪ የጡት በሽታ የወተትን ጥራት በመቀነስና በወተት ዉስጥ ለሚገኙ የመድሀኒት ቅሬቶች በማጋለጥና መድሐኒት ለተለማመዱ ባክቴሪያዎች መንስዔ በመሆን የህብረተሰብን ጤና ይጎዳል፡፡ የጡት በሽታ በኢትዮጵያ ዉስጥ የወተት ላሞችን በተደጋጋሚ ከሚያጠቁ ዋና ዋና በሽታዎች አንዱ ነዉ፡፡ ምንም እንኳ የጡት በሽታን መቆጣጠር የወተት ምርትን መጠንና ጥራት እና የወተት ላሞችን ደህንነት ለማሻሻል የሚያስችል ቢሆንም በአሁኑ ወቅት በኢትዮጵያ ለጡት በሽታ ቁጥጥር የተሰጠዉ ትኩረት አናሳ ነዉ፡፡ በሐገሪቱ ዉስጥ የጡት በሽታ ሁኔታ ሊሻሻል የሚቻልበትን እድል አሳንሶታል፡፡ በተጨማሪ የጡት በሽታ ቁጥጥር መርሃ ግብር ትግበራ የስነ-ምህዳርና የኢኮኖሚ መረጃ እንዲሁም የጡት በሽታ ቁጥጥር ለማከናወን አርቢዎችን የሚያበረታቱ ጉዳዮችን ማወቅ ይጠይቃል፡፡ በዚህ መጽሐፍ የመጀመሪያ ምራፍ እንደተገለጸዉ አጠቃላይ አላማዉ በሰሜን ምዕራብ ኢትዮጵያ የሆልስቲን ፍሮዥያንና የዜቡ ከብቶችን በማዳቀል በተገኙ ላሞች ለሚከናወኑ ገበያ ተኮር ለሆኑ የወተት እርባታ ጣቢያዎች የጡት በሽታ ቁጥጥር መርሃ ግብር ለመንደፍ እንዲቻል ስለጡት በሽታ እዉቀት እንዲየድግ አስተዋጽዖ ማድረግ ነዉ፡፡

በምዕራፍ 2 ከ167 የእርባታ ጣቢያዎችና 510 የሚያጠቡ ላሞች በተሰበሰበ መረጃ መሰረት በማድረግ የጡት በሽታ ክስተትና የጡት በሽታ መንስዔ የሆኑ ባክቴሪያዎች እንዲሁም በላምና በመንጋ ደረጃ ያሉ ለጡት በሽታ የሚያጋልጡ ሁኔታዎች ተገልጸዋል፡፡ ክሊኒካል ለሆነዉ የጡት በሽታ የግቱንና ወተቱን በማየት ንዑስ ክሊኒካል ለሆነዉ ካሊፎርኒያ የጡት በሽታ መመርመሪያ በመጠቀም ላሞች ለጡት በሽታ ተመርምረዋል፡፡ የጡት በሽታ መንስዔ የሆኑ ባክቴሪያዎችን ለመለየት ክሊኒካል ወይም ንዑስ ክሊኒካል የጡት በሽታ በአንዱ ወይም ከአንድ በላይ በሆኑ ጡቶች ከተገኘባቸዉ ላሞች ሁሉም የሚታለቡ ጡቶች የወተት ናሙና ተሰብስቧል፡፡ በተጨማሪ በካሊፎረኒያ የጡት በሽታ መመርመሪያ ከጡት በሽታ ነፃ ከሆኑ ላሞች 50% በዕጣ ተመርጠዉ የወተት ናሙና ተሰብስቦ የጡት በሽታ መነስዔ የሆኑ ባክቴሪያዎች ተለይተዋል፡፡ በመረጃዉ መሰረት 33% ጡቶች 62% ላሞች በካሊፎርኒያ የጡት በሽታ መመርመሪያ በጡት በሽታ የተጠቁ ሆነዉ ተገኝተዋል፡፡ ኳጉሌዝ የሌላቸዉ ስታፍሎኮካይ (31%) ስታፍሎኮከስ ኦርየስ (9%) ተደጋግመዉ በብዛት ተለይተዋል፡፡ የላሞችና የእረባታ ጣቢየዎች መረጣ እኩል እድል ባልተሰጠበት ሁኔታ የሚያገለግል የዳሰሳ ጥናት ሞዴል አማካኝነት ብዛት ያላቸዉ ለጡት በሽታ ያጋልጣሉ ተብለዉ በትገመቱ ፋክተሮቸና በንዑስ ክሊኒካል የጡት በሽታ፤ የጡት በሽታ መንስዔ ናቸዉ የተባሉ ባክቴሪያዎች፤ ኳጉሌዝ በሌላቸዉ ስታፍሎኮካይና ስታፍሎኮከስ ኦርየስ መካከል ስታቲስቲክሳዊ ግንኙነታቸዉ ተጠንቷል፡፡ ክሊኒካል በሆነዉ የጡት በሽታ ተጠቅቶ ማወቅ፤ ብዙ መዉልድ፤ ከ150 ቀን በላይ መታለብና የእርባታ ጣቢያዉ ባለቤቶች ከ10ኛ ክፍል በላይ የትምህርት ደረጃ ያላቸዉ መሆን ከንዑስ ክሊኒካል የጡት በሽታ ጋር ስታቲስቲክሳዊ ግኑኙነት አላቸዉ፡፡ የሆሊስቲይን ፍሬዥያን የደም መጠን 25% እና ከዚያ በላይ መሆን፤ ከ150 ቀን በላይ መታለብ፤ በረቱ ሊሾ የሆነ ከሆነና ጡቱን በመጨበጥና በመጨመቅ ማለብ የጡት በሽታ መንስዔ ናቸዉ የተባሉ ባክቴሪያዎችን ከመለየት ጋር ግንኙነት አላቸዉ፡፡ በተመሳሳይ የሆልስቲን የደም መጠናቸዉ ከ25-50% የሆኑ፤ ከ150 ቀን በላይ መታለብና ጡቱን በመጨበጥና በመጨመቅ ማለብ ኳጉሌዝ የሌላቸዉ ስታፍሎኮካይ ባክቴሪያዎችን ከመለየት ጋር ግንኙነት አላቸዉ፡፡ በክሊኒካል የጡት በሽታ ተጠቅቶ ማወቅና በመንጋዉ ዉስጥ ያሉት የላሞች ቁጥር መጨመር ስታፍሎኮከስ ኦርየስ ከመለየት ጋር ግንኙነት አላቸዉ፡፡ ግታቸዉ በጡት በሽታ መያዝ አለመያዙን መመልከት፤ ላሞች የፈልጉትን ያህል መመገብና ጥጆች ላሞችን እንዲጠቡ ማድረግ እንደቅደም ተከተላቸዉ ንዑስ ክሊኒካል የጡት በሽታን፤የጡት በሽታ መንስዔ ናቸዉ የተባሉ ባክቴሪያወችንና ኳጉሌዝ ከሌላቸዉ ስታፍሎኮካይ ባክቴሪያዎች ተቃራኒ ግንኙነት አላቸዉ፡፡ በወተት አምራች ማህበር አባላት በተያዙ የእርባታ ጣቢያዎች ንዑስ ክሊኒካል የጡት በሽታና ኳጉሌዝ የሌላቸዉ ስታፍሎኮካይን መለየት ከፍተኛ ስታቲስቲስቲካል ዖድ አላቸዉ፡፡ ግኝቱ በሰሜን መዕራብ ኢትዮጵያን ትኩረት ያደረገ የግት ጤና ቁጥጥር መርሀ ግብር ለማቀድ ጠቃሚ ነዉ፡፡

በኢትዮጵያ ዉስጥ ከሚገኙ የወተት እርባታ ጣቢያዎች የተለዩ ከጡት በሽታ ጋር ግንኙነት ያላቸዉ ስታፍሎኮከስ ኦርየስ የጀነቲክ ዝርያቸዉና መድሀኒቶችን መለማመድ አለመለማመዳቸዉ ከዚህ ቀደም ተጠንቶ አያዉቅም፡፡ በሦስተኛዉ ምዕራፍ 79 ስታፍሎኮከስ ኦርየስ በኤስ ፒ ኤ ታይፒንግ፤ መልቲ ሎከስ ስኩዌንስ ታይፒነግ፤ ቫይሩለንስ ፋክተር ታይፒንግና መድሃኒቶችን መለማመዳቸዉን ወይም አለመለማመዳቸዉን በመለካት ተተንትነዋል፡፡ ቫይሩለንስ ፋክተርን፤ ፒ ቪ ኤል ጅን፤ ሉክ ኤም ሉክ ኤፍና ከከብትና ከሰዉ ጋር ግንኙነት ያለዉ ሉኮሲዲን ኮድ የሚያደርገዉ ፒ ቪ ኤል ጅን፤ ቶክሲክ ሾክ ሲንድረም ቶክሲን 1 እና መቲሲሊን ለመቋቋም የሚያስችለዉን ሜክ ኤ ጅን ለማጥናት ፒ ሲ አር የተባለ መሳሪያ ጥቅም ላይ ዉሏል፡፡ የህክምና መድሀኒቶችን መለማመድ አለመለማመዳቸዉን ብሮዝ ማይክሮ ዳይሉሽን በተባለ ዘዴ ታይቱአል፡፡ ሀያ የተለያዩ የኤስ ፒ ኤ አየነቶች የተለዩ ሲሆን አብዛኛዎቹ ቲ042 (58%) እና ከዚህ ጋር የቀረበ ዝምድና ያለዉ ቲ15786 (11%) ናቸዉ፡፡ ሉክ ኤም ኤፍ፤ ቲ ኤስ ቲ እና ፒ ቪ ኤል በዝቅተኛ መጠን የተገኙ ሲሆን እንደ ቅደም ተከተላቸዉ 0.04፤ 0.10 እና 0.09 ድርሻ የነበራቸዉ ሲሆን ብዙ ጊዜ ሉክ ኤም ኤፍ ከቲ ኤስ ቲ ጋር አብሮ ሲገኝ ከፒ ቪ ኤል ጋር ግን አልተገኘም፡፡ ሜቲሲሊን የተለማመዱ ያልተገኙ ሲሆን ፔኒሲሊን/አምፒሲሊን (86%) እና ቴትራሳይክሊን (54%) የተለማመዱ ግን ተገኝተዋል፡፡ በስታፍሎኮከስ ኦርየሶች መካከል የታየዉ ከፍተኛ የሆነ መመሳሰል ብዙ ጊዜየተለመዱ ለጡት ህክምና የሚያገለግሉ መድሀኒቶችን የተለማመዱ ዝርያዎች በእርባታ ጣቢያዎች ዉስጥና በእርባታ ጣቢያዎች መካከል በንክኪ የሚዛመቱ መሆኑን አመላካች ነዉ፡፡ ይህ በኢትዮጵያ ውስጥ በስታፍሎኮከስ ኦርያስ ምክንያት የሚከሰተውን የጡት በሽታ ለመቆጣጠር መድሃኒት ከመጠቀም ይደልቅ የባክቴሪያዎችን መዛመትና አዲስ የጡት በሽታ ክስትትን ለማስቀረት መሞከር አፋጣኝ እርምጃ መሆኑን አመላካች ነው፡፡

በምዕራፍ 4 ና 5 በኢትዮጵያ የሆልስትን ፍሬዢያንና የዜቡ ድቅል ላሞችን በማርባት ገበያ ተኮር በሆኑ የእርባታ ጣቢያዎች የጡት በሽታ (ክሊኒካል እንዲሁም ንዑስ ክሊኒካል) የሚያስከትለው ኪሳራ ተሰልቷል፡፡ በምዕራፍ 4 ከዚህ ቀደም ስለጡት በሽታ የተፃፉ መረጃዎችን በመጠቀምና በእርባታ ጣቢዎች የላሞች መጠን 8 እንደሆነ በመውሰድ የጡት በሽታ የሚያስደከትለው ኢኮኖሚያዊ ኪሳራ ተገልጿል፡፡ የወተት ምርት መቀነስ፤ ምርት የሚሰጡበት ጊዜ ሳያልቅ ላሞች ከእርባታ ጣቢያ መወገድ፤የባለሙያ ክፍያ፤ የመድሃኒት ዋጋ፤ በጡት በሽታ ምክንያት የሚጣል ወተትና ተጨማሪ የጉልበት ኪሳራን በማካተት ተሰልቷል፡፡ የክሊኒካልና የንዑስ ክሊኒካል የጡት በሽታዎች ዓመታዊ አማካኝ ክስተት እንደቀደም ተከተላቸው 21.6% (ካለመከሰት እስከ 50% ይደርሳል) እና 36.2% (ካለመከሰት እስከ 75% ይደርሳል) ነበር፡፡ በአማካኝ በአንድ የእርባታ ጣቢያ የጡት በሽታ በአጠቃላይ 6፣709 የኢትዮጵያ ብር (ኢትብ) (1 ኢትብ =

0.0049 የአሜሪካን ዶላር) ኪሳራ የሚያስከትል ሲሆን በእርባታ ጣቢያዎች መካከል ከፍተኛ ልዩነት አለዉ፡ 5ኛና 95ኛ ፐርሰንታይል እንደቅደም ተከተላቸዉ 109 ኢትብ እና 22,009 ኢትብ ነዉ፡፡ ላሞች የምርት ጊዜአቸውን ሳይጨርሱ በመወገዳቸው ምክንያት የሚደርሰው ኪሳራ ከፍተኛዉን ድርሻ ይይዛል፡፡ አንድ ጡት በክሊኒካልና በንዑስ ክሊኒካል የጡት በሽታ ሲጠቃ እንደቅደምተከተላቸዉ 3,631 ኢትብ (ከምንም እስከ 12,401 ኢትብ ይደርሳል) በንዑስ ክሊኒካል ሲጠቃ 157 (ከምንም አስከ 412 ኢትብ ይደርሳል) ኪሳራ ይደርሳል፡፡ የክሊኒካል የጡት በሽታ ክስተትና ላሞች የምርት ጊዜአቸዉን ሳይጨርሱ ከእርባታ ጣቢያ መወገድ መለያት የሚደርስዉን ኪሳራ በቀላሉ ያለያየዋል፡፡

በምዕራፍ 5 የጡት በሽታ (ክሊኒካልና ንዑስ ክሊኒካል) ቁጥጥር ባለመደረጉ የሚያደርሰው ኪሳራ ኢኮኖሚክ ሞዴል በመጠቀም ተሰልቷል፡፡ የጡት በሽታ ቁጥጥር ባለመደረጉ የሚያደርሰው ኪሳራ በአማካኝ በእርባታ ጣቢያዎች መካከል ያለውን የኪሳራ ልዩነት ለማስላት በሰሜን ምዕራብ ኢትዮጵያ ከሚገኙ 150 የወተት አምራች ገበሬዎች መረጃ ተሰብስቧል፡፡ መረጃው አርቢወችን በግንባር በመገኘትና በመጤቅ፤ የእርባታ ጣቢዎችን በአካል በመጎብኘትና ካሊፎርኒያ የጡት በሽታ መመርመሪያ በመጠቀም እንዲሁም ንዑስ ክሊኒካል የጡት በሽታ ለሚያደርሰው የወተት ምርት መቀነስ ከዚህ ቀደም ከተጠኑ ጥናቶች መረጃ ተሰብስቧል፡፡ የጡት በሽታ ቁጥጥር ባለመደረጉ የሚደርሰው አማካኝ ኪሳራ በአንድ የእርባታ ጣቢያ በአመት 4,765 ኢትብ ሲሆን ከዚህ ውስጥ 54% የሚሆነው በንዑስ ክሊኒካል የጡት በሽታ ድርሻ ነዉ፡፡ የጡት በሽታ ቁጥጥር ባለመደረጉ ምክንያት በላም በአማካኝ 1,961 ኢትብ (ከምንም እስከ 7,357 ኢትብ ይለያያል) ኪሳራ ይደርሳል፡፡ የሚያደርሰው ኪሳራ ልዩነት በአብዛኛው በክሊኒካልና ንዑስ ክሊኒካል የጡት በሽታ ክስተት ልዩነት ነው፡፡ አብዛኛው ኪሳራ (80%) የጡት በሽታ የወተት ምርት እንዲቀንስ በማድረጉ ምክንት የሚደርስ ነው፡፡ ላሞች ያለጊዜአቸው እንዲወገዱ በማድረግ የሚደርሰው ኪሳራ ከ13-17% ድርሻ ያለው ሲሆን የባለሙያ ክፍያ፤ የመድሃኒት፤በጡት በሽታ ምክንያት የሚደፋ ወተትና ተጨማሪ የጉልበት ኪሳራ አጠቃላይ በጡት በሽታ ቁጥጥር ባለመደረጉ ለሚደርሰው ኪሳራ ያለው አስተዋጽኦ ዝቅተኛ ነው፡፡ በሰሜን መዕራብ ኢትዮጵያ በሚገኙ ገበያ ተኮር በሆኑ የወተት እርባታ ጣቢያዎች የጡት በሽታ አብዣኛዉ ኪሳራ የሚደርሰዉ የወተት ምርት እንዲቀንስ (በተለይ በንዑስ ክሊኒካል የጡት በሽታ ምክንያት) እና ላሞች ምርት የሚሰጡበት ጊዜ ተቋርጦ ከእርባታ ጣቢያ እነዲወገዱ በማድረግ ነዉ (መዕራፍ 4 እና 5)፡፡ ንዑስ ክሊኒክል የጡት በሽታ የሚያደርሰውን ኪሳራና በእርባታ ጣቢያዎች መካከል ያለውን ከፍተና የኪሳራ ልዩነት በአርቢዎች ለያ እዉቅና መፍጠር አርቢዎች የጡት በሽታ ቁጥጥር እንዲያደርጉ ለማበረታታት ጠቃሚ ሊሆን ይችላል፡፡

በምዕራፍ 6 የሰሜን ምዕራብ ኢትዮጵያ ወተት አምራች ገበሬዎች ስለጡት በሽታ ያላቸውን ፍላጎት ቲዎሪ ኦፍ ፕላንድ ቢሄቪየር የተባለ ሞዴል ከ134 የወተት አምራች ገበሬዎች በግንባር በመጠየቅ የተሰበሰበ መረጃ በመጠቀም ተጠንቷል፡፡ በጥቅሉ የጡት በሽታን ለመቆጣጠር የሚያገለግሉ እርምጃዎችን ለመተግበር ያላቸው ፍላጎትና ገበሬዎችን ለማበረታት ውሳኝ በሆኑ ጉዳዮች (አቲቲዉድ፤ ሰብጀክቲቭ ኖረምና ፐርሲብድ ቢሄቭየራል ኮንትሮል) መካከል ያለው ግንኙነት ተጠንቷል፡፡ በተጨማሪ ከአቲቲዉድ፤ ሰብጀክቲቭ ኖረምና ፐርሲብድ ቢሄቭየራል ኮንትሮል ጋር በመጎዳኘት ገበሬዎች የጡት በሽታን ለመቆጣጠር ባላቸው ፍላጎት ላይ ተፅዕኖ ይኖራቸዋል ተብለው የተገመቱ የህብረተሰብ መገለጫ የሆኑ ጉዳዮች ተጠንተዋል፡፡ በጥናቱ መሠረት ብዙዎቹ ገበሬዎች (93%) በጥቅሉ የጡት በሽታን ለመቆጣጠር የሚያገለግሉ እርምጃዎችን ለመተግበር ፖዘቲቭ እንቴንሽን አላቸው፡፡ ትንሽ አነስ ያሉ ግን ብዛት ያላቸዉ ገበሬዎች አንድ ወይም ከዚህ በላይ የሆኑ ሰብጀክቲቭ ኖረም አላቸው፡ የግት ንፅህናን ለማሻሻል (87%)፤ የበረቱን ንጽህና ለማሻሻል (78%)፤ የላሞችን አመጋገብ ለማሻሻል (76%) እና ፎርስተሪፒንግ (74%)፡፡ ከሚታወቁ የጡት በሽታን ለመቆጣተር ከሚያገለግሉ ዘዴዎች ይልቅ በጥቅሉ የጡት በሽታን ለመቆጣጠር ለሚያገለግሉ ዘዴዎች የበለጠ ፖዘቲቭ የሆነ አቲቲዉድ ሲኖራቸዉ ዝቅ ያለ ሰብጀክቲቭ ኖረምና ፐርሲብድ ቢሄቭየራል ኮንትሮል አላቸዉ፡፡ የበረቱን ንጽህና ለማሻሻል ሰብጀክቲቭ ኖረምና የላሞችን አመጋገብ ለማሻሻልፐርሲብድ ቢሄቭየራል ኮንትሮል ዝቅተኛ ናቸዉ፡፡ አቲቲዉድ በጥቅሉ የጡት በሽታን ለመቆጣጠር ከሚረዱ ዘዴዎች፤ የግት ንፅህናን ከማሻሻል፤ የበረቱን ንጽህና ከማሻሻልና ከፎርስተሪፒንግ ጋር ፖዘቲቭ ግንኙነት አላቸዉ፡፡ የግት ንፅህናን ለማሻሻልና ፎርስተሪፒንግ ለመተግበር ያላቸዉ እንቴንሽን ከነዚህ እርምጃዎች ሰብጀክቲቭ ኖረም ጋር ፖዘቲቭ ግንኙነት አላቸዉ፡፡ በሰሜን ምእራብ ኢትዮጵያ የጡት በሽታን ከመቆጣጠር ጋር በተያያዘ ጣልቃ ለመግባት ትኩቱ ቅድሚያ በገበሮዎች አቲቲዉድ ላይ ተጽዕኖ በመፍጠር የገበሬዎችን እንቴንሽን በማሻሻል መሆን ያለበት ይመስላል፡፡ የገበሬዎችን ሰብጀክቲቭ ኖረም ማሻሻል ሁለተኛዉ ትኩረት መሆን አለበት፡፡ ጣልቃ ገብነቱ ለምሳሌ በማሰልጠንና በሚድያ በማስተዋወቅ ሊሆን ይችላል፡፡

በመጽሀፉ ዉስጥ በተገለጹት ግኝቶች መሰረት የሚከተሉት መደምደሚያዎች ተሰጥተዋል፤

- በሰሜን ምዕራብ እትዮጵያ ከክልኒካል የጡት በሽታ የልቅ ንኡስ-ክሊኒካል የጡት በሽታ የበለጠ ተስፋፍቷል፤ በአብዛኛዉ በንክኪ በሚዛመቱ ጀርሞች ምክንያት ይከሰታል እናም ከከፍተኛ ኪሳራ ጋር ተያያዥነት አለዉ፡፡
- በሰሜን ምዕራብ እትዮጵያ በንክኪ በሚዛመቱ ጀርሞች ምክንያት አዲስ የሚከሰተቱ የጡት በሽታዎችን ለመከላከል የአለባን ንጽህናና የላሞችን አመጋገብ ማሻሻል በጣም ጠቃሚ ረምጃዎች ናቸዉ፡፡
- በሰሜን ምዕራብ ኢትዮጵያ አዲስ የሚከሰተቱ የጡት በሽታዎችን ለመከላከል የጡት በሽታን ለመቆጣጠር መድሁኒት ከመጠቀም የልቅ መሰራጨትን መግታት አላማ ያደረጉ ርምጃዎች የበለጠ ዉጤታማ የመሆን ዕድል አላቸዉ፡፡
- በጡት በሽታ ምክንያት ለሚደርሰዉ ኪሳራ ዝቅተኛ ግምት የወተት አምራች ገበሬዎች የጡት በሽታ መቆጣጠሪያ ርምጃዎችን ለመተግበር አለመበረታታት ምክንያት ሊሆን ይችላል፡፡
- የግትን ጤና ለመሻሻል በመጀምሪያ የገበሬዎችን አቲቲዉድ በመቀየር በሁለተኛ ደረጃ የነሱን ሰብጀክቲቭ ኖረም በማሻሻል የወተት አምራች ገበሬዎችን ማበረታታት በጣም ፍቱን ይመስላል፡፡

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Curriculum vitae

Sefinew Alemu Mekonnen was born on August 07, 1973 in Motta, Gojjam, Ethiopia. He attended primary education in Awuja elementary school and secondary school in Motta. In1994, he joined the Faculty of Veterinary Medicine, Addis Ababa University and graduated as Doctor of Veterinary Medicine in 1999. From 2000-2005 he worked as a veterinary practitioner under the Bureau of Agriculture of Amhara National Regional State, Ethiopia. From 2006-2007 he did his master's degree in veterinary microbiology at Addis Ababa University and he joined Faculty of Veterinary Medicine and Animal sciences, University of Gondar in the department of Veterinary Clinical Medicine.

In the Faculty of Veterinary Medicine and Animal sciences, University of Gondar, he served as head of the Department of Veterinary Clinical Medicine form 2010 to 2012. From September 2013 to April 2018, he did his PhD studies on mastitis management in urban and peri-urban dairy herds of North-Western Ethiopia at the department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands under the chair group of prof. Theo J. G. M. Lam. He is author and co-author of several publications in local and international peer-reviewed journals.

His research interest is on diseases in food animal production and their impact on economy and health of people.

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